Aus der Universitätsklinik und Poliklinik für Innere Medizin I

(Direktor: Prof. Dr. med. Th. Seufferlein)

Einfluss, Mediatoren und Bedeutung der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber

zur Erlangung des akademischen Grades

Dr. med. habil.

vorgelegt der Medizinischen Fakultät

der Martin-Luther-Universität Halle-Wittenberg

von Dr. med. Alexander Bernt Walter Zipprich

geboren am 23.Dezember 1972 in Halle (Saale)

Gutachter:

1.

2.

3.

Verteidigungsdatum Habilitationsschrift Dr. Alexander Zipprich:
16.10.2012

Gutachter:

Prof. Dr. F. Lammert, Universität Homburg

Prof. Dr. H. Schmidt, Universität Münster

Kurzreferat und bibliografische Gestaltung

Die Blutversorgung der Leber wird durch die Portalvene und die Leberarterie gewährleistet. Entwicklung einer Leberzirrhose führt zu intrahepatischen Veränderungen des Gefässwiderstandes mit Erhöhung des sinusoidalen und portalen Drucks. Die Veränderungen der hepatisch-arteriellen Durchblutung bei Zirrhose sind bisher nur unzureichend untersucht worden.

Die untersuchten Fragestellungen der vorliegenden Arbeit waren der Einfluss der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck, die Mediatoren der hepatisch-arteriellen Blutflussänderung und die Bedeutung der hepatisch-arteriellen Durchblutung für die mikrosomale Leberfunktion in der zirrhotischen Leber.

Die Ergebnisse zeigen, dass eine hepatisch-arterielle Vasodilatation bei Zirrhose vorliegt, wodurch der Anteil der hepatisch-arteriellen Durchblutung an der Lebergesamtdurchblutung steigt und somit auch zur Erhöhung des sinusoidalen und portalen Drucks beiträgt. Als Mediatoren der hepatisch-arteriellen Vasodilatation konnte im Tiermodell vor allem Stickstoffmonoxid identifiziert werden. Die Stickstoffmonoxid-abhängige Abnahme der Gefässmuskelschichtdicke (Remodeling) war in der Leberarterie zirrhotischer Ratten nachweisbar, was eine weitere hepatisch-arterielle Vasodilatation und eine verminderte Kontraktionsfähigkeit der Leberarterie zur Folge hat. Adenosin konnte als weiterer potenter Vasodilatator der Leberarterie im Tiermodell und bei Patienten mit Zirrhose nachgewiesen werden. Eine Adenosin-induzierte hepatisch-arterielle Vasodilatation und eine systemische Sauerstoffgabe erhöhten die mikrosomale Leberfunktion bei Patienten mit Zirrhose.

Zusammenfassend lässt sich darlegen, das die hepatisch-arterielle Durchblutung in der zirrhotischen Leber durch Stickstoffmonoxiderhöhung, Adenosinerhöhung und Remodeling gesteigert ist. Der hepatisch-arteriellen Durchblutung kommt somit eine grössere Rolle für die Lebergesamtdurchblutung bei Zirrhose zu. Künftige medikamentöse Therapien zur Änderung der Leberdurchblutung bei Zirrhose sollten auch den hepatisch-arteriellen Blutfluss beachten, zumal eine hepatisch-arterielle Blutflußsteigerung über eine vermehrte Sauerstoffzufuhr die mikrosomale Leberfunktion steigern kann.

Dr. med. Alexander Zipprich, Einfluss, Mediatoren und Bedeutung der hepatisch arteriellen Durchblutung in der zirrhotischen Leber. Habilitationsschrift, 2011, Martin-Luther-Universität Halle-Wittenberg

Verteidigungsdatum Habilitationsschrift Dr. Alexander Zipprich:
16.10.2012

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Verzeichnis der Abkürzungen und Symbole

ADMA	- Asymmetrisches Dimethylarginin
BKCa	- Kalzium-aktivierte Kalium-Kanäle
BH_4	- Tetrahydrobiopterin
CBS	- Cystathionin-b-Synthetase
CCl_4	- Tetrachlorkohlenstoff
cGMP	- zyklisches Guanosin-3‘,5‘-monophosphat
CSE	- Cythathionin-y-Lyase
COX	- Cyclooxygenase
DAG	- Diacylglycerol
eNOS	- endotheliale Stickstoffmonoxidsynthetase
ET_A	- Endothelin-A
ET_B	- Endothelin-B
ET_1	- Endothelin-1
GMP	- Guanosinmonophosphat
GRK_2	- G-Protein gekoppelte Rezeptor-Kinase
GTP	- Guanosintriphosphat
HABR	- Hepatisch-arterielle Pufferantwort
HSC	- Hepatische Sternzellen
H_2S	- Schwefelwasserstoff
IP	- Inositol-1-3-4-triphosphat
L-NMMA	- L-NG-Monomethylarginincitrat
MLCK	- Myosin-Leichtketten-Kinase
NAFLD	- Nicht alkoholische Fettlebererkrankung
NASH	- Nicht alkoholische Fettleberhepatits
NO	- Stickstoffmonoxid

NOS	- Stickstoffmonoxidsynthetase
PBC	- Primär biliäre Zirrhose
PDE-5	- Phosphodiesterase-5
PLC	- Phospholipase C
PSC	- Primär sklerosierende Zirrhose
ROCK	- Rho-assoziierte coild-coil forming protein kinase
TXA2	- Thromboxan A2
VOCC	- L-Typ Kalzium-Kanäle

I. Einleitung

1. Häufigkeit und Ursachen der Leberzirrhose

Die Leberzirrhose ist als Endstadium verschiedenster Lebererkrankungen charakterisiert durch das Vorhandensein von knotigen Umbauprozessen. Dadurch werden wesentliche Veränderungen in der Leberdurchblutung und der Leberarchitektur hervorgerufen. Hauptursache der Leberzirrhose ist in Westeuropa der übermäßige Alkoholkonsum, wobei die alkoholische Leberzirrhose etwa 60 - 70% aller Leberzirrhosen ausmacht. Weiter bedingen chronische Virushepatitiden, sowohl die chronische Virushepatitis B als auch die chronische Virushepatitis C, zusammen in etwa 15 – 20% eine Leberzirrhose. Zahlreiche andere seltenere Erkrankungen, wie beispielsweise die nicht-alkoholische Fettlebererkrankung (NAFLD), die nicht-alkoholische Fettleberhepatitis (NASH), die Autoimmunhepatitis, die biliären Zirrhosen (PBC, PSC), der Morbus Wilson oder die Hämochromatose führen ebenfalls zur Ausbildung einer Leberzirrhose.

Die jährliche Inzidenz der Leberzirrhose beträgt in Deutschland etwa 250 Neuerkrankungen pro 100.000 Einwohner. Die geschlechtspezifische Verteilung zwischen Männern und Frauen beträgt 2:1. Die Ein-Jahres-Überlebensraten schwanken je nach Schwere der Erkrankung und Vorliegen von Komplikationen zwischen 100% und 35% (1).

2. Portale Hypertension

Wesentliche Komplikation der Leberzirrhose ist das Entstehen einer portalen Hypertension (2). Diese ist definiert als Anstieg des portalen Drucks, gemessen als hepatisch-venöser Druckgradient, über 6 mm Hg. Tatsächlich klinisch relevant sind Druckgradienten von 10 mm Hg und höher (3). Dabei kommt es ab einem portalen Druck von 10 mm Hg zur Entstehung von Ösophagusvarizen und Aszites. Diese

beiden Komplikationen sind gleichzeitig die häufigsten Komplikationen der Leberzirrhose und gehen mit einer Abnahme des Überlebens einher (1). Patienten mit Leberzirrhose und klinisch nachweisbarem Aszites haben ein 6-fach erhöhtes Risiko innerhalb eines Jahres zu versterben im Vergleich mit Patienten ohne Aszites (1, 4). Ähnliches gilt für das Auftreten einer Ösophagusvarizenblutung als Komplikation der Ösophagusvarizen, welche in bis zu 30% der Fälle tödlich endet und auch beim Überleben der initialen Blutung im weiteren Verlauf mit einer mindestens 5-fach höheren Mortalität pro Jahr einhergeht (4).

3. Mechanismen, die zur portalen Hypertension führen

Die portale Druckerhöhung bei Zirrhose ist einerseits Folge des erhöhten portalen Gefässwiderstandes in der Leber und andererseits Folge eines vermehrten splanchnischen Bluteinstroms durch Dilatation der mesenterial-arteriellen Gefässe (2). Die Widerstandserhöhung in der Leber wird durch zwei Mechanismen verursacht. Zum einen führen die anatomischen Umbauprozesse bei der Entstehung der Zirrhose zu Veränderungen der Architektur, auf der anderen Seite ist der intrahepatische Gefässwiderstand durch intrahepatische Vasokonstriktion erhöht (5).

Eine zentrale Rolle bei der Entstehung der Zirrhose nimmt die hepatische Sternzelle ein. Hepatische Sternzellen sind Zellen mesenchymalen Ursprungs und ähneln sowohl morphologisch als auch funktionell Perizyten anderer Organe (6). Die Aktivierung der hepatischen Sternzellen ist das initiale Ereignis, welches zur Einlagerung von fibrotischem Material um die Sinusoide führt und somit die anatomischen Veränderungen einleitet (7). Zudem sind die hepatischen Sternzellen auch wesentlich an der Erhöhung des intrahepatischen Widerstandes beteiligt (6). Verschiedene Mechanismen wie die gesteigerte Konzentration von

Entzündungsmediatoren oder Hypoxie führen zur initialen Aktivierung der Sternzellen. Vor allem das Vorhandensein einer intrahepatischen Hypoxie konnte in zahlreichen Untersuchungen als initiales Ereignis in der Entstehung unterschiedlicher Ätiologien der Zirrhose nachgewiesen werden (8-10). Weiterhin scheinen auch andere intrahepatische Zelltypen in die initialen Veränderungen involviert zu sein, wobei neuere Untersuchungen vor allem die sinusoidalen Endothelzellen betreffen (11).

4. Anatomische Veränderungen bei portaler Druckerhöhung

Die Aktivierung der Sternzellen verursacht eine Einlagerung von fibrotischem Gewebe in den Disse'schen Raum (12). Dadurch kommt es zum Verschluss der Fenestrae in den Sinusoiden (sogenannte Kapillarisierung der Sinusoide) mit Zunahme der Diffusionsbarriere für nutritive Stoffe und vor allem für Sauerstoff (13). Andererseits führt die Einlagerung von fibrotischem Gewebe um die Sinusoide auch zur Einengung der sinusoidalen Strombahn mit Erhöhung des intrahepatischen Gefässwiderstandes. Zusätzlich weist die zirrhotische Leber thrombotische Verschlüsse von kleinen intrahepatischen Portalästen, Ballonierung von Hepatozyten und sinusoidalen Kollaps mit nachfolgendem Verschluss von Sinusoiden auf (14). Folge dieser Veränderungen ist eine weitere Verengung der Sinusoide und eine zunehmende Erhöhung des intrahepatischen Gefässwiderstandes (Abbildung 1). Diese morphologischen Veränderungen sind für etwa 70% der intrahepatischen Druckerhöhung verantwortlich und werden als statische Komponente der Druckerhöhung bezeichnet (12). Die restlichen 30% der intrahepatischen Widerstandserhöhung werden durch Erhöhung von Vasokonstriktoren und Verminderung von Vasodilatatoren verursacht und als dynamische Komponente des intrahepatischen Widerstandes bezeichnet (2).

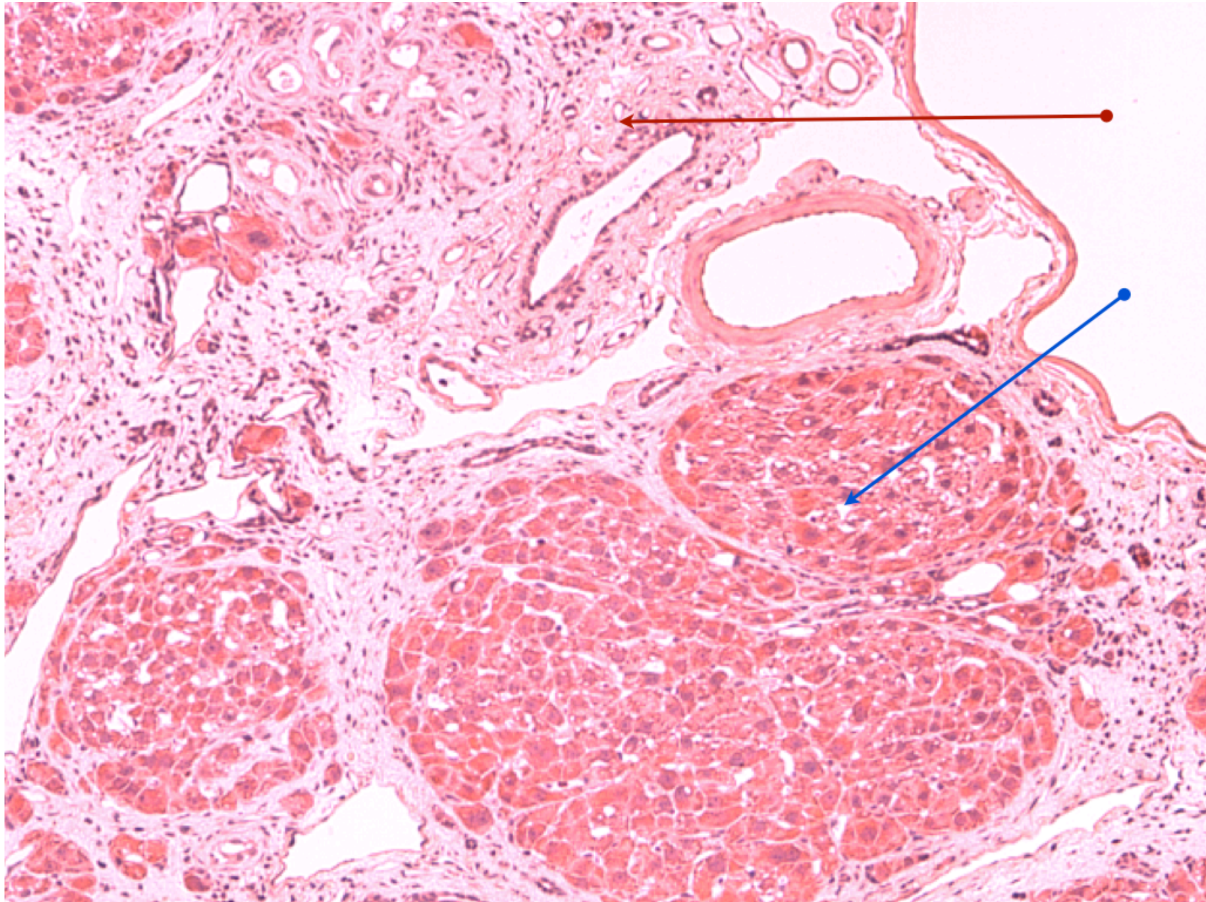


Abbildung 1: Histologische Abbildung einer makronodulären chemisch-induzierten zirrhotischen Leber mit ausgeprägter Fibrose (roter Pfeil) um die Gefässe und Ausbildung von zirrhotischen Knoten (blauer Pfeil; CCl4-induzierte Zirrhose einer Rattenleber, 20x Vergrößerung; unveröffentlichte eigene Abbildung)

5. Rolle der hepatischen Sternzellen für die intrahepatische Widerstandserhöhung

Eine zentrale Rolle bei der intrahepatische Vasokonstriktion nimmt wiederum die hepatische Sternzelle (HSC) ein. Hepatische Sternzellen sind um die Sinusoide lokalisiert und normalerweise in der gesunden Leber in einem ruhenden Zustand. Diese Zellen werden, wie oben beschrieben, aktiviert und dadurch in einen Myofibroblasten-ähnlichen Phänotyp überführt (6). Aktivierte Sternzellen haben im Gegensatz zu ruhenden Sternzellen Gefässmuskelzellen und exprimieren Myosin und zytosolische Proteine, welche für die Kontraktilität notwendig sind (15).

Zusätzlich zu den aktivierten kontraktilen Fähigkeiten der Sternzellen werden verschiedene vasokonstriktorisch wirkende Rezeptoren und Kanäle exprimiert. Beispielsweise werden L-Typ Kalzium-Kanäle (VOCC) nicht in ruhenden HSC vorgefunden, konnten aber in isolierten aktivierten HSC aus zirrhotischen Lebern nachgewiesen werden (16). Weiterhin wurden auch Kalzium-aktivierte Kalium-Kanäle (BKCa) in aktivierten HSC gefunden (17). BKCa-Kanäle scheinen bei der Vermittlung der konstriktiven Wirkung verschiedenster Mediatoren wie Endothelin und Stickstoffmonoxid eine Rolle zu spielen (18).

6. Vasokonstriktorische Mediatoren

Die Erhöhung des intrahepatischen Gefässtonus in der zirrhotischen Leber ist ein multifaktorieller Prozess, der durch Endothelin, Eicosanoide, Angiotensin II, Vasopressin und RhoA vermittelt wird. In den nachfolgenden Absätzen wird auf die einzelnen Mediatoren näher eingegangen.

6.1. Endothelin

Ein wichtiger Vasokonstriktor ist das Aminopeptid Endothelin. Drei verschiedene Endotheline (1 - 3) sind bekannt, jeweils bestehend aus 21 Aminosäuren (19). Die Plasmakonzentrationen von Endothelin-1 und -3 sind erhöht in der intrahepatischen Zirkulation der zirrhotischen Leber. Endothelin bindet an zwei verschiedenen Rezeptoren, den Endothelin-A- und dem Endothelin-B-Rezeptor. Für die vasokonstriktorische Wirkung bei Leberzirrhose ist die Bindung von Endothelin an den Endothelin-A-Rezeptor verantwortlich, der normalerweise auf der Gefässmuskulatur lokalisiert ist (20). Der Endothelin-A-Rezeptor entfaltet seine vasokonstriktorische Wirkung über einen G-Protein-vermittelten Signalweg, wodurch es letztlich zu einer intrazellulären Kalzium-Konzentrationserhöhung und

somit Vasokonstriktion kommt (Abbildung 2) (7, 20). Aktivierte Sternzellen exprimieren einerseits den Endothelin-A-Rezeptor, andererseits wird Endothelin auch in der zirrhotischen Leber von aktivierten Sternzellen und von Endothelzellen produziert (21). Endothelin nimmt somit eine zentrale Rolle einerseits in der Aktivierung der Sternzellen als auch andererseits in der intrahepatischen Vasokonstriktion der zirrhotischen Leber ein.

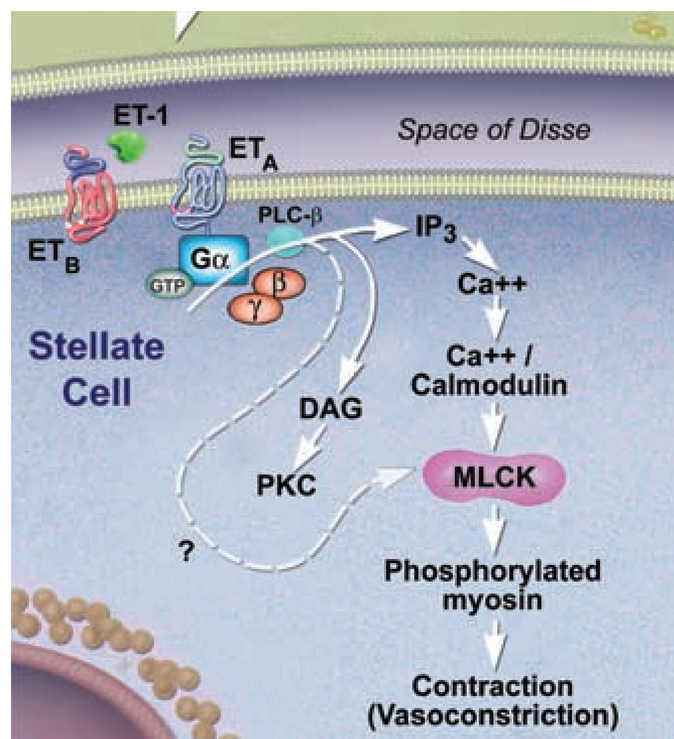


Abbildung 2: Schematische Darstellung des Endothelinsignalwegs als Mechanismus der intrahepatischen Vasokonstriktion in der zirrhotischen Leber [aus Referenz (19)].

Zeichenerklärung: ET-1=Endothelin-1; ET-A=Endothelin-A-Rezeptor; ET-B=Endothelin-B-Rezeptor; G_{alpha}=G-Protein; GTP=Guanosintriphosphat; IP_3=Inositol-1-3-4-triphosphat; Ca^{++} =Kalzium; Ca^{++}/Calmodulin=Kalzium-Calmodulin; MLCK=Myosin-Leichtkette-Kinase; DAG=Diacylglycerol; PLC_{-beta}=Phospholipase C-Beta

6.2. Prostaglandine

Ein weiterer Mechanismus der zur Widerstandserhöhung in der zirrhotischen Leber beiträgt, ist die Erhöhung der sogenannten Eicosanoide. Eicosanoide sind eine Gruppe von zum Teil vasokonstriktorischen Stoffen (Prostaglandinen,

Leukotrienen und Thromboxanen). Die Leber hat eine hohe Konzentration an Leukotrienen, da diese normalerweise in der Leber abgebaut werden (22). Cyclooxygenase-1 (COX-1) ist dabei das entscheidende Enzym in der Biosynthese von Arachidonsäure zu Prostaglandinen und Thromboxanen (23). Die Aktivierung der zytosolischen Phospholipase A2 (PLA2) über einen G-Protein-vermittelten Rezeptor führt zu einer Freisetzung von Arachidonsäure aus den Membranphospholipiden. Arachidonsäure wird durch das COX-Enzym weiter zu Prostaglandin H2 (PGH2) metabolisiert. PGH2 wiederum ist die Vorstufe von Prostaglandinen und Thromboxanen. Vor allem dem Thromboxan A2 (TXA2) wird in der zirrhotischen Leber eine wesentliche vasokonstriktorische Rolle zugeschrieben (24). Sinusoidale Endothelzellen zeigen eine vermehrte Expression von COX-1, woraus gefolgert wurde, dass diese Zellen für die vermehrte Produktion von Thromboxan A2 in der zirrhotischen Leber verantwortlich sind (25). Neuere Daten zeigen , dass wahrscheinlich auch Kupfferzellen teilweise für die vermehrte Produktion von Thromboxanen in der zirrhotischen Leber verantwortlich sind (26).

6.3. Angiotensin II

Neben der Erhöhung der Endotheline und Prostaglandine ist bei Vorliegen einer Zirrhose auch die Konzentration von Angiotensin II erhöht. Der vasokonstriktive Effekt von Angiotensin II ist vermittelt durch den Angiotensin II-Typ I-Rezeptor auf aktivierten Sternzellen (27). Die Stimulation dieses Rezeptors durch Angiotensin II führt zu einem Anstieg der intrazellulären Kalzium-Konzentration durch den L-Typ Kalziumkanal und nachfolgend zur Kontraktion, ein Effekt, der durch den Angiotensin-Hemmer Losartan inhibiert werden konnte (27, 28). Interessanterweise weisen aktivierte Sternzellen nicht nur alle Komponenten des

Renin-Angiotensin-Systems (u.a. Angiotensinogen, Renin, Angiotensin Converting Enzym) auf, sondern haben auch nachgewiesene Aktivitäten dieser Enzyme. Sie sind daher in der Lage, Angiotensin II zu produzieren, sodass nicht nur zirkulierendes, sondern auch lokal produziertes Angiotensin II zur Widerstandserhöhung in der intrahepatischen Zirkulation bei Zirrhose beiträgt (29).

6.4. RhoA/RhoA-Kinase

Ein weiterer Signalweg, der in den erhöhten intrahepatischen Gefässwiderstand bei Leberzirrhose involviert ist, ist der RhoA/RhoA-Kinase Signalweg. RhoA ist ein Guanosintriphosphat-(GTP) bindendes Protein aus der Ras-Familie. Die RhoA-Aktivität ist ein wichtiger Faktor bei der Regulation der Aktinorganisation, der Zellmorphologie, der Chemotaxis und der Kontraktion in verschiedensten Zellen und auch in den hepatischen Sternzellen (30). RhoA stimuliert die Rho-assoziierte coiled-coil forming protein kinase (ROCK), welche die Anordnung von zytoplasmatischen Filamenten aus Aktin triggert (31, 32). Der RhoA/RhoA-Kinase Signalweg ist ein Mechanismus, der die in der zirrhotischen Leber nachgewiesene Hyperkontraktilität vermittelt (33). Verglichen mit gesunden Kontrolllebern, war bei zirrhotischen Lebern eine dreifach höhere Konzentration eines RhoA-Kinase-Hemmers zur Inhibierung der alpha-1-Adrenorezeptor vermittelten Vasokonstriktion notwendig, was auf eine Aktivierung in der zirrhotischen Leber hinweist und durch eine Therapie mit dem Multikinase-Inhibitor Sorafenib vermindert werden konnte (34, 35). Tatsächlich wiesen diese Tiere einen geringen portalen Widerstand auf, wodurch eine Beteiligung des RhoA/RhoA-kinase Signalwegs bei der Entstehung des portalen Hypertonus nachgewiesen werden konnte (35).

7. Vasodilatatorische Mediatoren

Auf der anderen Seite wird die Widerstandserhöhung in der zirrhotischen Leber durch eine endotheliale Dysfunktion und verminderte Produktion von Vasodilatatoren mitverursacht. Der wichtigste Vasodilatator in diesem Zusammenhang ist Stickstoffmonoxid (NO), ein weiterer Vasodilatator ist Schwefelwasserstoff (H_2S). Auch diese Stoffwechselwege werden in den folgenden Abschnitten näher dargelegt.

7.1. Stickstoffmonoxid

Stickstoffmonoxid (NO) wird synthetisiert durch die endotheliale, die neuronale und die induzierbaren Stickstoffmonoxidsynthetasen (NOS). In der zirrhotischen Leber ist die endotheliale (eNOS) die hauptsächlich für die Synthese von NO verantwortliche Synthetase (36). Stickstoffmonoxid verursacht eine Aktivierung der löslichen Guanylzyklase. Dadurch wird die Synthese von zyklischen Guanosin-3‘, 5‘-monophosphat (cGMP) erhöht und eine Gefässmuskelrelaxation und schlussendlich Vasodilatation hervorgerufen (2). Der vasodilatatorische Effekt von Stickstoffmonoxid ist normalerweise durch Phosphodiesterasen begrenzt, die den Abbau von cGMP in die inaktive Form Guanosin-monophosphat (GMP) katalysieren (37).

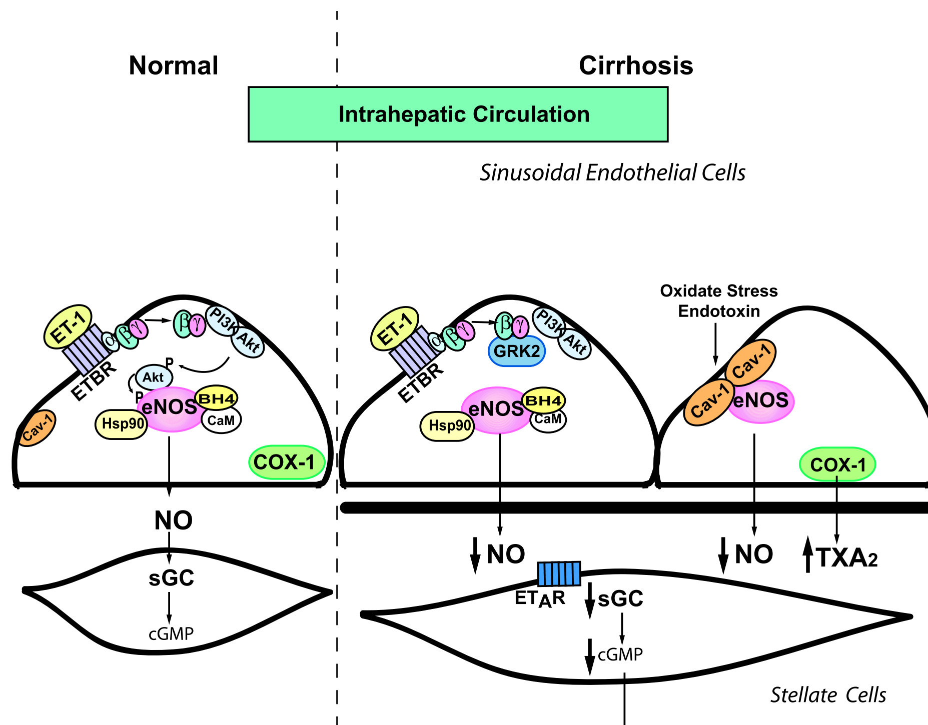


Abbildung 3: Schematische Darstellung des Stickstoffmonoxidsignalwegs in der Normalleber (linke Seite) und mit den entsprechenden Veränderungen (siehe Text) in der zirrhotischen Leber (rechte Seite) [aus Referenz (38)].
Zeichenerklärung: ET1=Endothelin-1; ETRA=Endothelin-A-Rezeptor; ETBR=Endothelin-B-Rezeptor; Cav-1=Caveolin-1; eNOS=endotheliale Stickstoffmonoxidsynthetase; BH_4=Tetrahydrobiopterin; CaM=Calmodulin; Hsp90=Heat shock Protein 90; COX-1=Cyclooxygenase-1; TXA_2=Thromboxan-A2; sGC=lösliche Guanylzyklase; cGMP=zyklisches Guanosin-monophosphat; GRK2=G-protein-gekoppelte Rezeptor-Kinase

Endothelzellen aus der zirrhotischen Leber haben eine gestörte Stickstoffmonoxidsynthese (39-41). Caveolin und Calmodulin sind zwei Proteine, welche die Sickstoffmonoxidsynthese regulieren, wobei Caveolin-1 die Aktivität der endothelialen Stickstoffmonoxidsynthetase hemmt und Calmodulin die Trennung von Caveolin von der eNOS bewirkt (40). In der zirrhotischen Leber konnte eine gesteigerte Caveolin-1-Expression und eine gesteigerte Interaktion mit Stickstoffmonoxid nachgewiesen werden, wodurch die Stickstoffmonoxidsynthese vermindert wird (Abbildung 3)(39).

Ein weiter Mechanismus, der in die Regulation der endothelialen Stickstoffmonoxidsynthetaseaktivität involviert ist, ist der Akt-Signalweg (42, 43). In

der zirrhotischen Leber ist die Akt-abhängige Phosphorylierung der endothelialen Stickstoffmonoxidsynthetase vermindert, wodurch die Aktivität der Synthetase abnimmt (43, 44). Als Mechanismus konnten neuere Untersuchungen eine Abnahme der Akt-abhängigen Phosphorylierung durch GRK2 (G-protein-gekoppelte Rezeptor-Kinase) nachweisen (Abbildung 3)(45). GRK2 ist vermehrt in der zirrhotischen Leber nachweisbar und scheint somit einen entscheidenden Effekt in der Akt-vermittelten Regulation der endothelialen Stickstoffmonoxidsynthethase zu haben (46). Zusätzlich konnten weitere aktivitätshemmende Faktoren wie Asymmetrisches Dimethylarginin (ADMA) und Tetrahydrobiopterin (BH_4) in der zirrhotischen Leber identifiziert werden (47-49).

In der zirrhotischen Leber ist nicht nur die Synthese von Stickstoffmonoxid vermindert, sondern auch dessen Wirkung sowie der Abbau verändert (36, 50). Tatsächlich konnte in der zirrhotischen Leber eine höhere Konzentration von Phosphodiesterase-5 (PDE-5), ein Enzym, dass den Abbau von Stickstoffmonoxid reguliert, gefunden werden (37).

Der verminderte Effekt von Stickstoffmonoxid in der zirrhotischen Leber ist somit eine Kombination aus verminderter Synthese, verminderter Wirkung und vermehrtem Abbau.

7.2. Schwefelwasserstoff und Homozystein

In der zirrhotischen Leber konnten durch veränderte Funktionen von Cystathionin-Synthetase (CBS) und Cystathionin-Lyase (CSE) erhöhte Serumspiegel von Homozystein nachgewiesen werden (51). Hyperhomozystämie verursacht eine endotheliale Dysfunktion und beeinträchtigt die endotheliale Vasodilatation in normalen Kontrolltieren und in zirrhotischen Tieren (52). Weiterhin ist durch die Funktionsänderung der CBS- und CSE-Enzyme die

Synthese von Schwefelwasserstoff (H_2S) vermindert, ein Endprodukt des Homozystein/L-Zystein-Stoffwechselwegs (51, 52). H_2S ist ein gasförmiger Neuromodulator mit vasodilatatorischen Eigenschaften. Bei der Perfusion der zirrhotischen Leber konnte H_2S den verminderten Effekt von Stickstoffmonoxid kompensieren. Es konnte weiterhin gezeigt werden, dass die Homozystein-induzierte Kontraktion der Sternzellen durch H_2S-Gabe entgegengewirkt werden konnte, so dass die hepatischen Sternzellen als Effektorzelle vermutet werden kann (52).

Zusammenfassend lässt sich schlussfolgern, dass die intrahepatische Widerstandserhöhung eine Kombination aus morphologischen Veränderungen mit Verengung der Sinusoide und aus einer intrahepatischen Vasokonstriktion der portal-venösen bzw. sinusoidalen Gefässe durch vermehrte Konzentration von vasokonstriktorischen Mediatoren und verminderte Konzentration von vasodilatatorischen Mediatoren ist (Abbildung 4).

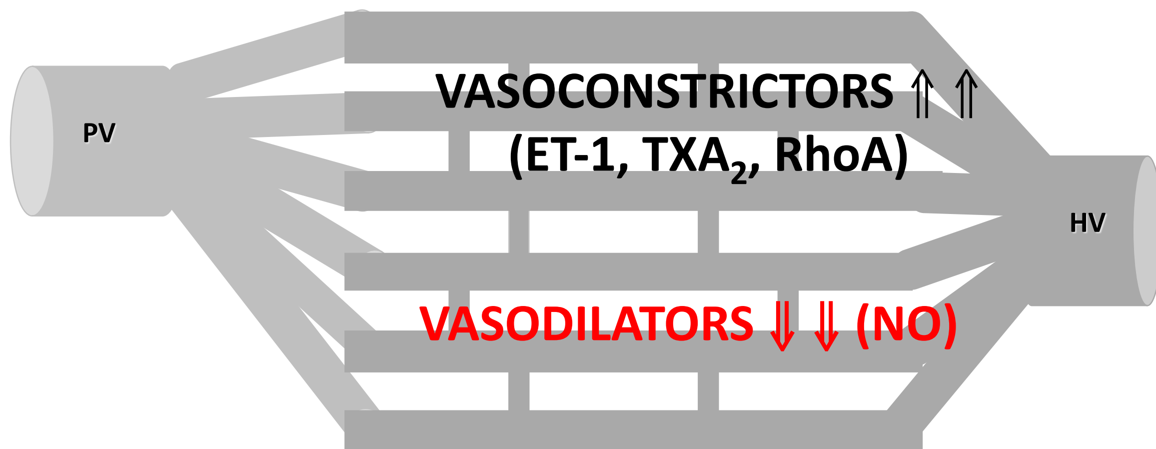


Abbildung 4: Schematische Darstellung der intrahepatischen (sinusoidalen und post-sinusoidalen) Veränderungen bei Zirrhose. Es kommt zur vermehrten Produktion von Vasokonstriktoren (vasoconstrictors) und verminderten Produktion von Vasodilatatoren (vasodilators)[aus Referenz (53)].
Zeichenerklärung: ET-1-Endothelin-1; TXA_2-Thromboxan A_2; RhoA-RhoA-Kinase; NO-Stickstoffmonoxid; PV-Pfortader; HV-Lebervene

8. Regulation der hepatisch-arteriellen Durchblutung

Die Durchblutung der Leber ist einzigartig durch eine bivaskuläre Blutzufuhr sowohl durch die Portalvene (normalerweise etwa 70 Prozent) als auch durch die Leberarterie (normalerweise etwa 30 Prozent). Die Regulation der hepatisch-arteriellen Durchblutung scheint dabei ganz wesentlich durch die Portalvenendurchblutung gesteuert, ein Phänomen das als hepatisch-arterielle buffer response (HABR) bezeichnet wird (54). Dabei führt eine Abnahme der portal-venösen Durchblutung zu einer kompensatorischen Zunahme der hepatisch-arteriellen Durchblutung und umgekehrt. Das Vorhandensein der HABR ist sowohl in der gesunden als auch in der zirrhotischen Leber nachgewiesen

worden (55, 56). Die hepatisch-arterielle Durchblutungsreserve ersetzt nach diesen Daten vor allem den nutritiven Ausfall der verminderten Portaldurchblutung, nicht aber die komplette Blutflussverminderung (57). Der wesentliche vasodilatatorisch-wirkende Mediator der HABR scheint Adenosin zu sein (55). Nach Lautt et al. wird dieses Adenosin kontinuierlich an dem Zusammenfluss der Portalvene und der Leberarterie produziert und durch den Portalblutfluss ausgewaschen. Kommt es zu einer Abnahme des Portalflusses wird weniger Adenosin ausgewaschen und kumuliert. Dadurch kommt es zur Dilatation der arteriellen Gefäße und zur Zunahme der arteriellen Durchblutung (55). Tatsächlich konnte gezeigt werden, dass Adenosin über den A_2-Adenosin-Rezeptor eine Dilatation der Leberarterie hervorruft (58). Eigene Arbeiten haben gezeigt, dass auch bei Patienten mit Zirrhose eine intra-arterielle Infusion von Adenosin in die Leberarterie zu einer Blutflusssteigerung führt (14). Andere Studien konnten zeigen, dass wahrscheinlich auch Stickstoffmonoxid (NO) in die Regulation der hepatisch-arteriellen Durchblutung involviert ist (59, 60). Diese Experimente wurden in einem Tiermodell der biliären Zirrhose, also einem Modell mit hauptsächlich prä-sinusoidaler Initiierung der Zirrhose, durchgeführt (60). Dabei ist von besonderer Bedeutung, dass der Gefässwiderstand der Pfortader im sinusoidalen und post-sinusoidalen Gefässbett determiniert ist, während der Gefässwiderstand in der Leberarterie in den prä-sinusoidalen Gefässabschnitten bestimmt wird (61). Eine Erhöhung der Stickstoffmonoxidkonzentration in diesem Gefässabschnitt könnte somit auf einen spezifischen Mediator zur Dilatation der Leberarterie hindeuten. Allerdings wird in dem in dieser Studie untersuchten Zirrhosemodell eine prä-sinusoidale Entzündung durch den Verschluss der Gallenwege provoziert, so dass Veränderungen in diesem Bereich auch modellbedingt und nicht zirrhosetypisch sein könnten.

9. Wahl des Tiermodells

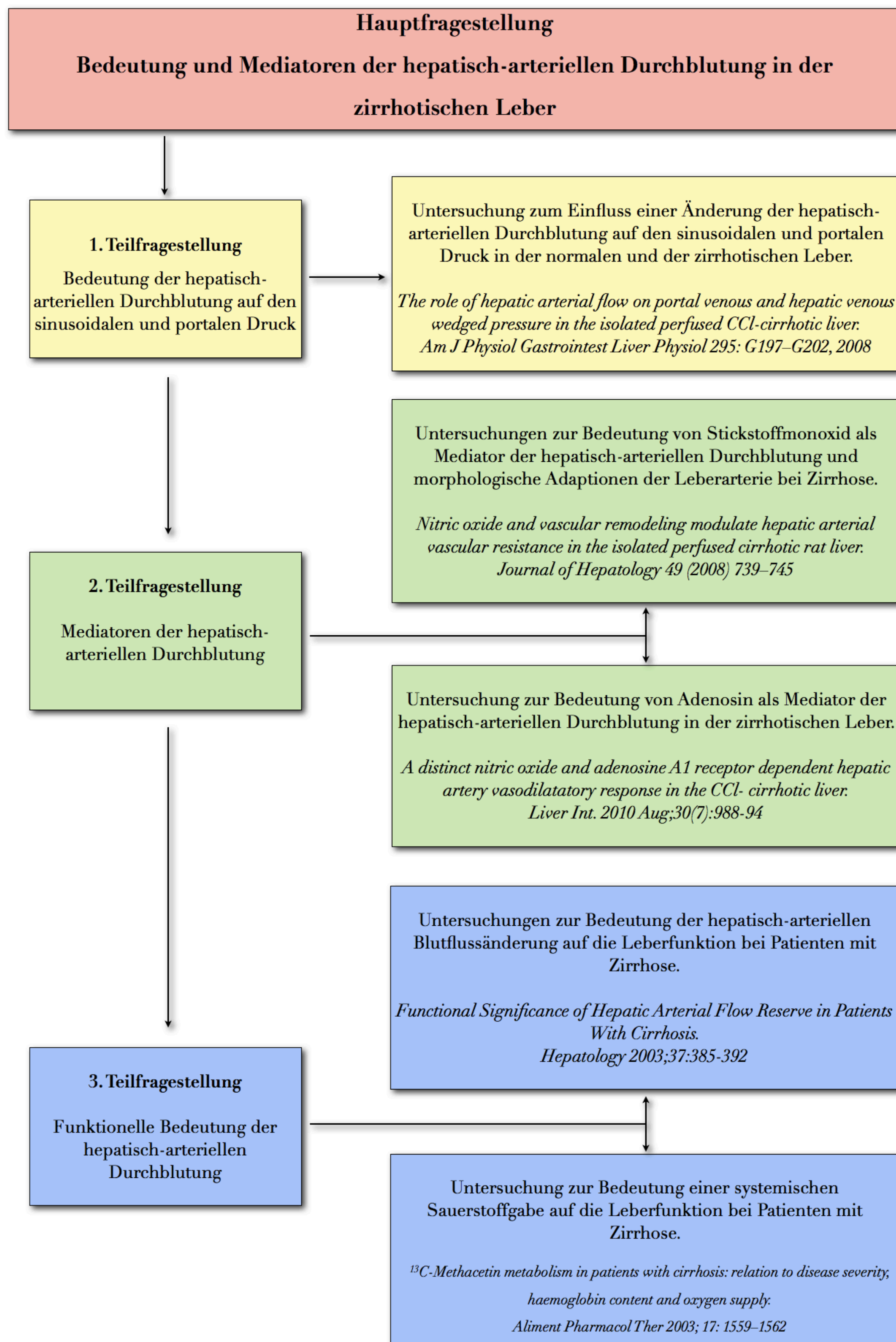
Es ist bisher nicht umfassend geklärt, inwieweit Stickstoffmonoxid tatsächlich ein Mediator der hepatisch-arteriellen Durchblutung ist. Die Untersuchungen dazu sollten in einem Tiermodell erfolgen, dass der wesentlich häufiger vorkommenden alkoholischen Zirrhose (sinusoidale und post-sinusoidale Initiierung der Zirrhose) entspricht, um modellbedingte mögliche Veränderungen soweit wie möglich zu eliminieren. Solch ein Tiermodell wäre die Tetrachlorkohlenstoff-induzierte (CCl_4) Zirrhose, ein Modell das in Bezug auf die portal-venöse Durchblutung sehr gut untersucht ist. Die Entstehung der Zirrhose wird in diesem Modell über eine initiale Schädigung in den sinusoidalen und post-sinusoidalen Bereichen induziert (62). Dieses Modell repräsentiert dadurch die Veränderungen der alkoholischen Leberzirrhose am Besten.

Das Zirrhosemodell der Gallengangsligatur (BDL) ist ein Modell, dass darauf beruht, dass der extrahepatische Gallengang verschlossen wird (63). Dadurch kommt es zum intrahepatischen Gallenstau und einer Entzündungsreaktion um die Gallengänge. Durch die Entzündungsreaktion im Bereich der Gallengänge sind in diesem Modell die inflammatorischen Mediatoren stärker als im CCl_4-induzierten Zirrhosemodell erhöht (64). Dies führt zu einem höheren Anteil an Stickstoffmonoxid (NO) durch die induzierbare Stickstoffmonoxidsynthetase (iNOS)(64). Die Zirrhose im BDL-Modell entwickelt sich primär prä-sinusoidal durch Inflammation der Gallenwege, d.h. in den Abschnitten der Leber die vor den Sinusoiden lokalisiert sind (65). Für die Untersuchung der Leberarterie ist dies von entscheidender Bedeutung, da der Gefässwiderstand der Leberarterie in den prä-sinusoidalen Abschnitten determiniert ist (66). Zusammenfassend lässt sich feststellen, dass beide Modelle unterschiedliche Krankheitsätiologien

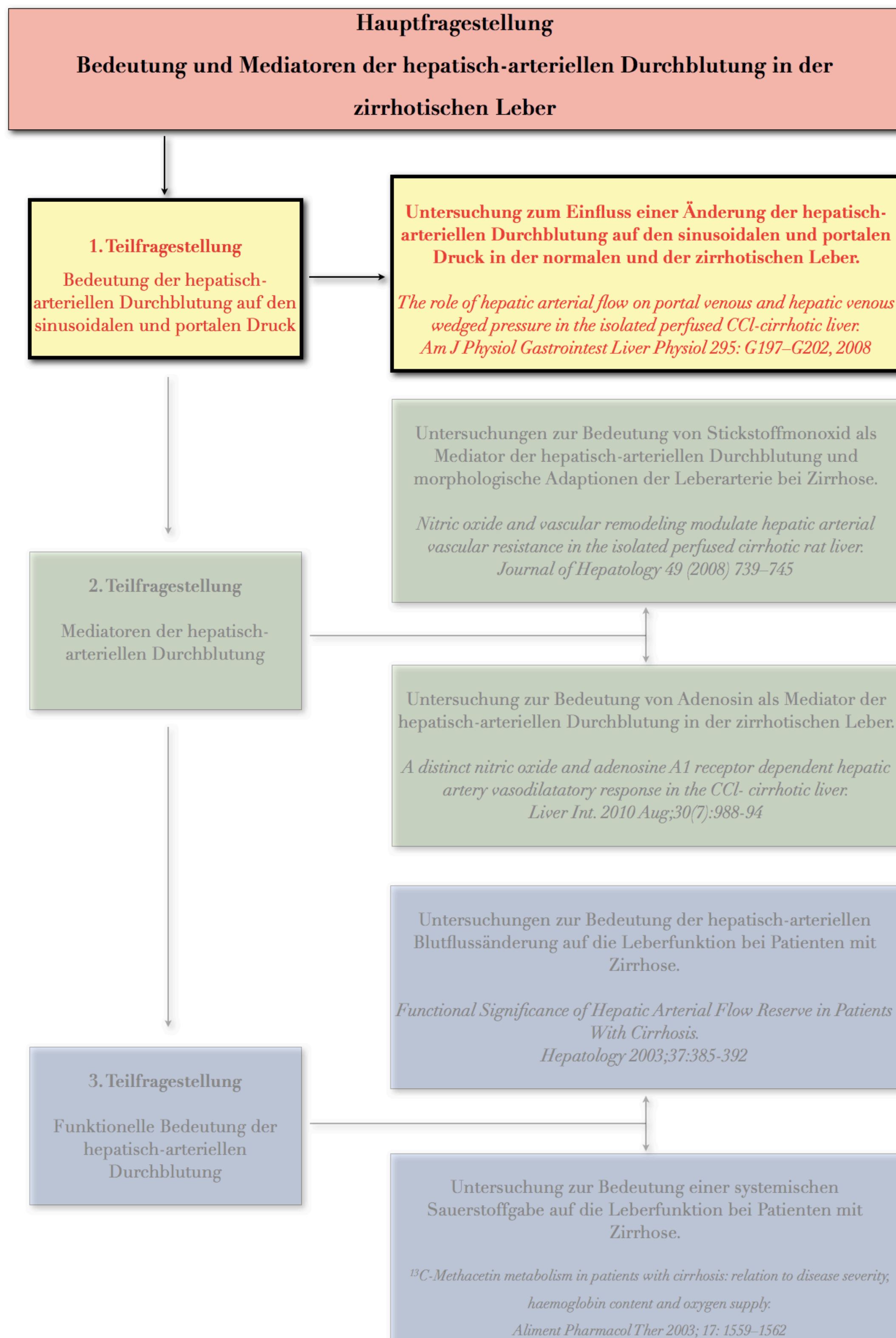
repräsentieren und speziell in Bezug auf die Veränderungen der Hämodynamik der Leberarterie signifikante Unterschiede zu erwarten sind.

Tatsächlich sind alle oben aufgeführten strukturellen und enzymatischen Veränderungen, die zur Beeinflussung des intrahepatischen Widerstands in der zirrhotischen Leber führen, bisher nur für die portal-venöse Durchblutung untersucht. Ausserdem wurden fast alle Mechanismen trotz der bekannten Unterschiede der Zirrhosemodelle nur in jeweils einem Modell nachgewiesen. Inwieweit diese portal-venösen Mechanismen auch einen Einfluss auf die hepatisch-arterielle Durchblutung haben und ob andere Mediatoren, wie beispielsweise Adenosin, ebenfalls eine Rolle in der Regulation der hepatisch-arteriellen Durchblutung bei Zirrhose spielen, ist nur unzureichend untersucht. Die vorliegende Arbeit untersucht daher die Bedeutung, die Regulation und die Veränderungen der hepatisch-arteriellen Durchblutung im zirrhotischen Tiermodell und bei Patienten mit Zirrhose.

II. Fragestellungen



III. Verschiedene Publikationen/Methoden/Ergebnisse



1. The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl-cirrhotic liver. Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008

Die Fragestellung der Arbeit war, ob eine Änderung des hepatisch-arteriellen Blutflusses den sinusoidalen Druck und den portal-venösen Druck ändert und ob es Unterschiede zwischen lebergesunden und zirrhotischen Versuchstieren diesbezüglich gibt.

Normale und zirrhotische Rattenlebern wurde mit einer bivaskulären Leberperfusion (Abbildung 5) unter gleichzeitiger Messung des arteriellen, portal-venösen und sinusoidalen (gemessen als Lebervenenverschlußdruck) Drucks untersucht. Nach Start der Leberperfusion wurde zunächst eine 20-minütige Stabilisierungsphase abgewartet. Danach wurde der Fluss (initialer Fluss 32 ml/min) in der Pfortader schrittweise alle 2 Minuten auf einen minimalen Fluss von 20 ml/min gesenkt (Experiment 1). Nach einer weiteren 15-minütigen Stabilisierungsphase mit den initialen Flüssen, wurde im zweiten Versuch der arterielle Fluss zunächst für 2 Minuten auf 5 ml/min gesenkt und anschliessend für jeweils 2 Minuten auf 10 und 15 ml/min erhöht (Experiment 2).

Die Steigerung des hepatisch-arteriellen Flusses (Experiment 2) führte zu einer signifikanten Zunahme des portal-venösen (p=0,002) und des sinusoidalen Drucks (p<0,001), wobei die absoluten Änderungen des hepatisch-arteriellen Flusses mit den absoluten Änderungen des portal-venösen Drucks (Zirrhoseleber: r=0,64, p<0,001; Normalleber: r=0,67, p<0,001) und des sinusoidalen Drucks (Zirrhoseleber: r=0,71; p<0,001; Normalleber: r=0,82, p<0,001) korrelierten. Die Änderungen des portal-venösen und des sinusoidalen Widerstandes, induziert durch die hepatisch-arterielle Flussänderung, korrelierten eng miteinander (Zirrhoseleber: r=0,92, p<0,001; Normalleber: r=0,77, p<0,001).

Die Änderung des portal-venösen Flusses (Experiment 1) führte zu einer signifikanten Änderungen des sinusoidalen Drucks bei Zirrhoselebern ($p<0{,}001$) und Normallebern ($p<0{,}001$), aber zu keinen Änderungen des hepatisch-arteriellen Drucks.

Schlußfolgerung: Zunahme und Abnahme des hepatisch-arteriellen Blutflusses führt zu gleichsinnigen signifikanten Änderungen des sinusoidalen und des portal-venösen Drucks. Die Änderungen werden höchstwahrscheinlich verursacht durch einen direkten Einfluss der hepatisch-arteriellen Durchblutung auf die sinusoidale Perfusion.

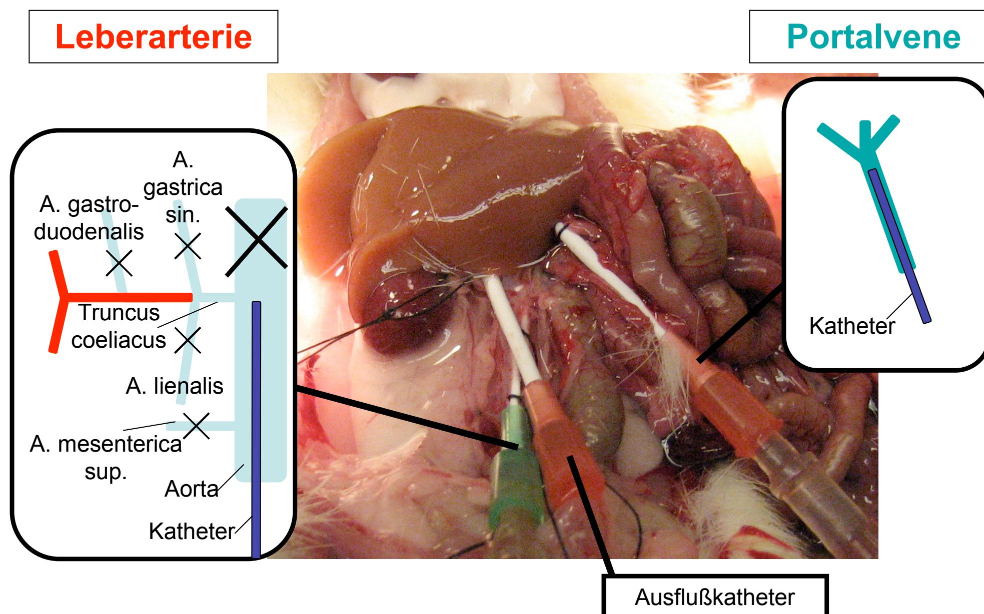


Abbildung 5: Schematische Darstellung einer bivaskulären Leberperfusion mit entsprechender Angabe der Ligatur der Gefäße. Die Messung des Lebervenenverschlußdrucks ist nicht in der Abbildung dargestellt. (Unveröffentlichte eigene Abbildung)

Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008.
First published May 22, 2008; doi:10.1152/ajpgi.00190.2007.

The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl_4-cirrhotic liver

Alexander Zipprich,[1,2] Mauricio R. Loureiro-Silva,[1,2] Irita D'Silva,[2] and Roberto J. Groszmann[1,2]

[1]*Digestive Disease Section, Yale University School of Medicine, New Haven, Connecticut; and* [2]*Hepatic Hemodynamic Laboratory, Veterans Affairs Medical Center, West Haven, Connecticut*

Submitted 30 April 2007; accepted in final form 15 May 2008

Zipprich A, Loureiro-Silva MR, D'Silva I, Groszmann RJ. The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl_4-cirrhotic liver. *Am J Physiol Gastrointest Liver Physiol* 295: G197–G202, 2008. First published May 22, 2008; doi:10.1152/ajpgi.00190.2007.—In cirrhosis, hepatic venous pressure gradient is used to measure portal venous and sinusoidal pressures, as well as drug-induced decreases of elevated pressures. The aim of this study was to investigate the influence of hepatic arterial flow (HAF) changes on portal venous perfusion (PVPP) and wedged hepatic venous pressure (WHVP). Normal and CCl_4-cirrhotic rats were subjected to a bivascular liver perfusion with continuous measurements of PVPP, WHVP, and hepatic arterial perfusion pressure. Flow-pressure curves were performed with the use of different flows either through the portal vein (PVF: 20–32 ml/min) or HAF (5–15 ml/min). Increases in HAF lead to significant absolute and relative increases in PVPP ($P = 0.002$) and WHVP ($P < 0.001$). Absolute changes in HAF correlated to absolute changes in PVPP (cirrhosis: $r = 0.64$, $P < 0.001$; control: $r = 0.67$, $P < 0.001$) and WHVP (cirrhosis: $r = 0.71$, $P < 0.001$; control: $r = 0.82$, $P < 0.001$). Changes in PVPP correlated to changes in WHVP due to changes in PVF only in cirrhosis ($r = 0.75$, $P < 0.001$), whereas changes in HAF correlated in both cirrhosis ($r = 0.92$, $P < 0.001$) and control ($r = 0.77$, $P < 0.001$). In conclusion, increases and decreases in HAF lead to respective changes in PVPP and WHVP. This suggests a direct influence of HAF on PVPP and WHVP most likely due to changes in sinusoidal perfusion.

sinusoidal; CCl_4-cirrhotic rats; liver perfusion

THE LIVER HAS A DUAL BLOOD supply through the portal vein and the hepatic artery. Portal venous blood flow corresponds to ~70–80% and hepatic arterial blood flow to ~20–30% of the total liver blood flow in humans. In cirrhosis, intrahepatic vascular resistance is increased due to structural and functional changes (31). Furthermore, vasodilation in splanchnic and systemic circulation leads to an increase of portal venous inflow, the total amount of blood entering the portal system (31). A large part of this flow escapes through portal-systemic collaterals. However, both increased intrahepatic vascular resistance and increased portal venous inflow cause an increase in portal venous pressure, which has been defined as the interaction between portal venous flow and the resistance the liver is offering to this flow (12). On the other hand, these intrahepatic and systemic hemodynamic changes in patients with cirrhosis lead to changes of hepatic arterial flow that is also influenced by local factors (15, 18).

The wedge hepatic venous pressure, a reflection of sinusoidal pressure, as well as hepatic venous pressure gradient, i.e., the difference between wedged hepatic venous pressure and free hepatic venous pressure, are used to estimate elevated sinusoidal and portal venous pressures in patients with cirrhosis (12, 25). Most pharmacological therapies used to reduce portal pressure are based on the reduction of portal flow, and hepatic venous pressure gradient is used to monitor drug efficacy (2, 6, 9, 20). Indeed, it has been demonstrated that both hepatic venous pressure gradient and drug-induced reduction in hepatic venous pressure gradient are excellent predictors of survival and development of complications in patients with cirrhosis (2, 6, 9, 10, 20, 27, 28).

Data showing the influence of hepatic arterial blood flow on the hepatic venous pressure gradient in patients with cirrhosis are limited. In fact, these data are controversial, and the influence from hepatic arterial blood flow on the hepatic venous pressure gradient is difficult to conclude from these studies (23, 24). However, most of these studies compared baseline hepatic arterial blood flow and hepatic venous pressure gradient, whereas the influence of changes in hepatic arterial flow on hepatic venous pressure gradient has not been investigated. With the use of intravascular Doppler sonography it has been shown that adenosine-induced hepatic arterial vasodilation leads to an increase in hepatic venous pressure gradient (15). Although this was investigated only in a small number of patients with cirrhosis, it suggested a direct influence of changes in hepatic arterial flow on hepatic venous pressure gradient. However, the above-described concept of measuring drug efficacy by using hepatic venous pressure gradient assumes no significant contribution from the hepatic arterial flow to portal venous pressure and hepatic venous wedge pressure (16, 29, 30). Therefore, the aim of this study was to investigate the influence of different hepatic arterial and portal venous flows on wedged and portal pressure.

MATERIALS AND METHODS

Twenty-two control and cirrhotic male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) underwent in situ liver perfusion. The American Physiological Society guide principles for the care and use of animals were followed. The Institutional Animal Care and Use Committee at the Veterans Affairs Media Center previously approved all procedures involving animals.

Induction of cirrhosis. Rats weighing 75–100 g underwent inhalation exposure of CCl_4 three times a week. Phenobarbital (0.35 g/l) was added to the drinking water as described previously (19). Treatment was given for ~14 wk. Perfusions were performed 6 to 10 days after the last doses of CCl_4 and phenobarbital. Age-matched rats were used as a control group.

Address for reprint requests and other correspondence: R. J. Groszmann, Digestive Disease Section/111H, VA Healthcare System, 950 Campbell Ave., West Haven, CT 06516 (e-mail: roberto.groszmann@yale.edu).

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In vivo measurement of portal pressure. Rats were weighed and anaesthetized with ketamine hydrochloride (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA; 100 mg/kg body wt) and diazepam (10 mg/kg body wt). Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilum. The abdomen was opened with a midline incision, and the ileocolic vein was cannulated. After a 10-min stabilization period, the in vivo portal venous pressure was measured. Portal hypertension was defined by a portal pressure higher than 10 mmHg (1).

In situ rat liver perfusion. After measurement of the portal pressure in vivo, xylazine (Rompum; Bayer, Shawnee Mission, KS; 40 mg/animal) was added, and an in situ bivascular liver perfusion via the portal vein and the hepatic artery was performed as previously described (7). Briefly, the opening of the abdomen was extended, and loose ligatures were placed around the aorta cranial of the celiac artery, around the mesenteric artery immediately after branching from the aorta, and the aorta caudal of the mesenteric artery. Left gastric and splenic artery were tied at its origin of the celiac artery. Left and right renal arteries, as well as gastroduodenal artery (branch of the common hepatic artery), were ligated. The bile duct was cannulated with a polyethylene tube (PE-10). Loose ligatures were placed around the inferior vena cava and the portal vein. The portal vein was cannulated with a 14-gauge Teflon catheter, and the perfusion with 32 ml/min of oxygenated (carbon gas, 95% O_2-5% CO_2) Krebs-Henseleit solution containing dextrose (11 mM) in a nonrecirculating mode was started. The inferior vena cava was cut immediately. The ligatures around the portal vein were closed. The aorta was cannulated with an 18-gauge Teflon catheter, and the ligature around the mesenteric artery was closed. The perfusion of the hepatic artery with 8 ml/min of oxygenated (carbon gas, 95% O_2-5% CO_2) Krebs-Henseleit solution containing dextrose (11 mM) in a nonrecirculating mode was started. The tip of the catheter was placed close to the branch of the celiac artery, and all ligatures around the aorta were closed. A 14-gauge catheter was introduced in the inferior vena cava, and the thorax was opened.

To measure the sinusoidal pressure, a PE-60 catheter was guided from the right atrium, through the thoracic segment of the inferior vena cava into the left hepatic lobe, and wedged in the hepatic vein. The ligature around the superior vena cava was closed to secure the wedged catheter. The preparation was transferred to a temperature-controlled (37°C) Plexiglas perfusion chamber (Yale University Medical Instruments). The perfusion system was changed to a recirculating mode (100 ml Krebs-Henseleit solution containing dextrose), initiating the stabilization period.

During the stabilization and the experimental period, the perfusion pressure of the portal vein and the hepatic artery were measured constantly using two independent strain-gauge transducers (P23XL; Spectramed, Oxnard, CA), respectively. The wedged pressure was measured during the experimental period using a third independent strain-gauge transducer (P23XL; Spectramed). The free hepatic venous pressure was taken as zero in the outflow of the perfusion system, which allows us to use the wedged hepatic venous pressure as a continuous measurement and also as a proof of a wedged position during the entire experiment since a nonwedged position measurement would be closer to the zero reference point. Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilium. Perfusion and sinusoidal pressure were continuously recorded by Chart 3.6 program with the use of MacLab/4e hardware (AD instruments, Colorado Springs, CO). During the stabilization and experimental period, the perfusate was oxygenated with a Silastic tubing lung interposed between the perfusate reservoir and the peristaltic pump (13).

Experimental design. Normal and cirrhotic livers were perfused with constant flows during the stabilization period, and the flow through the wedged catheter was maintained. After the stabilization period, the wedged catheter outflow was interrupted to allow the measurement of the wedged hepatic venous pressure. During *Experiment 1*, the initial portal venous flow of 32 ml/min was reduced 2 ml/min every 2 min to a final flow of 20 ml/min. Next portal venous flow was reset to 32 ml/min, initiating a second 15-min period of stabilization. After this second stabilization period (*Experiment 2*), the hepatic arterial flow was first reduced from 8 ml/min to 5 ml/min and then increased to 10 and 15 ml/min with a 2-min interval between flows.

Portal venous vascular resistance and hepatic arterial vascular resistance were calculated by portal venous perfusion pressure and portal venous flow and by hepatic arterial perfusion pressure and hepatic arterial flow, respectively. Sinusoidal vascular resistance was calculated by wedge pressure and by total liver perfusion flow, i.e., the sum of portal venous and hepatic arterial flow.

Liver global viability was assessed by gross appearance of the liver, stable perfusion pressure, and bile production during the stabilization periods (>0.4 µl/min per g liver). After the experiment, liver and spleen were removed and weighed. Liver tissue samples were collected and fixed in formalin.

Statistics. Data are presented as means ± SE. Mann-Whitney *U*-test was used for comparisons of different groups at baseline level. Comparison for repeated measurements was assessed using the Friedman test to detect changes in each group (within group effects). Multivariate analysis of repeated measurements (ANOVA) was used to detect differences between control and cirrhotic groups (between group effect). The association between continuous variables was assessed with the Spearman rank correlation test. *P* values ≤ 0.05 were considered significant.

Table 1. *Absolute and relative changes in PVPP, HAPP, and WHVP due to changes in PVF (Experiment 1)*

ΔPVF, ml/min (Δ% total perfusion flow, %)	Animal Condition	ΔPVPP, mmHg (Δ%PVPP, %)	ΔHAPP, mmHg (Δ%HAPP, %)	ΔWP, mmHg (Δ%WP, %)
−2	Control	−0.22±0.02 (−3.44±0.34)	0.12±0.10 (0.10±0.10)	−0.08±0.04 (−2.33±1.34)
(−5)	Cirrhosis	−0.21±0.06 (−3.28±0.92)	−0.02±0.70 (−0.02±0.11)	−0.11±0.04 (−2.88±1.10)
−4	Control	−0.40±0.04 (−6.21±0.57)	0.32±0.34 (0.25±0.29)	−0.13±0.07 (−3.55±2.44)
(−10)	Cirrhosis	−0.45±0.08 (−6.68±1.29)	−0.15±0.11 (−0.21±0.16)	−0.21±0.08 (−5.25±2.14)
−6	Control	−0.63±0.05 (−9.65±0.67)	0.64±0.54 (0.56±0.45)	−0.19±0.09 (−5.38±3.11)
(−15)	Cirrhosis	−0.65±0.11 (−9.81±1.64)	−0.18±0.16 (−0.27±0.25)	−0.29±0.16 (−7.17±3.19)
−8	Control	−0.82±0.05 (−12.63±0.74)	0.89±0.76 (0.79±0.63)	−0.25±0.10 (−7.17±3.66)
(−20)	Cirrhosis	−0.85±0.12 (−12.68±1.68)	−0.18±0.16 (−0.26±0.24)	−0.37±0.14 (−8.84±3.92)
−10	Control	−1.02±0.06 (−15.70±0.95)	1.12±0.91 (1.00±0.76)	−0.29±0.11 (−8.57±3.64)
(−25)	Cirrhosis	−1.03±0.15 (−15.27±2.07)	−0.10±0.14 (−0.15±0.22)	−0.41±0.17 (−9.81±4.60)
−12	Control	−1.24±0.07 (−19.10±1.05)	1.22±0.91 (1.15±0.78)	−0.31±0.12 (−9.28±3.93)
(−30)	Cirrhosis	−1.27±0.15 (−18.83±1.97)	0.003±0.16 (0.02±0.24)	−0.47±0.19 (−11.20±5.30)

Applicable values are means ± SE. Δ, Absolute change; Δ%, relative change; PVPP, portal venous perfusion pressure; HAPP, hepatic arterial perfusion pressure; WHVP, wedged hepatic venous pressure; PVF, portal vein flow. PVPP in control and cirrhosis conditions: $P < 0.001$ (within-group effect). WP: control, $P = 0.002$ (within-group effect); cirrhosis: $P = 0.025$ (within-group effect).

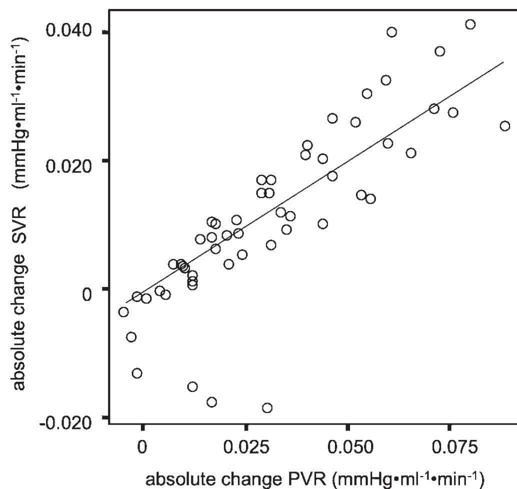

Fig. 1. Correlation between absolute changes of portal venous vascular resistance (PVR) and sinusoidal vascular resistance (SVR) in cirrhotic animals due to changes in portal venous flow (r = 0.92, P < 0.001).

RESULTS

In vivo portal pressure. Cirrhosis was confirmed by histological examination in all CCl_4-treated animals (n = 9). Cirrhotic animals (12.4 ± 0.8 mmHg) had a significantly higher in vivo portal pressure than control animals (6.6 ± 1.0 mmHg; P < 0.001). Body weight was not different between cirrhotic (449 ± 9.6 g) and control (474 ± 12.8 g; n = 13) animals, whereas ratios of liver weight and spleen weight to body weight were higher in cirrhotic animals (P < 0.05).

Experiment 1

Change of portal venous flow. The decrease of portal venous flow induced an increase of sinusoidal vascular resistance in cirrhotic (P < 0.001) as well as control (P < 0.001) animals (see Table 1). Furthermore, this decrease of portal venous flow correlated with the flow-induced increase of sinusoidal vascular resistance in cirrhotic (r = −0.63, P < 0.001) as well as in control (r = −0.52, P < 0.001) animals. However, this observed increase of sinusoidal vascular resistance was not significantly different between both groups. Decrease of portal venous flow did not lead to significant changes of hepatic arterial perfusion pressure in both groups.

Changes of portal venous vascular resistance. Decrease of portal venous flow caused significant changes of portal venous perfusion pressure and portal venous vascular resistance in cirrhotic (P < 0.001) and control (P < 0.001) animals but without significant differences between both groups.

Flow-induced changes of portal venous perfusion pressure correlated with changes of wedged pressure in cirrhotic animals (r = 0.75, P < 0.001). Furthermore, changes of portal venous vascular resistance correlated well with changes of sinusoidal vascular resistance in cirrhotic animals (r = 0.92, P < 0.001, Fig. 1) and in control animals (r = 0.67, P < 0.001).

Experiment 2

Change in hepatic arterial flow. Changes of hepatic arterial flow lead to significant changes of portal venous perfusion pressure and portal venous vascular resistance (P < 0.01; Fig. 2). The changes of hepatic arterial flow were, in addition, correlated with changes of portal venous perfusion pressure as well as portal venous vascular resistance in cirrhotic (r = 0.64, P < 0.001) and in control (r = 0.66, P < 0.001) animals (see Table 2). Interestingly, we also observed changes of wedged pressure due to changes in hepatic arterial flow. Changes in hepatic arterial flow caused changes of wedged pressure and sinusoidal vascular resistance in cirrhotic and control animals (P < 0.01; Fig. 2). Moreover, we found a correlation between changes of hepatic arterial flow and changes of wedged pressure in both groups (cirrhosis: r = 0.71, P < 0.001; control: r = 0.82, P < 0.001). However, changes of portal venous perfusion pressure, portal venous vascular resistance, wedged pressure, and sinusoidal vascular resistance were not significant different between cirrhotic and control animals due to changes of hepatic arterial flow (Fig. 2). Although there may be a trend to have different slopes between normal and cirrhotic livers, the slopes shown in Fig. 2 are not statistically different. Interestingly, the changes of portal venous perfusion pressure and wedged pressure in response to changes of hepatic arterial flow were highly correlated in both groups (cirrhosis: r = 0.92, P < 0.001, Fig. 3; control: r = 0.77, P < 0.001).

Change in hepatic arterial vascular resistance. In response to changes of hepatic arterial flow, cirrhotic animals had significantly smaller changes of hepatic arterial perfusion pressure (15.7 ± 0.8 vs. 20.6 ± 1.3 mmHg; ANOVA: P = 0.01) and hepatic arterial vascular resistance (−2.71 ± 0.06 vs.

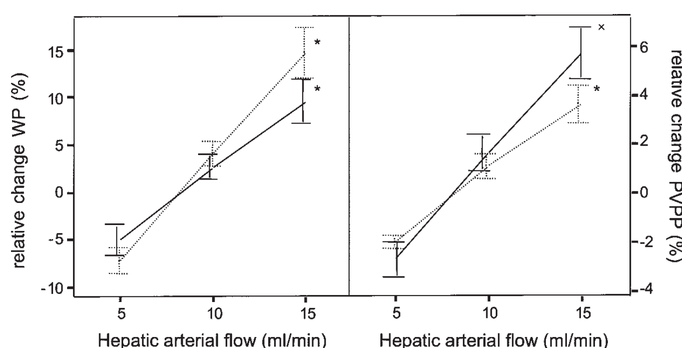

Fig. 2. Relative changes of wedge pressure (WP, *left*) and portal venous perfusion pressure (PVPP, *right*) due to different hepatic arterial flows in cirrhotic (solid line, n = 9) and control (dotted line, n = 13) animals (within-group effect: *P < 0.001, ×P = 0.002). Error bars indicate ± 1 SE.

Table 2. *Absolute and relative changes in PVPP, HAPP, and WHVP due to changes in HAF (Experiment 2)*

ΔHAF, ml/min (Δ% total flow, %)	Animal Condition	ΔPVPP, mmHg (Δ%PVPP, %)	ΔHAPP, mmHg (Δ%HAPP, %)	ΔWP, mmHg (Δ%WP, %)
−3	Control	−0.10±0.03 (−1.4±0.4)	−10.5±0.74 (−13.4±0.3)	−0.18±0.03 (−7.1±1.3)
(−7.5)	Cirrhosis	−0.14±0.06 (−1.93±0.86)	−7.93±0.30 (−12.28±0.20)	−0.19±0.07 (−5.00±1.64)
+2	Control	+0.06±0.05 (+0.8±0.6)	+6.7±0.45 (+8.5±0.3)	+0.12±0.04 (+4.1±1.3)
(+5)	Cirrhosis	+0.06±0.09 (+1.14±0.98)	+5.12±0.26 (+7.92±0.25)	+0.07±0.09 (+2.71±1.31)
+7	Control	+0.19±0.08 (+2.6±1.0)	+20.6±1.25 (+26.4±0.6)	+0.40±0.10 (+14.8±2.7)
(+17.5)	Cirrhosis	+0.28±0.11 (+4.10±1.33)	+15.70±0.76 (+24.27±0.63)	+0.32±0.11 (+9.64±2.34)

Applicable values are means ± SE. HAF, hepatic arterial flow. HAPP: $P < 0.001$ (within-group effect); $P = 0.01$ (ANOVA cirrhosis vs. control, between-group effect). PVPP: $P \leq 0.002$ (within-group effect). WP: $P < 0.001$ (within-group effect).

-3.17 ± 0.19 mmHg·ml^{-1}·min^{-1}; $P = 0.03$) than control animals. In cirrhotic animals, changes of hepatic arterial perfusion pressure correlated to changes of portal venous perfusion pressure ($r = 0.60$, $P = 0.001$) and wedged pressure ($r = 0.72$, $P < 0.001$). In addition, changes of hepatic arterial resistance correlated with changes of portal venous vascular resistance ($r = -0.66$, $P < 0.001$) and sinusoidal vascular resistance ($r = 0.58$, $P = 0.002$) in cirrhotic animals. These correlations were also present in control animals (portal venous perfusion pressure: $r = 0.66$, $P < 0.001$; wedged pressure: $r = 0.77$, $P < 0.001$; portal venous vascular resistance: $r = -0.63$, $P < 0.001$).

Comparison of Experiment 1 and 2

To estimate the effect of portal venous flow with the effect of hepatic arterial flow on wedged pressure, we compared the results from *Experiment 1*, reduction of portal venous flow, with the results from *Experiment 2*, reduction of hepatic arterial flow. Interestingly, changes of wedged pressure and sinusoidal vascular resistance were similar in response to reduction of portal venous flow or to reduction of hepatic arterial flow.

DISCUSSION

The hepatic venous wedged pressure gives an excellent approximation to actual portal pressure and is used to monitor the effect of drug efficacy in portal hypertension (2, 12, 20).

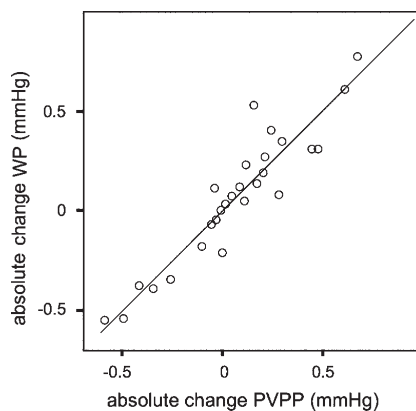

Fig. 3. Correlation between absolute changes of PVPP and WP in cirrhotic animals due to changes in hepatic arterial flow ($r = 0.92$, $P < 0.001$).

Moreover, at the moment, it is the only method that can define the portal pressure response to pharmacological therapy since other clinical or radiological parameters do not reliably reflect this response (12). However, this concept of measuring drug efficacy using hepatic venous pressure gradient assumes that the decrease achieved on portal pressure and hepatic venous wedged pressure is all mediated by a decrease of portal venous inflow (16, 29, 30).

In the present study, we show a significant influence of hepatic arterial flow on portal venous and wedged pressure, as well as portal venous resistance and sinusoidal resistance in cirrhotic and normal animals. Moreover, we found a correlation between flow-induced changes of portal venous perfusion pressure and wedged pressure due to both changes in portal venous and hepatic arterial flow. Several studies have shown an excellent correlation between portal venous and hepatic venous wedged pressure in animals, as well as in humans (11, 12, 21).

In our study, portal venous perfusion pressure correlated well with the wedged pressure due to changes in portal venous and hepatic arterial flow. Animal studies investigating the influence of hepatic arterial flow on portal venous pressure found different results. Decreasing or stopping hepatic arterial flow modifies the portal venous pressure over a wide range (3, 8, 14, 22). Reduction in hepatic arterial flow caused decreases in portal venous pressure probably due to alterations in total blood flow through the sinusoids (3, 22). However, all of these studies were performed in normal animals and did not measure the sinusoidal resistance. Our study was performed in both cirrhotic and normal animals, and we measured the wedged pressure, a reflection of sinusoidal resistance. We found that changes of portal venous pressure and resistance in response to decreased and increased hepatic arterial flows were similar in cirrhotic and control animals. Our results with equal changes in sinusoidal resistance in both models support the hypothesis that alteration in total blood flow through the sinusoids is the main mechanism of change in portal resistance. Furthermore, the same response on wedged pressure and sinusoidal resistance following changes in flow either in the portal vein or in the hepatic artery support this hypothesis. Moreover, it indicates that, in the liver perfusion model, the portal venous vascular resistance is located in the sinusoids (3). On the other hand, changes in hepatic arterial flow were smaller in cirrhotic animals. Smaller increases in hepatic arterial flow caused equal increases in wedged pressure when we compared cirrhotic with control livers. This could be interpreted as a greater influence of hepatic arterial flow on wedged pressure in cirrhosis.

In cirrhotic animals, as well as in patients, hepatic arterial vascular resistance has been shown to be higher than, equal to, or lower than that without liver disease (5, 15, 23, 26, 30, 32, 33). We found a significantly smaller increase in hepatic arterial pressure in CCl_4-induced cirrhotic animals compared with normal animals in response to increase in hepatic arterial flow. The mechanisms involved in this vasodilatation have not been completely elucidated, but it has been shown that the hepatic artery is under local as well as systemic influence (15, 17). Therefore, an involvement of different locally and systemically produced vasodilatory factors like nitric oxide and adenosine are possible (4, 32).

To investigate the influence of the hepatic arterial flow on portal venous and wedged pressure, we used a bivascular liver perfusion system. Although this preparation is established, there have been differences with in vivo measurements in recent years. The viscosity of the Krebs-Henseleit solution is lower than the viscosity of blood, which leads to a lower shear stress and subsequent to a lower perfusion pressure and vascular resistance. Furthermore, we could not observe a change of hepatic arterial vascular resistance due to changes of portal venous perfusion flow. Therefore, it seems that this preparation lacks the hepatic arterial buffer response. It was described by other investigators that the perfusion system does not show the normal hepatic arterial buffer response (3). However, in preliminary experiments (data not shown) with a greater decrease of portal venous flow, we found a marked decrease of hepatic arterial vascular resistance, showing the presence of the hepatic arterial buffer response in the used perfusion system.

In conclusion, this study demonstrates that changes in hepatic arterial flow lead to respective changes in portal venous and wedged pressure. Our findings indicating a direct influence of hepatic arterial flow on portal venous and wedged pressure most likely due to changes in total flow through the sinusoids. This was observed in cirrhotic and normal animals, and a similar reduction of portal venous and hepatic arterial flow lead to comparable reduction in wedged pressure.

GRANTS

A. Zipprich was supported by the Wilhelm-Roux-Programm of the Martin-Luther-University Halle-Wittenberg. M. Loureiro-Silva was supported by the American Liver Foundation through the Liver Scholar Award and by the Pilot Project Grant from the Liver Center Yale University. R. J. Groszmann was supported by the Veterans Affairs Merit Review.

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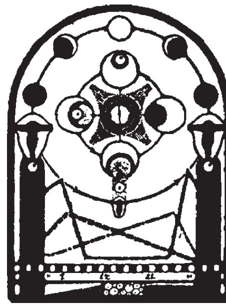

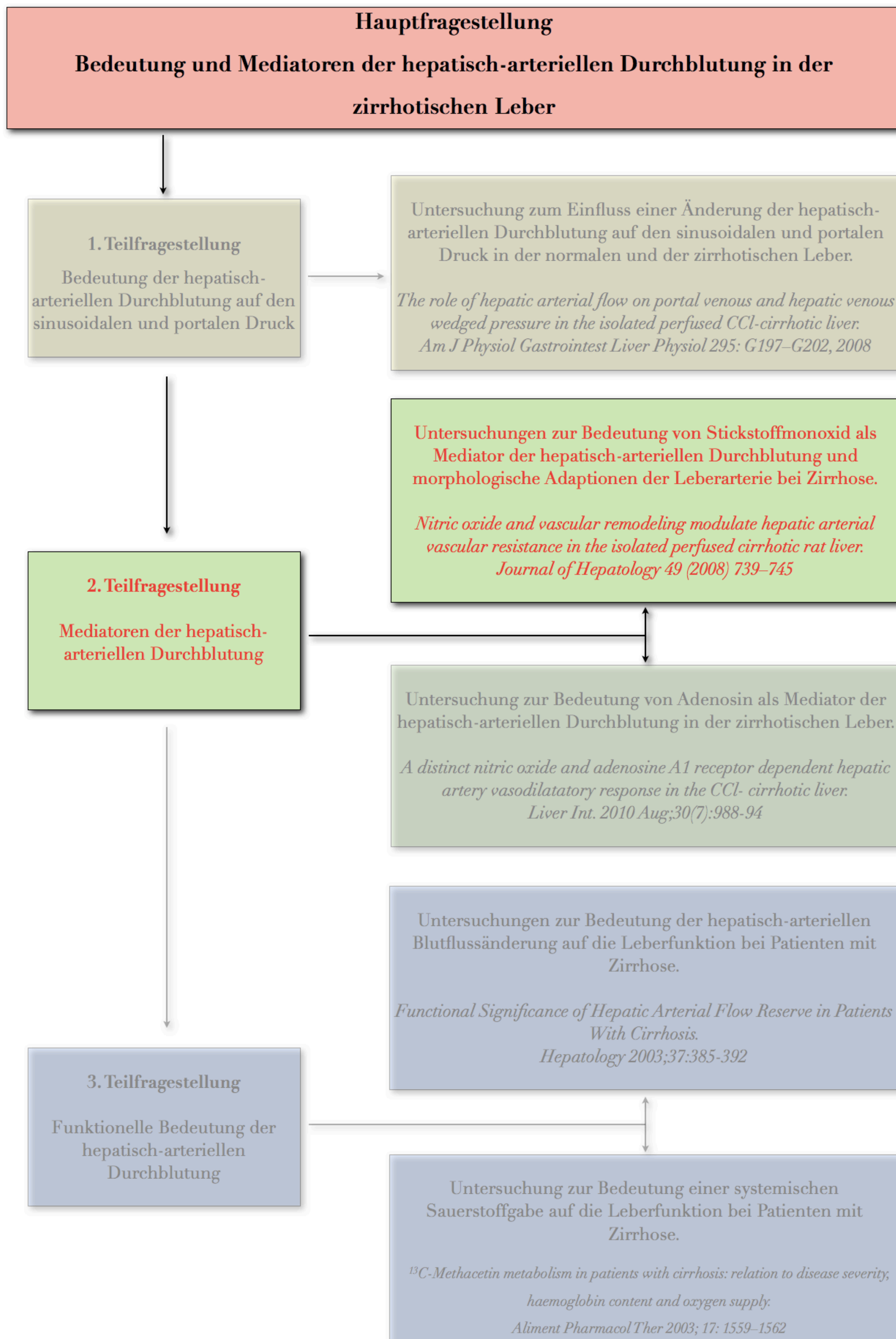
Hauptfragestellung
Bedeutung und Mediatoren der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber
1. Teilfragestellung
Bedeutung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck
Untersuchung zum Einfluss einer Änderung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck in der normalen und der zirrhotischen Leber.
The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl-cirrhotic liver.
Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008
Untersuchungen zur Bedeutung von Stickstoffmonoxid als Mediator der hepatisch-arteriellen Durchblutung und morphologische Adaptionen der Leberarterie bei Zirrhose.
Nitric oxide and vascular remodeling modulate hepatic arterial vascular resistance in the isolated perfused cirrhotic rat liver.
Journal of Hepatology 49 (2008) 739–745
2. Teilfragestellung
Mediatoren der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung von Adenosin als Mediator der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber.
A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatatory response in the CCl- cirrhotic liver.
Liver Int. 2010 Aug;30(7):988-94
Untersuchungen zur Bedeutung der hepatisch-arteriellen Blutflussänderung auf die Leberfunktion bei Patienten mit Zirrhose.
Functional Significance of Hepatic Arterial Flow Reserve in Patients With Cirrhosis.
Hepatology 2003;37:385-392
3. Teilfragestellung
Funktionelle Bedeutung der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung einer systemischen Sauerstoffgabe auf die Leberfunktion bei Patienten mit Zirrhose.
13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply.
Aliment Pharmacol Ther 2003; 17: 1559–1562

2. Nitric oxide and vascular remodeling modulate hepatic arterial vascular resistance in the isolated perfused cirrhotic rat liver. Journal of Hepatology 49 (2008) 739–745

Die Bedeutung von Stickstoffmonoxid für die hepatisch-arterielle Vasodilatation und stickstoffmonoxid-abhängige morphologische Veränderungen der Gefäßwand, wie Remodeling der Leberarterie, sind unklar und wurden in der vorliegenden Arbeit untersucht.

Normalratten (eine Kontrollgruppe zur CCl_4-induzierten Zirrhose und eine Sham-operierte Gruppe als Kontrollgruppe der BDL) und zwei verschiedene Zirrhosemodelle (gallengangsligierte Ratte - BDL und CCl_4-induzierte Zirrhose) wurden einer bivaskulären Leberperfusion mit Messung des portal-venösen, des hepatisch-arteriellen und des sinusoidalen Drucks unterzogen. Dosis-Wirkungs-Kurven der Leberarterie wurden durch Gabe des Vasokonstriktors Methoxamin (alpha1-Agonist) jeweils ohne oder unter vorheriger Inkubation mit L-NMMA (einem Stickstoffmonoxidsynthetase-Hemmer) erhalten. Weiterhin wurde durch semi-quantitative Auswertung der histologischen Präparate der Gefässdurchmesser, die Gefässwanddicke und die Anzahl der Gefässmuskelzellen in den Leberarterien bestimmt und die Gefässwanddicke bzw. die Gefässmuskelanzahl im Verhältnis zum Gefässdurchmesser berechnet.

Der hepatisch-arterielle Widerstand ($p<0,001$) und die Vasokonstriktion nach Gabe von Methoxamin ($p<0,04$) waren niedriger in den Leberarterien von zirrhotischen Ratten verglichen mit den Normalratten (Abbildung 6). Weiterhin zeigten BDL-zirrhotische Ratten einen geringeren hepatisch-arteriellen Widerstand ($p<0,001$) und eine geringere Vasokonstriktion nach Methoxamingabe verglichen mit CCl_4-zirrhotischen Ratten ($p=0,011$).

Die Inkubation mit L-NMMA führte bei beiden Zirrhosemodellen zu einem signifikanten Anstieg der Vasokonstriktion nach Methoxamingabe ($p<0,05$; Abbildung 6). Während die erhaltenen Dosis-Wirkungs-Kurve der CCl_4-induzierten Zirrhoseleber keinen signifikanten Unterschied zur Normalleber unter L-NMMA-Inkubation zeigte, war die Dosis-Wirkungs-Kurve der BDL-Ratte weiterhin signifikant niedriger verglichen zur Normalratte ($p<0,001$; Abbildung 6) und zur CCl_4-Zirrhoseratte ($p<0,003$).

Das Verhältnis von Gefässwanddicke und Gefässdurchmesser war signifikant ($p<0,02$) geringer in beiden Zirrhosemodellen verglichen mit den entsprechenden Normalratten, aber nicht unterschiedlich zwischen beiden Zirrhosemodellen. Weiterhin war das Verhältnis von Gefässmuskelzellen und Gefässdurchmesser in den Normalratten höher im Vergleich zu den Zirrhosemodellen ($p<0,05$) und höher in den CCl_4-zirrhotischen Arterien verglichen mit den BDL-zirrhotischen Arterien ($p=0,005$).

Schlussfolgerung: Stickstoffmonoxid ist ein wichtiger Modulator der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber mit größerer Bedeutung in der CCl_4-induzierten Zirrhose. Die BDL-induzierte Zirrhose weisst demgegenüber stärker ausgeprägte strukturelle Gefäßwandveränderungen im Sinne eines Remodelings auf.

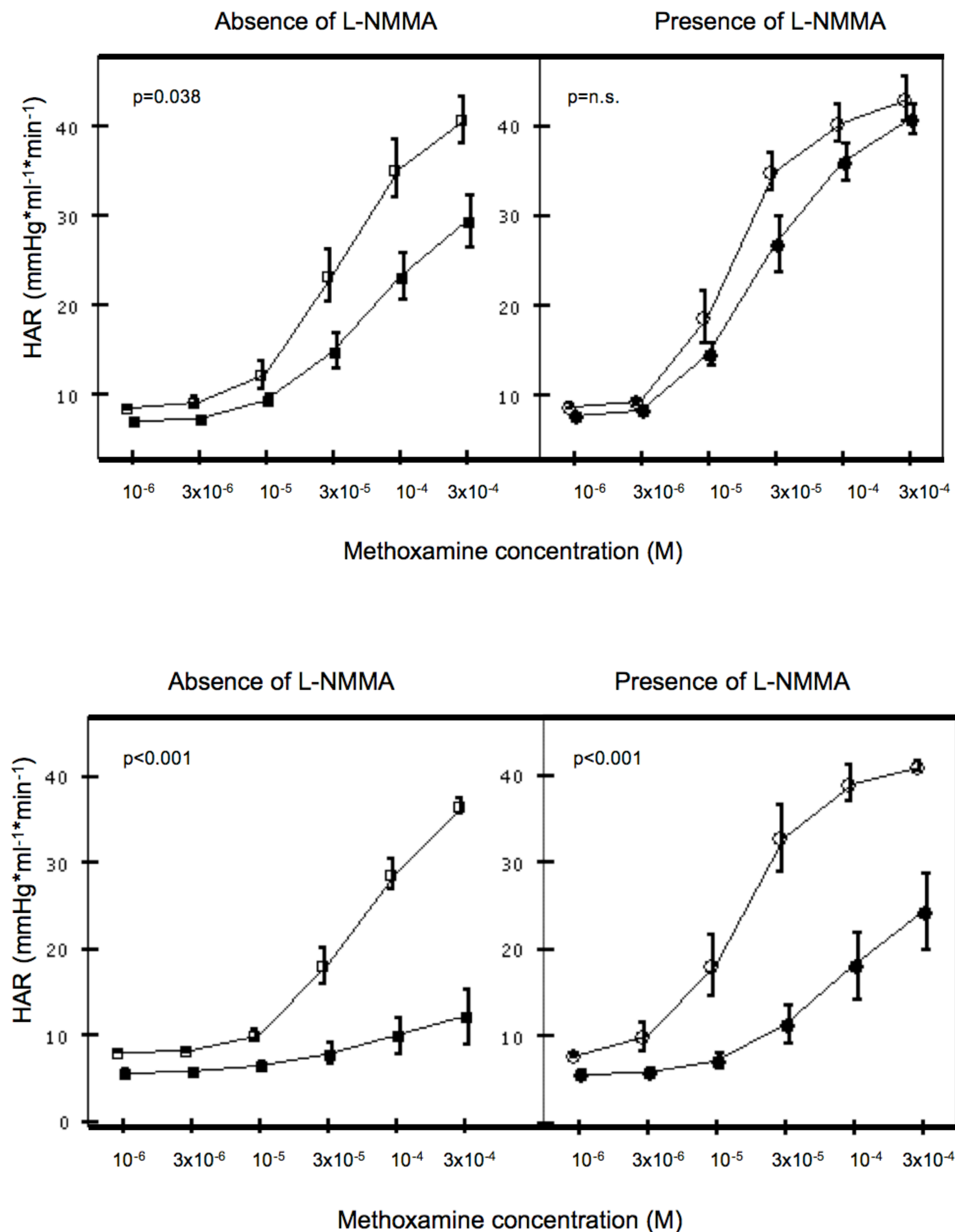

Abbildung 6: Dosis-Wirkungs-Kurve des Einflusses von Methoxamin auf die Vasokonstriktion der Leberarterien (HAR) ohne (absence) und mit (presence) Inkubation des Stickstoffmonoxidsyntheseblockers L-NMMA in Lebern normaler (weiße Symbole) und CCl_4-zirrhotischer (schwarze Symbole; oberes Diagramm) sowie in Lebern Sham-operierter (weiße Symbole) und BDL-zirrhotischer (schwarze Symbole; unteres Diagramm) Ratten [aus Referenz (67)].

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Nitric oxide and vascular remodeling modulate hepatic arterial vascular resistance in the isolated perfused cirrhotic rat liver☆

Alexander Zipprich[1,2], Mauricio R. Loureiro-Silva[1,2], Dhanpat Jain[3], Irita D'Silva[2], Roberto J. Groszmann[1,2,*,†]

[1]*Digestive Disease Section, Yale University School of Medicine, New Haven, CT, USA*
[2]*Hepatic Hemodynamic Laboratory, Veterans Affairs Medical Center, West Haven, CT, USA*
[3]*Department of Pathology, Yale University School of Medicine, New Haven, CT, USA*

***Background/Aims*: Hepatic arterial resistance is modulated by the hepatic arterioles but the role of NO and vascular remodeling in hepatic arterial resistance in cirrhosis is unknown.**

***Methods*: Cirrhosis was induced by CCl_4 or BDL. Using a bivascular liver perfusion dose-responses curves to methoxamine were obtained from the hepatic artery in absence and presence of L-NMMA. Lumen-diameter, wall thickness and number of smooth muscle nuclei were quantitated in the arteries using image analysis.**

***Results*: Hepatic arterial resistance and the response to methoxamine were lower in cirrhosis compared to controls ($p \leqslant 0.04$) and lower in BDL compared to CCl_4 ($p \leqslant 0.01$). L-NMMA increased the response to methoxamine in CCl_4 ($p = 0.002$) and BDL ($p = 0.05$) but corrected the response only in CCl_4 (p = n.s. vs. control). Wall thickness and the number of smooth muscle nuclei were significantly smaller in cirrhosis compared to controls ($p < 0.05$) and the number of nuclei was also lower in BDL compared to CCl_4 ($p = 0.005$).**

***Conclusions*: NO is the main modulator of hepatic arterial resistance in CCl_4 but not in BDL. Intrahepatic arterial remodeling is present in both cirrhotic models but is greater in BDL. This indicates a larger role of structural changes in the control of hepatic arterial resistance in BDL.**
Published by Elsevier B.V. on behalf of European Association for the Study of the Liver.

***Keywords*: Hepatic artery; Rat liver perfusion; Cirrhosis; Bile duct ligation; Carbon tetrachloride; Nitric oxide; Vascular remodeling**

Received 7 January 2008; received in revised form 2 June 2008; accepted 4 June 2008; available online 6 August 2008
Associate Editor: J. Bosch
☆ The authors declare that they do not have anything to disclose regarding funding from industries or conflict of interest with respect to this manuscript. This study was funded by Department of Veterans' Affairs.
* Corresponding author. Tel.: +1 203 932 5711x4696; fax: +1 203 932 3873.
E-mail address: roberto.groszmann@yale.edu (R.J. Groszmann).
† Address: Digestive Disease Section/111H, VA Connecticut Healthcare System, 950 Campbell Avenue, West Haven, CT 06516, USA.
Abbreviations: CCl4, carbon tetrachloride; NO, nitric oxide; HAR, hepatic arterial resistance; BDL, bile duct ligated; PVR, portal venous vascular resistance; SiVR, sinusoidal vascular resistance; PreSiVR, presinusoidal vascular resistance; PBS, phosphate buffered saline; EVG, Elastic Van-Geisson; C, circumference; LD, lumen-diameter; WT, wall thickness; N, nuclei; N/LD, nuclei to diameter; L-NMMA, nitric oxide synthase inhibitor.

1. Introduction

Increased intrahepatic vascular resistance is the main cause of the development of portal hypertension in cirrhosis [1]. Although structural changes are the main cause of this increased intrahepatic vascular resistance, an enhanced intrahepatic vascular tone is also involved in the pathophysiology of this hemodynamic abnormality [1]. Increased production of vasoconstrictors and decreased production of vasodilators, mainly nitric oxide (NO), are the main factors leading to this enhanced vascular tone [1,2]. We have previously shown that the increased vascular tone observed in CCl_4-induced cirrhotic rat livers is located in the sinusoidal and post-sinusoidal areas, which is caused, at least in part, by a deficient

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doi:10.1016/j.jhep.2008.06.027

NO production. On the other hand, we observed a preserved NO production in the pre-sinusoidal portal venous segment in these cirrhotic rat livers [3].

In contrast to the findings in the intrahepatic circulation, the vascular resistance in the splanchnic and systemic circulation in cirrhosis is decreased and the production of NO increased [4]. The liver has a dual blood supply via the portal vein and the hepatic artery. Contrary to the portal circulation the hepatic arterial vascular resistance (HAR) seems to be influenced by systemic as well as local factors. It has been known for many years that while the portal blood flow entering the liver is progressively decreased in cirrhosis the hepatic arterial flow is increased [5,6]. The HAR is located mainly in the hepatic arterioles (pre-sinusoidal). We have recently shown that the HAR is decreased in CCl_4-cirrhotic rats [7]. However, the mechanisms involved in this hepatic arterial vasodilatation are not completely elucidated. Different investigators have explored the participation of an increased NO production on this abnormality but the results are controversial and inconclusive [7–11].

On the other hand, the reduction in HAR may be, in part, due to structural adaptation in the vessels, a process known as vascular remodeling. In fact, vascular remodeling has been shown to occur in pulmonary and superior mesenteric arteries of cirrhotic animals [12,13]. Interestingly, NO may act as mediator of vascular structural changes by inhibiting vascular smooth muscle cell proliferation and migration, and by stimulating endothelial cell migration and reorganization [14]. However, it is unknown if the hepatic arterioles undergo remodeling in cirrhosis.

Therefore, the aims of this study were to investigate the role of NO in modulating the decreased hepatic arterial vascular resistance and also to investigate possible structural adaptations of the intrahepatic arterial vessels to the chronic increased hepatic arterial blood flow observed in liver cirrhosis.

2. Methods

Eighty male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were included in this study. The American Physiological Society guide principles for the care and use of animals were followed. The appropriate Institutional Animal Care and Use committee previously approved all procedures involving animals. In this study we utilized two different models of cirrhosis: The CCl_4 model with a sinusoidal and post-sinusoidal involvement and the bile duct ligated model (BDL) with portal fibrosis and pre-sinusoidal–sinusoidal portal hypertension.

2.1. Induction of CCl_4-cirrhosis

Rats underwent inhalation exposure of carbon tetrachloride (CCl_4) three times a week. Phenobarbital (0.35 g/l) was added to the drinking water as described previously [3]. Treatment was given for at least 12 weeks. Perfusions were performed 6–10 days after the last doses of CCl_4 and Phenobarbital. Age-matched rats were used as control group.

2.2. Surgical procedure for BDL-cirrhosis

Bile duct ligation (BDL) was performed as described before [15]. Briefly, the abdomen was opened through a midline incision and the common bile duct was exposed. The common bile duct was ligated twice with 3-0 silk and resected between ligatures. Muscle and skin were sutured separately using 3-0 silk. The sham group underwent the same procedure including manipulation of the common bile duct but without ligation and resection of the common bile duct. Sham and BDL animals were treated with Vitamin K (Hospira, Lake Forest, IL, USA) once a week [16].

2.3. In situ rat liver perfusion

Rats were anesthetized with ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA; 100 mg/kg body weight) and xylazine (Rompum, Bayer, Shawne Mission, KS, USA; 40 mg/animal). A bivascular liver perfusion was performed as described before [17]. Briefly, after the abdomen was opened loose ligatures were placed around the aorta cranial to the celiac artery, around the superior mesenteric artery immediately after branching from the aorta, and the aorta caudal to the mesenteric artery. Left gastric and splenic arteries were tied at its origin of the celiac artery and a lose ligature placed around the esophagus. Left and right renal arteries as well as gastroduodenal artery (branch of the common hepatic artery) were ligated. Except in the BDL rat, the bile duct was cannulated with a polyethylene tube (PE 10). The portal vein was cannulated with a 14-gauge Teflon catheter and the perfusion with 32 ml/min of oxygenated (95% O_2, 5% CO_2) Krebs–Henseleit solution containing dextrose (11 mM) in a non-recirculating mode was started. The inferior vena cava was cut immediately. The aorta was cannulated with an 18-gauge Teflon catheter and the ligatures around the superior mesenteric artery and the esophagus were closed. The perfusion of the hepatic artery with 8 ml/min of oxygenated (95% O_2, 5% CO_2) Krebs–Henseleit solution containing dextrose (11 mM) in a non-recirculating mode was started. The tip of the catheter was placed close to the branch of the celiac artery and all ligatures around the aorta were closed. A 14-gauge catheter was introduced in the inferior vena cava and the thorax was opened.

In order to measure the sinusoidal pressure, a PE-60 catheter was guided from the right atrium, through the thoracic segment of the inferior vena cava into the left hepatic lobe and wedged in the hepatic vein. The ligature around the inferior vena cava was closed to secure the wedged catheter. The preparation was transferred to a temperature-controlled (37 °C) Plexiglas perfusion chamber (Yale University Medical Instrument) initiating the stabilization period.

During the stabilization and the experimental period the perfusion pressure of the portal vein and the hepatic artery were measured constantly using two independent strain-gauge transducers (P23XL, Spectramed, Oxnard, CA, USA), respectively. The wedged pressure was measured during the experimental period using a third independent strain-gauge transducer (P23XL, Spectramed, Oxnard, CA, USA). Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilium. Perfusion and sinusoidal pressure were continuously recorded by Chart 3.6 program using MacLab/4e hardware (AD instruments). During the stabilization and experimental period the perfusate was oxygenated using a silastic tubing lung interposed between the perfusate reservoir and the peristaltic pump [18].

2.4. Experimental design

All livers were perfused with constant flow during the stabilization period flow through the wedged catheter was also maintained. The stabilization period was performed in a recirculating mode in absence or presence of the NO-production inhibitor L-NMMA (4×10^{-4} M; Sigma Chemicals Co., St. Louis, MO, USA). After the stabilization period the wedged catheter outflow was interrupted, allowing the measurement of the wedged pressure and the perfusion was changed to an open mode in presence and absence of L-NMMA, respectively. A

dose-response curve using six consecutive doses of the α1-agonist methoxamine (10^{-6} to 3×10^{-4} M; Sigma Chemicals Co., St. Louis, MO, USA) infused in the hepatic artery were performed.

Liver global viability was assessed by gross appearance of the liver, stable perfusion curves and bile production (except in the BDL rats) during the stabilization period (>0.4 μl min^{-1} g^{-1} liver). After the experiment liver and spleen were removed and weighed. Liver tissue samples were collected and fixed in formalin.

2.5. Calculations of vascular resistances

HAR was calculated from the hepatic arterial flow and the hepatic arterial perfusion pressure. Portal venous vascular resistance (PVR) was calculated from the portal venous perfusion pressure and portal venous flow. Sinusoidal vascular resistance (SiVR) was calculated from the wedge pressure and the total flow, i.e. portal venous plus hepatic arterial flow. Portal venous pre-sinusoidal vascular resistance (PreSiVR) was calculated from the difference between portal venous resistance and sinusoidal resistance.

2.6. Tissue harvest, histology, and morphometry

Tissue harvest and morphometric analysis were performed as previously described [14]. Rats were anesthetized with ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA; 100 mg/kg body weight) and xylazine (Rompum, Bayer, Shawne Mission, KS, USA; 40 mg/animal). The abdomen was opened and two ligatures were placed around the portal vein. The portal vein was cannulated with a 14-gauge Teflon catheter and the perfusion with 40 ml/min of oxygenated (95% O_2, 5% CO_2) phosphate buffered saline (PBS, pH 7.4) in a non-recirculating mode was started. The inferior vena cava was cut immediately. The thorax was opened and an additional perfusion at a constant pressure via the left ventricle with PBS was started. Perfusion was maintained until obtaining a clear outflow-perfusate. Thereafter, the perfusion medium was changed to PBS containing adenosine (0.1 mM; Sigma Chemicals Co., St. Louis, MO, USA), papaverine (0.1 mM; Sigma Chemicals Co., St. Louis, MO, USA), and heparin sodium (50 U/ml) to relax vascular smooth muscle followed by perfusion fixation with freshly depolymerised, 4% paraformaldehyde in PBS [13,14]. Liver tissue samples from the right hepatic lobe were carefully excised and stained using hematoxylin and eosin (HE). On representative tissue blocks from each group sections were stained with Elastic Van-Geisson (EVG) and desmin by indirect immunoperoxidase method. The EVG stain highlights the elastic tissue in the vessels while desmin stain helps to identify smooth muscle in the vessel wall.

Morphometric analyses of arterial vessels from the entire sample were performed using video microscopy. The image was captured and displayed on a computer monitor using an image analysis program (Bioquant Nova Prime, Bioquant Image Analysis Corporation, Nashville, TN, USA). The perimeter of the vessel lumen was measured twice in every vessel and the average of these measurements was taken as the circumference (C) of a circle. Lumen-Diameter (LD) was determined from the equation $LD = C/\pi$ assuming that the cross section of the vessel was circular *in vivo* [14]. Wall thickness (WT) was measured twice eight times in every vessel (i.e. every 45°) as the linear distance between endothelium and adventitia and the values were averaged. Hematoxylin positive nuclei (N) were counted in each vessel. In order to compare different vessels, the ratio of wall thickness to diameter (WT/LD) and number of nuclei to diameter (N/LD) were calculated and used for comparison between the different groups [19]. Morphometric analyses were performed by two independent investigators, one blinded to the perfusion results.

2.7. Statistics

Data are presented as means ± SEM. Mann–Whitney test was used for comparisons of two different groups and One-Way-ANOVA for comparison of more than two groups followed by a preplanned contrast test to compare cirrhotic and control groups. Comparison for repeated measurements was assessed using multivariate analysis of repeated measurements followed by Bonferroni correction to detect differences between groups, p-values ⩽0.05 were considered significant.

3. Results

Cirrhosis was histologically confirmed in both CCl_4- and BDL-cirrhotic animals and ascites was present in all cirrhotic animals. Differences in body and liver weights among models are summarized in Table 1.

3.1. Basal vascular resistances

Hepatic arterial vascular resistance (HAR) was lower in CCl_4 ($p < 0.001$) and BDL ($p < 0.001$) compared to control and sham. Furthermore, HAR was lower in BDL compared to CCl_4($p < 0.001$; Table 1). Portal venous vascular resistance (PVR) was higher in CCl_4 ($p < 0.001$) and BDL ($p = 0.013$) compared to control groups. Additionally, sinusoidal vascular resistance (SiVR) was higher in CCl_4 ($p = 0.002$) and BDL ($p = 0.011$) compared to appropriate control group (Table 1). However, neither PVR nor SiVR were significantly different between CCl_4 and BDL. In contrast to portal venous and sinusoidal vascular resistance, pre-sinusoidal vascular resistance (PreSiVR) was higher in BDL compared to CCl_4 ($p = 0.007$; Table 1). Moreover, PreSiVR was also higher in BDL compared to sham ($p = 0.013$) but was not different between CCl_4 and control.

Presence of L-NMMA did not change basal perfusion pressures.

3.2. Dose-response to α1-agonist methoxamine in the hepatic artery

Absolute increases in hepatic arterial vascular resistance (HAR) were lower in CCl_4 ($p = 0.038$; $n = 7$) and BDL ($p = 0.001$; $n = 7$) compared to control ($n = 7$) and sham ($n = 7$; Figs. 1 and 2). Furthermore, the absolute increase in HAR was lower in BDL compared to CCl_4 ($p = 0.011$). In contrast, absolute increases in PVR and SiVR were not different among the different groups in response to methoxamine administration in the hepatic artery.

Presence of the NO-production inhibitor L-NMMA significantly increases the response to methoxamine in all four groups (Figs. 1 and 2). Interestingly, in cirrhotic livers presence of L-NMMA increased the response to MTX in both CCl_4 ($p = 0.002$, Fig. 1) and BDL ($p = 0.05$; Fig. 2) but completely corrected the response only in the CCl_4 model (p = n.s. vs. control; Fig. 1). In that regard, the observed absolute increase in hepatic arterial vascular resistance (HAR) in presence of L-NMMA was lower in BDL compared to sham ($p < 0.001$) and CCl_4 ($p = 0.003$).

Comparing the absolute change in pressure response at the highest dose of methoxamine induced by L-NMMA we found similar results in BDL and CCl_4 cirrhotic animals.

Table 1
Description of animal characteristics, liver perfusion parameters, and the absolute values of the morphological parameters in both cirrhotic groups (CCl_4 and BDL) as well as both normal groups (control and sham)

	Control	CCl_4	Sham	BDL
Weight (g)	413.4 ± 9.7	350.5 ± 9.6[*,&]	420.8 ± 13.2	401.9 ± 14.9
Liver weight (g)	13.5 ± 0.8	13.5 ± 0.8	12.2 ± 0.2	20.5 ± 1.1[**,$]
Spleen weight (g)	1.3 ± 0.04	2.3 ± 0.2[*]	1.2 ± 0.03	2.5 ± 0.2[**]
HAR ($mmHg\ ml^{-1}\ min^{-1}$)	8.62 ± 0.19	7.26 ± 0.12[*]	8.16 ± 0.13	5.75 ± 0.24[**,$]
PVR ($mmHg\ ml^{-1}\ min^{-1}$)	0.20 ± 0.01	0.27 ± 0.01[*]	0.18 ± 0.01	0.38 ± 0.05[***]
SiVR ($mmHg\ ml^{-1}\ min^{-1}$)	0.08 ± 0.008	0.14 ± 0.01[*]	0.07 ± 0.006	0.12 ± 0.01[**]
PreSiVR ($mmHg\ ml^{-1}\ min^{-1}$)	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.25 ± 0.05[***,$$]
Lumen-Diameter (μm)	28.99 ± 5.51	53.63 ± 17.71	47.03 ± 12.54	60.89 ± 14.17
Wall thickness (μm)	0.47 ± 0.03	0.40 ± 0.04	0.52 ± 0.06	0.35 ± 0.04[***]
Nuclei per vessel	55.11 ± 10.07	64.43 ± 17.48	75.89 ± 19.89	30.55 ± 6.65

HAR, hepatic arterial vascular resistance; PVR, portal venous vascular resistance; SiVR, sinusoidal vascular resistance; PreSiVR, pre-sinusoidal vascular resistance.
[&] $p = 0.008$ vs. BDL.
[$] $p < 0.001$.
[$$] $p = 0.007$ vs. CCl_4.
[*] $p \leqslant 0.002$ vs. control.
[**] $p < 0.001$.
[***] $p < 0.02$ vs. sham.

3.2.1. Remodeling of the intrahepatic arterial vessels

The absolute values of lumen diameter (LD) and wall thickness (WT) of the different groups are indicated in Table 1. In order to compare the different vessels ratios between WT and LD as well as the number of smooth muscle nuclei (N) and LD were calculated. WT/LD was significantly smaller in CCl_4 (0.0009 ± 0.0001; $p = 0.011$) as well as BDL (0.0008 ± 0.0002; $p = 0.006$) compared to control (0.0023 ± 0.0005) and sham (0.0015 ± 0.0002) group but not different between CCl_4 and BDL. N/LD was larger in control (0.0023 ± 0.0004) and sham (0.0018 ± 0.0004) livers compared to CCl_4 (0.0014 ± 0.0002; $p = 0.043$) and BDL (0.0006 ± 0.0001; $p < 0.001$) and, moreover, smaller in BDL compared to CCl_4 ($p = 0.005$) (Fig. 3).

4. Discussion

The findings of the present study indicate that in the hepatic artery of both cirrhotic models the vascular resistance is significantly reduced. This conclusion is based on a lower basal hepatic arterial vascular resistance and decreased hepatic arterial vasoconstriction in response to the vasoconstrictor methoxamine. Moreover, we observed an even lower basal HAR and a lesser vasoconstriction in BDL- compared to CCl_4-induced cirrhosis. In fact, these two models are markedly different. While the CCl_4 model of cirrhosis presents mainly with sinusoidal and post-sinusoidal involvement, in the BDL model there is significant inflammation and fibrosis that originates mainly in the portal space, with signif-

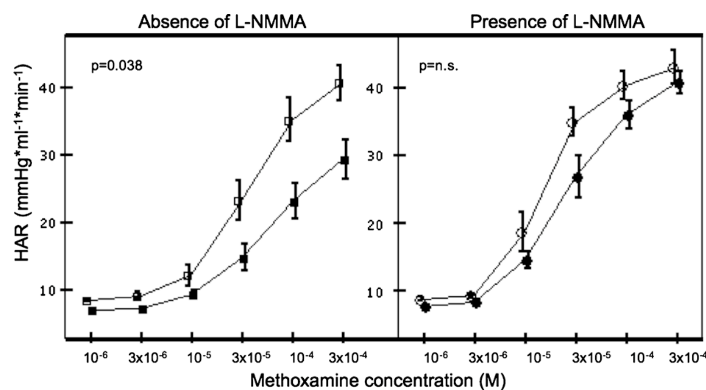

Fig. 1. Dose–response curves of the hepatic artery in response to methoxamine (α1-agonist) in control (open symbols) and CCl_4-cirrhotic (filled symbols) rats in the absence (left panel) and presence (right panel) of L-NMMA (Multivariate analysis for repeated measurements and Bonferroni test).

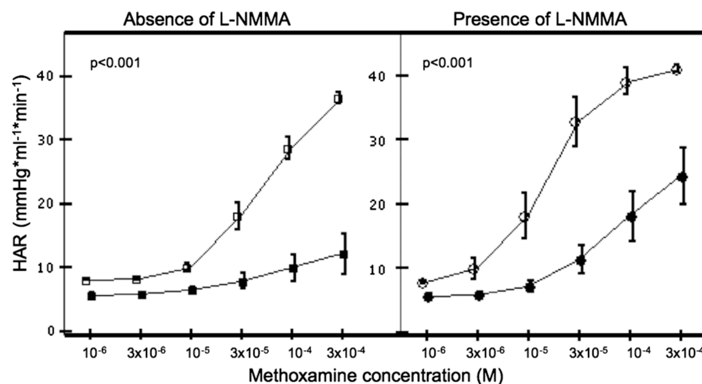

Fig. 2. Dose-response curves of the hepatic artery in response to methoxamine (α1-agonist) in sham (open symbols) and BDL-cirrhotic (filled symbols) rats in the absence (left panel) and presence (right panel) of L-NMMA (Multivariate analysis for repeated measurements and Bonferroni test).

icant pre-sinusoidal portal hypertension (Table 1). The underlying mechanisms are likely related to both NO availability and structural changes in the vascular bed as suggested by our data.

The NO synthase inhibitor (L-NMMA) increases the response to methoxamine in the hepatic artery of both cirrhotic models. This finding indicates an increase availability of NO in the hepatic artery of these two models. However, the presence of L-NMMA completely corrects the response to methoxamine in CCl_4-cirrhosis but not in the BDL-cirrhotic model. Our results in BDL are similar to the findings of Yang et al. who have shown an improved response to noradrenaline (α1-adrenoreceptor agonist) in the hepatic artery of BDL-cirrhotic rats in presence of L-NMMA [11].

The site of main vascular resistance of the hepatic arterial tree resides in the hepatic arterioles, which are located in the pre-sinusoidal area [20]. In that respect, our findings of an increased NO availability in the hepatic arterioles in both cirrhotic models contrasts with the

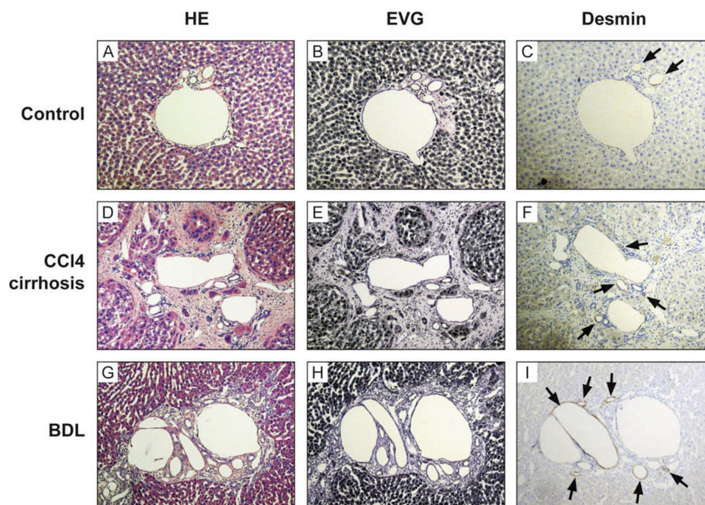

Fig. 3. Vascular remodeling in CCl_4 (middle panel) and BDL-animals (lower panel) compared to controls (top panel). HE (A,D,G) and EVG (B,E,H) stains highlight the vessels in each group. In addition, desmin stain (C,F,I) highlights the smooth muscle in the arteriolar walls. Increase in the number of arteriolar profiles is easily evident in both models (arrows). Also note that few of the arterioles in the BDL-model have markedly increased luminal diameter. The differences in wall thickness are subtle and difficult to appreciate in the photomicrographs. [This figure appears in colour on the web.]

known reduction in NO production in the sinusoidal bed from CCl_4-cirrhotic animals [21]. On the other hand, these results reinforce our previous observation that NO availability in the CCl_4-cirrhotic livers is preserved in the pre-sinusoidal area while it is decreased in the sinusoidal/post-sinusoidal areas [3].

The basal HAR in BDL-cirrhosis was lower and the response to methoxamine was also lower after inhibition of NO generation. However, our result of similar change of HAR at the highest dose of methoxamine, induced by inhibition of NO, suggests similar NO production in both cirrhotic models. Therefore, differences in NO production cannot explain the differences in HAR between the two models.

An explanation for even more severe decrease of hepatic arterial vascular resistance in BDL cirrhosis is likely due to structural changes in the hepatic arterioles i.e. remodeling of the vessel wall. Indeed, several investigators have shown structural changes in other arterial vessels in chronically low arterial blood pressure conditions in normal and cirrhotic animals [13,19,22,23]. Our results also indicate intrahepatic vascular remodeling of the hepatic arterioles in both cirrhotic models. These changes are reflected by the increase in luminal diameter, decrease in wall thickness and less smooth muscle cells in both animal models (Table 1; Fig. 3). These data suggest an increase in the numbers of thin walled dilated arterioles in both models compared to controls and explains decreased HAR also based on structural basis. The changes of luminal dilatation and wall thinning were significantly more prominent in BDL-animals compared to CCl_4-animals. More structural changes in BDL-animals also supports the observed lower HAR compared to CCl_4-animals. Although we did not quantitatively measure the number of vessels there was an increase in the number of arteriolar vessels in the portal areas in both models, indicating neo-angiogenesis. Furthermore, BDL-cirrhotic rats have less smooth muscle cells compared to CCl_4-cirrhotic rats explaining the lower response to methoxamine in absence and presence of L-NMMA [23].

BDL-cirrhotic rats show a greater activity of disease in the pre-sinusoidal area and a more severe hyperdynamic circulatory dysfunction compared to CCl_4-cirrhotic rats [24,25]. Since the hepatic artery is under local as well as systemic control both factors could influence the grade of remodeling. Indeed, it has been shown that the hepatic arterial blood flow is more than twice as high in the BDL than in the CCl_4 model [26,27]. It is possible that the initial lesion that leads to cirrhosis in the BDL-model, which is predominantly in the pre-sinusoidal area, cause these differences in hepatic arterial blood flow. Therefore, we hypothesize that the chronic decrease in HAR in the pre-sinusoidal arterioles during the development of cirrhosis is an essential factor leading to vascular remodeling [28]. This could explain the different degrees of vascular remodeling in BDL compared to CCl_4-cirrhotic livers [14,29].

In conclusion, the present study shows a lower HAR and lesser response to methoxamine of the hepatic artery in both CCl_4- and BDL-cirrhotic livers compared to control livers. Furthermore, BDL-cirrhotic livers had a significant lower HAR and lesser response to methoxamine compared to CCl_4-cirrhotic livers. Presence of the NO inhibitor L-NMMA completely corrected the response in CCl_4-cirrhosis but not in BDL-cirrhosis. Both cirrhotic models show vascular remodeling in the intrahepatic hepatic arterioles. Interestingly, BDL-cirrhotic animals have greater degree of remodeling, which is probably one mechanism in a multifactorial process explaining the difference in response to methoxamine either in absence or in presence of L-NMMA.

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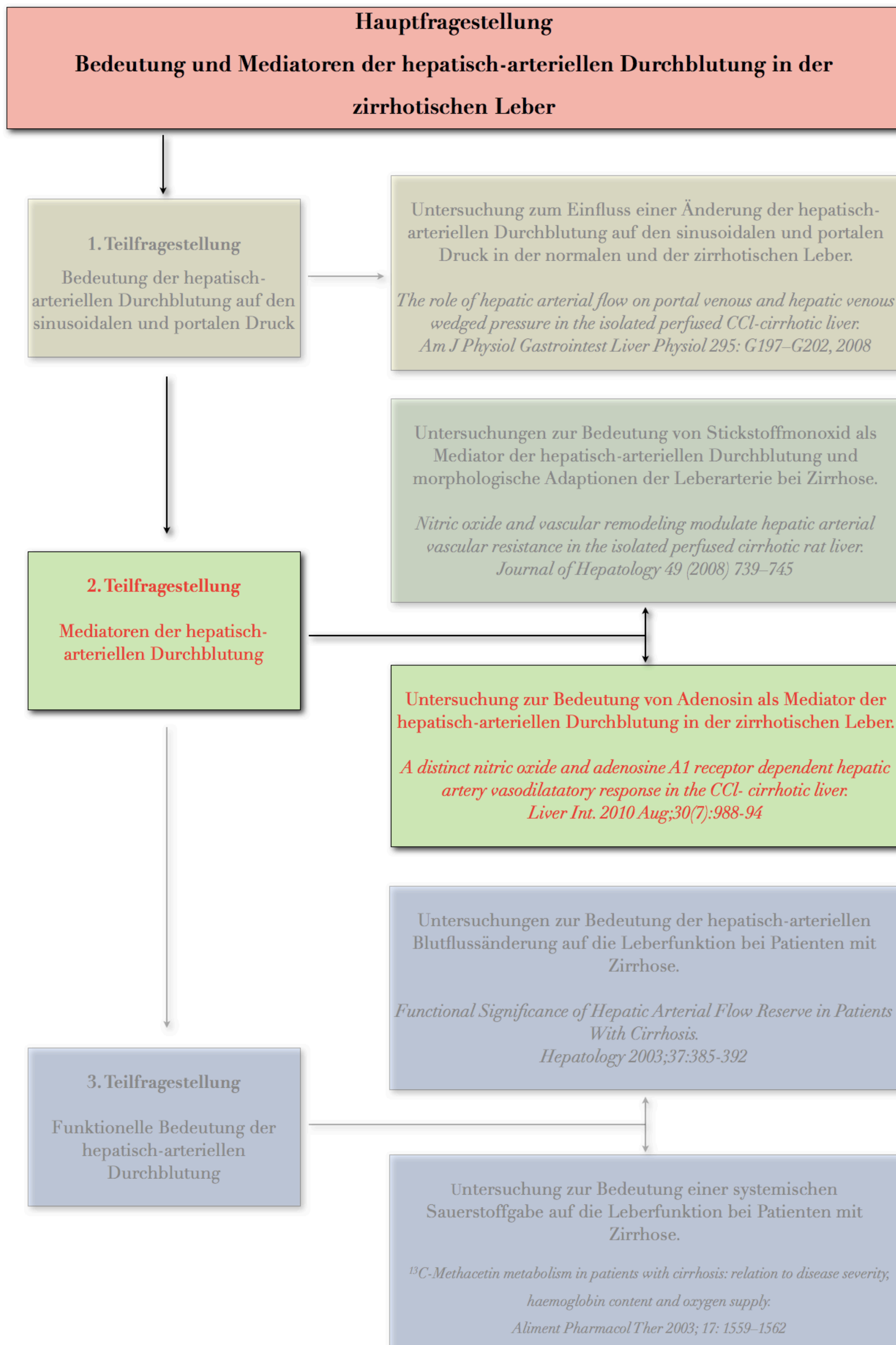
Hauptfragestellung
Bedeutung und Mediatoren der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber
1. Teilfragestellung
Bedeutung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck
Untersuchung zum Einfluss einer Änderung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck in der normalen und der zirrhotischen Leber.
The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl-cirrhotic liver.
Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008
Untersuchungen zur Bedeutung von Stickstoffmonoxid als Mediator der hepatisch-arteriellen Durchblutung und morphologische Adaptionen der Leberarterie bei Zirrhose.
Nitric oxide and vascular remodeling modulate hepatic arterial vascular resistance in the isolated perfused cirrhotic rat liver.
Journal of Hepatology 49 (2008) 739–745
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Mediatoren der hepatisch-arteriellen Durchblutung
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Hepatology 2003;37:385-392
3. Teilfragestellung
Funktionelle Bedeutung der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung einer systemischen Sauerstoffgabe auf die Leberfunktion bei Patienten mit Zirrhose.
13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply.
Aliment Pharmacol Ther 2003; 17: 1559–1562

3. A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatatory response in the CCl- cirrhotic liver. Liver Int. 2010 Aug;30(7): 988-94.

Die Bedeutung und der Wirkmechanismus von Adenosin für die Blutflussregulierung in der Leberarterie der zirrhotischen Leber wird in der nächsten Fragestellung untersucht.

Dosis-Wirkungs-Kurven für den Vasodilatator Adenosin wurden, nach vorheriger Vasokonstriktion mit Methoxamin, in Normal- und Zirrhoseratten (CCl_4-induzierte Zirrhose) ohne und nach Inkubation mit einem unselektiven Adenosinblocker (8-SPT), einem Stickstoffmonoxidsyntheseblocker (L-NMMA) und einem selektiven A1-Blocker (Coffein) durchgeführt. Die Rezeptorenexpression wurde mittels Western Blot bestimmt.

Adenosin führte zur Vasodilatation in normalen und in zirrhotischen Lebern, welche durch Inkubation mit 8-SPT in beiden Modellen signifikant verringert wurde ($p<0,02$). Die Vasodilatation durch Adenosin war interessanterweise im Zirrhosemodell signifikant stärker ausgeprägt verglichen mit der Normalleber ($p=0,016$). Die Inkubation der Leber sowohl mit Coffein als auch mit L-NMMA (Abbildung 7) verringerte die Vasodilatation in der zirrhotischen Leberarterie, aber nicht in der normalen Leberarterie. Dementsprechend zeigten die zirrhotischen Leberarterien eine höhere Dichte von Adenosin A1-Rezeptoren (Stickstoffmonoxid-abhängiger Rezeptor) und eine geringere Dichte von Adenosin A2-Rezeptoren (Stickstoffmonoxid-unabhängiger Rezeptor).

Schlussfolgerung: Die Adenosin-abhängige Vasodilatation ist erhöht in der zirrhotischen Leber. Dieser Mehreffekt wird durch den Stickstoffmonoxid-abhängigen Adenosin-A1 Rezeptor vermittelt.

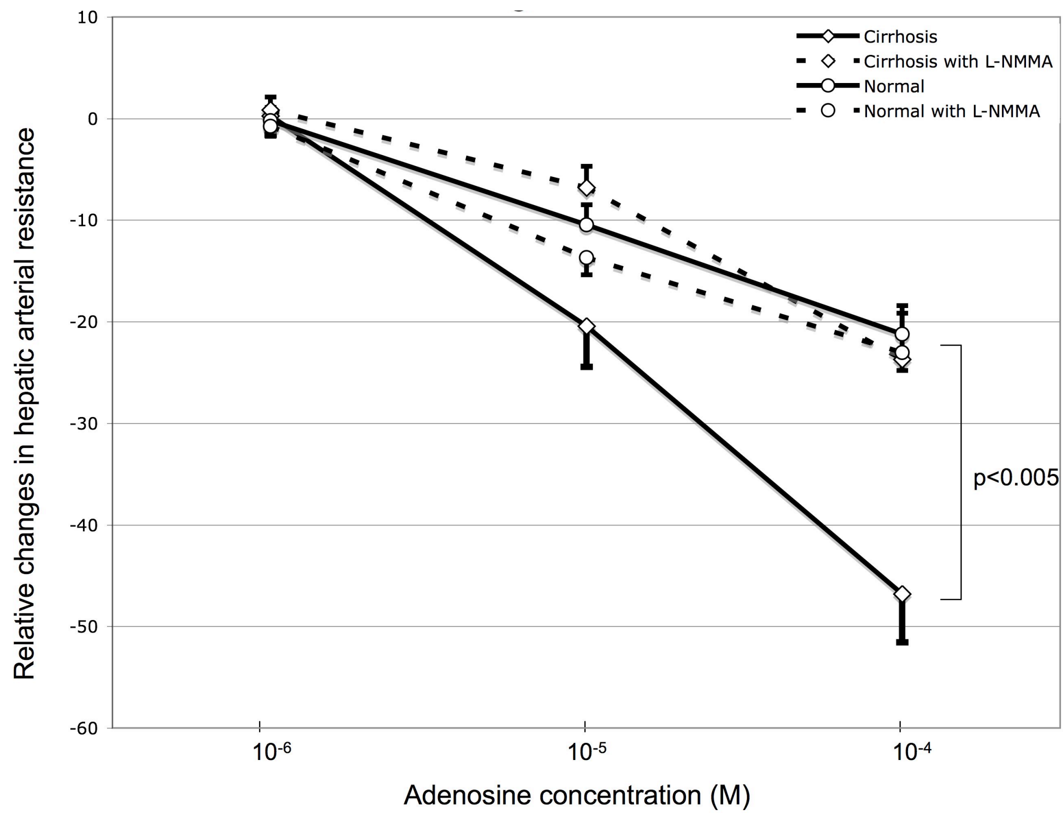

Abbildung 7: Relative Veränderungen des Widerstandes in der Leberarterie hervorgerufen durch Adenosin ohne und mit dem Stickstoffmonoxidsynthesehemmer L-NMMA in normalen und zirrhotischen Tieren [aus Referenz (68)].

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

BASIC STUDIES

A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatatory response in the CCl_4-cirrhotic liver

Alexander Zipprich[1,2], Wajahat Z. Mehal[1], Cristina Ripoll[1,2] and Roberto J. Groszmann[1,2]

1 Digestive Disease Section, Yale University School of Medicine, New Haven, CT, USA
2 Hepatic Hemodynamic Laboratory, Veterans Affairs Medical Center, West Haven, CT, USA

Keywords
adenosine – cirrhosis – hepatic artery – liver perfusion

Correspondence
A. Zipprich, MD, Department of Internal Medicine I, Ernst-Grube-Str. 40, Martin-Luther-University Halle-Wittenberg, 06120 Halle/Saale, Germany
Tel: +493 455 572665
Fax: +493 455 572253
e-mail: alexander.zipprich@medizin.uni-halle.de

Received 16 October 2009
Accepted 24 April 2010

DOI:10.1111/j.1478-3231.2010.02278.x

Abstract
Increase of portal venous vascular resistance is counteracted by decrease of hepatic arterial vascular resistance (hepatic arterial buffer response). This process is mediated by adenosine in normal livers. In cirrhosis, hepatic arterial vascular resistance is decreased but the involvement of adenosine in this process is unknown. The aim of our study was to identify the signalling pathway responsible for the decreased hepatic arterial resistance in cirrhotic livers. *Methods:* Cirrhosis was induced by CCl_4. Using a bivascular liver perfusion dose–response curves to adenosine of the HA were performed in the presence and the absence of pan-adenosine blocker (8-SPT), A1 blocker (caffeine) or nitric oxide synthase-blocker (L-NMMA) after preconstriction with an α1-agonist (methoxamine). Western blot of the HA were used to measure the density of the A1 and A2a receptors. *Results:* Adenosine caused a dose dependent relaxation of the hepatic artery of both cirrhotic and control animals that were blocked in both groups by 8-SPT ($P < 0.02$). The response to adenosine was greater in cirrhotic rats ($P = 0.016$). Both L-NMMA ($P = 0.003$) and caffeine reduced the response to adenosine in cirrhotic but not in control animals. Western blot analysis showed a higher density of A1 and a lower density of A2a receptor in cirrhotic animals ($P < 0.05$). *Conclusion:* The adenosine-induced vasodilatation of the HA is increased in cirrhotic rats suggesting a role for adenosine-NO in the decreased hepatic arterial vascular resistance found in cirrhosis. This significantly greater response in cirrhosis by the A1 receptor follows the same pathway that is seen in hypoxic conditions in extra-hepatic tissues.

The liver is unique in having an arterial and venous blood supply. Liver perfusion is a function of combination of these, and the two blood supplies are interregulated (1, 2). In the healthy liver an experimental reduction in portal blood flow results in a reduction in the arterial vascular resistance with increase in arterial flow (hepatic arterial buffer response) and vice versa. The signalling pathway for this response is local, with the reduction of liver perfusion resulting in an increase in concentration of the vasodilatator adenosine (2–4). In general, vasodilatatory effects of adenosine are mediated by four different adenosine receptors but mainly by A1 and A2 receptors (5). The responsible receptor for adenosine-mediated vasodilatation in the hepatic artery of normal livers is the adenosine A2 receptor (6).

The situation in the cirrhotic liver is partially analogous to the experimental reduction of portal blood flow in that there is a reduction in portal flow, and a corresponding decrease in hepatic vascular resistance and increase in arterial blood flow (7, 8). The increased hepatic arterial blood flow in cirrhosis can be inhibited by a pan-adenosine receptor antagonist suggesting that the mechanism present in the healthy liver is operative in the cirrhotic liver (9, 10). This is further suggested by adenosine-mediated vasodilatation of the hepatic artery in cirrhotic patients (11).

An understanding of the pathways regulating vascular resistance and flow in the cirrhotic liver are of great interest. The assumption that the adenosine A2 receptor mechanism identified as responsible for the hepatic arterial buffer response in the healthy liver is also responsible for the increased hepatic arterial blood flow in the cirrhotic liver has not been directly tested. Furthermore, in other vascular beds different pathways come into play in disease states (5, 12, 13). The aim of our study was to identify the signalling pathway responsible for the increased hepatic arterial flow in the cirrhotic liver.

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Methods

Fifty-nine male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were included in this study. The American Physiological Society guide principles for the care and use of animals were followed. The appropriate Institutional Animal Care and Use committee previously approved all procedures involving animals.

Induction of CCl_4-cirrhosis

Rats underwent inhalation exposure of carbon tetrachloride (CCl_4) three times a week. Phenobarbital (0.35 g/L) was added to the drinking water as described previously (8). Treatment was given for at approximately 12 weeks. Perfusions were performed 6–10 days after the last doses of CCl_4 and phenobarbital. Age-matched rats were used as control group.

In situ rat liver perfusion

Rats were anaesthetized with ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA; 100 mg/kg body wt) and xylazine (Rompun, Bayer, Shawne Mission, KS, USA; 40 mg/animal). A bivascular liver perfusion was performed as described before (7, 14). Briefly, after the abdomen was opened loose ligatures were placed around the aorta cranial to the celiac artery, around the superior mesenteric artery immediately after branching from the aorta, and the aorta caudal to the mesenteric artery. Left gastric and splenic arteries were tied at its origin of the celiac artery and a loose ligature placed around the oesophagus. Left and right renal arteries as well as gastroduodenal artery (branch of the common hepatic artery) were ligated. The bile duct was cannulated with a polyethylene tube (PE 10). The portal vein was cannulated with a 14 G teflon catheter and the perfusion with 32 ml/min of oxygenated (carbon gas, 95% O_2, 5% CO_2) Krebs–Henseleit solution containing dextrose (11 mM) in a non-recirculating mode was started. The inferior vena cava was cut immediately. The aorta was cannulated with an 18 G teflon catheter and the ligatures around the superior mesenteric artery and the oesophagus were closed. The perfusion of the hepatic artery with 8 ml/min of oxygenated (carbon gas, 95% O_2, 5% CO_2) Krebs–Henseleit solution containing dextrose (11 mM) in a non-recirculating mode was started. The tip of the catheter was placed close to the branch of the celiac artery and all ligatures around the aorta were closed. A 14 G catheter was introduced in the inferior vena cava and the thorax was opened.

In order to measure the sinusoidal pressure, a PE-60 catheter was guided from the right atrium, through the thoracic segment of the inferior vena cava into the left hepatic lobe and wedged in the hepatic vein (7). The ligature around the inferior vena cava was closed to secure the wedged catheter. The preparation was transferred to a temperature-controlled (37 °C) Plexiglas perfusion chamber (Yale University Medical Instrument, New Haven, CT, USA) initiating the stabilization period.

During the stabilization and the experimental period the perfusion pressure of the portal vein and the hepatic artery were measured constantly using two independent strain-gauge transducers (P23XL, Spectramed, Oxnard, CA, USA) respectively. The wedged pressure was measured during the experimental period using a third independent strain-gauge transducers (P23XL, Spectramed). Before each experiment, all pressure measurement systems were calibrated with the zero point at the level of the hepatic hilum. Perfusion and sinusoidal pressure were continuously recorded by CHART 3.6 program using MacLab/4e hardware (AD instruments Inc., Colorado Springs, CO, USA). During the stabilization and experimental period the perfusate was oxygenated using a Silastic tubing lung interposed between the perfusate reservoir and the peristaltic pump (15).

Experimental design

All livers were perfused with constant flows during the stabilization period and the flow through the wedged catheter was maintained. The stabilization period was performed in a recirculating mode in absence or presence of the NO-production inhibitor L-NMMA (4×10^{-4} M; Sigma Chemicals Co., St Louis, MO, USA) or pan-adenosine receptor inhibitor 8-sulphophenyltheophylline (8-SPT; 10^{-5} M; Sigma Chemicals Co.). After the stabilization period the wedged catheter outflow was interrupted, allowing the measurement of the wedged pressure and the perfusion was changed to an open mode in presence and absence of L-NMMA or 8-SPT respectively. In an additional set of rats ($n = 8$) the same experimental setting was used but instead of L-NMMA or 8-SPT the adenosine A1 receptor blocker caffeine (10^{-4} M; Sigma Chemicals Co.) was administered during the entire perfusion.

This open mode was kept until the end of the experiment allowing the selective measurement of the drugs effect in the hepatic artery, the portal vein, and the sinusoidal area. The liver perfusion system is known as a vasodilatated system. To investigate the effects of vasodilatators a preconstriction is needed. Therefore, after the stabilization period a preconstriction with the α_1-agonist methoxamine (10^{-4} M; Sigma Chemicals Co.) was performed followed by a dose–response curve using three consecutive doses of adenosine (10^{-6}–10^{-4} M; Sigma Chemicals Co.) infused in the hepatic artery.

Liver global viability was assessed by gross appearance of the liver, stable perfusion curves and bile production during the stabilization period ($> 0.4\,\mu$l/min/g liver). After the experiment liver and spleen were removed and weighed. Liver tissue sample were collected and fixed in formalin.

Western blot of the hepatic artery

Additional animals ($n = 8$) were used for collection of the extrahepatic part of the hepatic artery. Rats were

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anaesthetized with ketamine hydrochloride (Ketaset, Fort Dodge Animal Health; 100 mg/kg body wt) and xylazine (Rompun, Bayer; 40 mg/animal). The abdomen was opened and the extrahepatic part of the hepatic artery as well as the upper part of celiac artery was carefully released from the surrounding tissue. The vessel was dissected and washed in Krebs solution and immediately frozen using liquid nitric oxygen, and stored at $-80\,^{\circ}C$. Samples were homogenized in an appropriate lysis buffer containing 50 mM Tris–HCl, 1 mmol 4-(2-aminoethyl)-benzenesulphonyl fluoride, protease inhibitor cocktail tablet (Roche Diagnostics GmbH, Mannheim, Germany), and 1% (v/v) Nonidet PK40, pH 7.5. Protein content in the supernatants was quantified using the Lowry method with bovine serum albumin as standard. The supernatants were subjected to the SDS-PAGE gel electrophoresis of protein (20 mg), and Western blotting was performed using antibodies that recognized adenosine A1 (Sigma Chemicals Co.), Adenosine A2a (Sigma Chemicals Co.), and eNOS (Transduction Laboratories, Lexington, KY, USA). Enhanced chemiluminescence was used for protein detection. Intensity of the bands corresponding to the protein of interest was measured using densitometry.

Calculations of vascular resistances

Hepatic arterial vascular resistance (HAR) was calculated from the hepatic arterial flow and the hepatic arterial perfusion pressure. Portal venous vascular resistance (PVR) was calculated from the portal venous perfusion pressure and portal venous flow. Sinusoidal vascular resistance (SiVR) was calculated from the wedge pressure and the total flow, i.e. portal venous and hepatic arterial flow.

Statistics

Data are presented as means ± SEM. Mann–Whitney test was used for comparisons of two different groups and one-way analysis of variance for comparison of more than two groups followed by a preplanned contrast test to compare cirrhotic and control groups. Comparison for repeated measurements was assessed using multivariate analysis of repeated measurements followed by Bonferroni's test to detect differences between groups. *P*-values ≤ 0.05 were considered significant.

Results

Liver perfusion

Liver weight was equal (11.7 ± 0.6 vs. 11.6 ± 0.2 g) and spleen weight higher (2.1 ± 0.09 vs. 1.2 ± 0.02 g; $P < 0.001$) in cirrhotic compared with control animals. Basal PVR (0.28 ± 0.008 vs. 0.24 ± 0.005 mmHg/ml/min; $P < 0.001$) and SiVR (0.13 ± 0.009 vs. 0.08 ± 0.005 mmHg/ml/min; $P < 0.001$) were higher and, in contrast, basal HAR (6.46 ± 0.25 vs. 8.62 ± 0.37 mmHg/ml/min) lower in cirrhotic compared with control animals. PVR, SiVR and HAR did not change during incubation neither with 8-SPT nor with L-NMMA.

Effect of adenosine on hepatic arterial resistance

Incubation with methoxamine in absence of 8-SPT and L-NMMA lead to a lower increase of HAR in cirrhotic animals compared with control animals (24.52 ± 1.70 vs. 32.87 ± 0.87 mmHg/ml/min; $P = 0.004$). Because the response to methoxamine was different in both groups the response to adenosine is shown as percentage of the methoxamine-induced increase.

Adenosine caused a dose-dependent decrease in HAR in cirrhotic and control livers and, moreover, this decrease was significantly higher in cirrhosis ($P = 0.005$; Fig. 1). Presence of 8-SPT inhibited significantly the response to adenosine in both cirrhosis ($P < 0.001$) as well as controls ($P = 0.014$). Interestingly, presence of L-NMMA inhibited the response to adenosine in cirrhotic ($P = 0.003$) but not in control livers (Fig. 1). Furthermore, in presence of L-NMMA the response to adenosine was not different between cirrhotic and control livers (Fig. 1).

The adenosine A1 receptor blocker caffeine decreased the response to adenosine in hepatic arteries of cirrhotic livers but not in hepatic arteries of normal livers. Therefore, the previously detected different response to adenosine in cirrhotic and normal animals was not observed in the presence of caffeine as there were no significant different in the dose–response curves between cirrhotic and normal animals (Fig. 2).

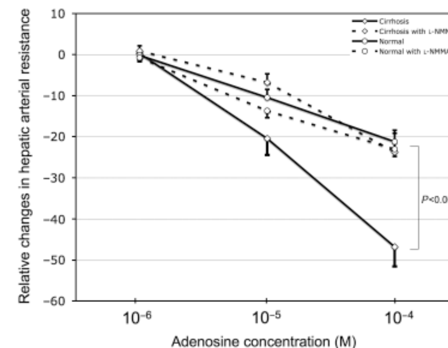

Fig. 1. Dose–response curves to adenosine of the hepatic artery in cirrhotic (diamond; $n = 13$) and normal (circle; $n = 14$) rats in absence (solid line) and presence (dashed lines) of the nitric oxide production inhibitor L-NMMA. The vasodilatatatory effect of adenosine in cirrhotic livers in the absence of L-NMMA was significantly higher compared with normal livers and to the presence of L-NMMA ($P < 0.005$). It is concluded that the greater response in cirrhotic livers is because of the nitric oxide dependent adenosine A1 receptor.

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Effects of adenosine of the portal venous and sinusoidal resistance

The vascular resistance of the portal vein is determined in the sinusoids and the confluence of the hepatic artery is located in zone 1 of the sinusoids (16). Therefore, infusion of vasoactive drugs into the hepatic artery could also change the sinusoidal and portal venous resistance.

Presence of 8-SPT did not influence the methoxamine-induced increase in PVR in cirrhotic nor in control livers compared with the methoxamine-induced increased in absence of 8-SPT. In contrast, presence of L-NMMA caused a greater increase in PVR because of methoxamine in cirrhotic ($P = 0.007$) as well as control livers ($P = 0.003$). Interestingly, adenosine infusion via the hepatic artery caused an increase in PVR in control livers but not in cirrhotic livers ($P = 0.001$; Fig. 3). This effect was blocked with 8-SPT in controls ($P = 0.001$). However, L-NMMA did not inhibit the response to adenosine (via hepatic artery) in controls and neither did the presence of 8-SPT nor L-NMMA modify the effect to adenosine in cirrhosis (Fig. 3). The presence of caffeine did not change the response to adenosine in control nor in cirrhotic livers.

As seen in PVR, presence of 8-SPT did not change the response to methoxamine in SiVR of cirrhotic nor of control livers compared with absence of 8-SPT. In contrast, presence of L-NMMA increased the response to methoxamine in cirrhotic ($P = 0.007$) but not in control livers. Adenosine administration via the hepatic artery did not changed SiVR in cirrhosis and controls neither in presence nor in absence of 8-PST, caffeine or L-NMMA.

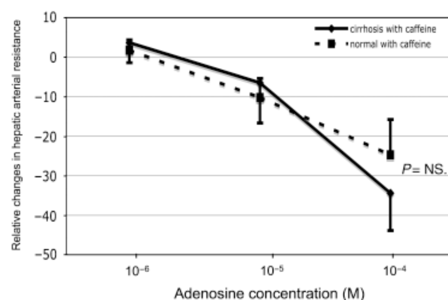

Fig. 2. Dose–response curve to adenosine of the hepatic artery in cirrhotic (solid line; $n = 4$) and normal (dashed; $n = 4$) line in presence of caffeine (adenosine A1 receptor blocker). The effect of caffeine was only present in cirrhosis and the curves were not significant different.

Western blot analyses of the adenosine receptors

Hepatic arteries from cirrhotic rats showed a significant higher eNOS expression compared with the control group ($P < 0.05$; Fig. 4). In both, cirrhotic and control animals, adenosine A2a receptors and adenosine A1 receptors were present. However, cirrhotic animals showed a significant lower relative density of adenosine A2a receptors ($P < 0.01$) and, in contrast, expressed significantly more adenosine A1 receptors compared with control animals ($P < 0.05$, Fig. 4).

Discussion

The hepatic arterial resistance in cirrhosis is decreased because of increased nitric oxide production and vascular remodeling (8). The vasodilatation of the hepatic artery in cirrhosis leads to an absolute and relative increase of the proportion of the hepatic arterial perfusion on the total liver perfusion and knowledge about the mediators

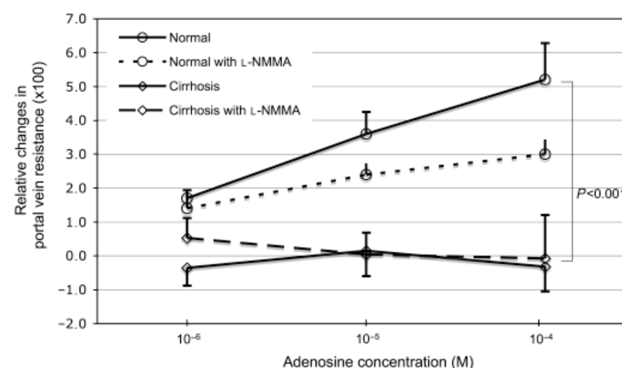

Fig. 3. Dose–response curves to adenosine (administered into the hepatic artery) of the portal vein in cirrhotic (diamond) and normal (circle) rats in absence (line) and presence (dashed lines) of the nitric oxide production inhibitor L-NMMA. The effect of adenosine was only present in normal livers.

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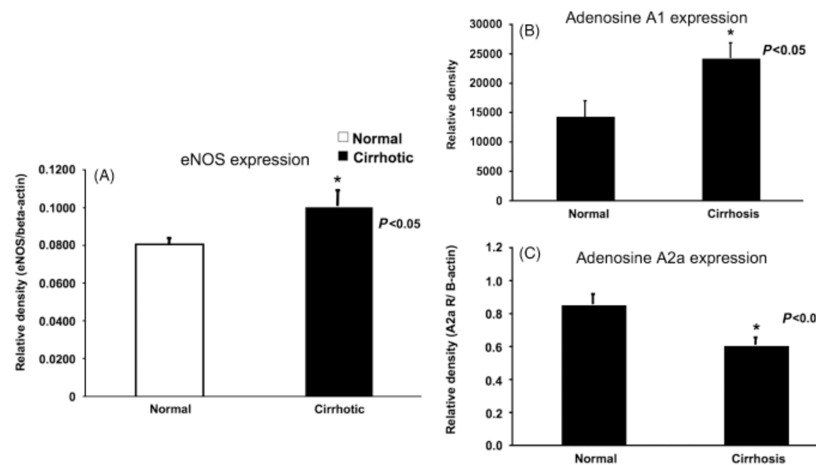

Fig. 4. Results of the Western blots ($n = 8$). (A) eNOS expression of the hepatic artery of normal and cirrhotic rats. eNOS expression was significantly higher in hepatic arteries of cirrhotic animals compared with normal animals ($P < 0.05$). (B) Adenosine A1 expression of the hepatic artery of normal and cirrhotic rats. The adenosine A1 expression was significantly higher in cirrhotic animals compared with normal animals ($P < 0.05$). (C) Adenosine A2a expression of the hepatic artery of normal and cirrhotic rats. The adenosine A2a expression was significantly higher in normal animals compared with cirrhotic animals ($P < 0.01$).

of the hepatic artery are necessary for possible future therapies. The results of the present study suggest that adenosine could be an additional mediator of the hepatic arterial vasodilatation in cirrhosis. Previous studies showed that adenosine is a potent vasodilatator of the hepatic artery in normal livers (2, 17). This adenosine-mediated vasodilatation in normal livers is mediated by the adenosine A2 receptor in a nitric oxide independent fashion (6, 18). Our results in normal livers with vasodilatation because of adenosine, inhibition of this effect by the pan-adenosine blocker 8-SPT, and the lack of any effect of both, the adenosine A1 receptor blocker caffeine and the nitric oxide blocker L-NMMA, support these results. Although we also found in normal hepatic arteries expression of the adenosine A1 receptor, a nitric oxide-dependent receptor, the lack of any effect of blocking nitric oxide synthesis and the lack of any effect of the adenosine A1 blocker caffeine suggests that the main effect in normal livers is mediated through the nitric oxide independent adenosine A2 receptor.

In this study adenosine shows a significantly greater vasodilatatory effect in hepatic arteries of cirrhotic compared with control livers. Inhibition of nitric oxide production corrected the response to adenosine in cirrhosis to a level of normal livers. That implicates that the additional effect of adenosine in cirrhosis is mediated by an additional receptor because the adenosine A2 receptor is nitric oxide independent (6, 18). Indeed, our results with lower effect of adenosine during nitric oxide inhibition, the effect of the adenosine A1 receptor blocker that was present only in cirrhotic hepatic arteries and the greater expression of the nitric oxide dependent adenosine A1 receptor in cirrhotic hepatic arteries suggest that this receptor is the responsible receptor for mediating the effect of adenosine in cirrhosis. Furthermore, we have previously shown in two different models of cirrhosis that nitric oxide production is upregulated (8). The adenosine A1 receptor is located on the endothelium and since we also found an upregulated eNOS expression in the hepatic arteries of cirrhotic animals this further suggests a greater role of that receptor in cirrhosis (12, 19). Taken together, our results implicate that in cirrhotic animals the hepatic arterial vasodilatation caused by adenosine is mainly mediated by the adenosine A1 receptor.

Adenosine is an important mediator involved in the regulation of the intrahepatic circulation that is the main mediator of the hepatic arterial buffer response in normal livers (2). The hepatic arterial buffer response is a phenomenon that counteracts changes in portal venous blood flow. Decrease in portal venous blood flow leads to increase in hepatic arterial flow (3). It has been proposed that the decreased portal venous flow caused accumulation of adenosine and therefore hepatic arterial vasodilatation (3, 20). The functional role of the hepatic arterial buffer response seems mainly to maintain part of the oxygen supply to the liver than to stabilize the blood supply (10, 17). Adenosine is produced in tissues under hypoxic conditions and the increase of high-oxygenated arterial blood would counteract therefore hypoxia.

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In this regard, vasodilatatory response to adenosine under systemic hypoxia in skeletal muscle is mediated by the adenosine A1 receptor although both, the adenosine A1 as well as the adenosine A2 receptor are present (12, 21). Inhibition of nitric oxide production diminished the response to adenosine under hypoxic conditions demonstrating also an involvement of nitric oxide (12, 21). Therefore, our findings of a greater influence of the adenosine A1 receptor in cirrhosis and lowering of the effect with inhibition of nitric oxide production are comparable with findings under systemic hypoxic conditions in other tissues. Hypoxaemia is a well-known condition in cirrhosis and the presence of the hepatic arterial buffer response in cirrhosis has been shown previously (9, 21). In that context our results clarify the responsible receptor of the adenosine-mediated vasodilatation and implicate a role of adenosine in mediating vasodilatation in cirrhosis. Furthermore, we speculate that hypoxia could be the initial trigger leading to a greater influence of the adenosine A1 receptors in hepatic arteries of cirrhotic livers (22–24). This is further supported by previous results showing that adenosine is an excellent vasodilator of the hepatic artery in cirrhotic patients and that this leads to an improvement of the oxygen-dependent liver function (11, 25).

On the other side we found a vasoconstriction because of adenosine on the portal venous side in normal livers. This vasoconstriction was not blocked by L-NMMA nor by caffeine. However, we infused the adenosine to the hepatic artery and not to the portal vein. To draw conclusions from these findings one should perfuse the adenosine through the portal vein to achieve higher concentration.

In conclusion, we demonstrated a greater vasodilatatory effect of adenosine in hepatic arteries of cirrhotic livers. Both, the over-expression of the adenosine A1 receptor in hepatic arteries of cirrhotic animals as well as decreased response to adenosine because of inhibition of nitric oxide production in cirrhotic animals but not in control animals, lead to the conclusion that the response of adenosine in hepatic arteries of cirrhotic livers is mediated mainly by the adenosine A1 receptor.

Acknowledgements

A. Zipprich was supported by the Wilhelm-Roux-Stipendium of the Martin-Luther-University of Halle-Wittenberg, Germany. Cristina Ripoll was supported by the Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III) (CM 03/00037). R. Groszmann was supported by the VA Merit Review. W. Mehal was supported by %R01DK076674-4.

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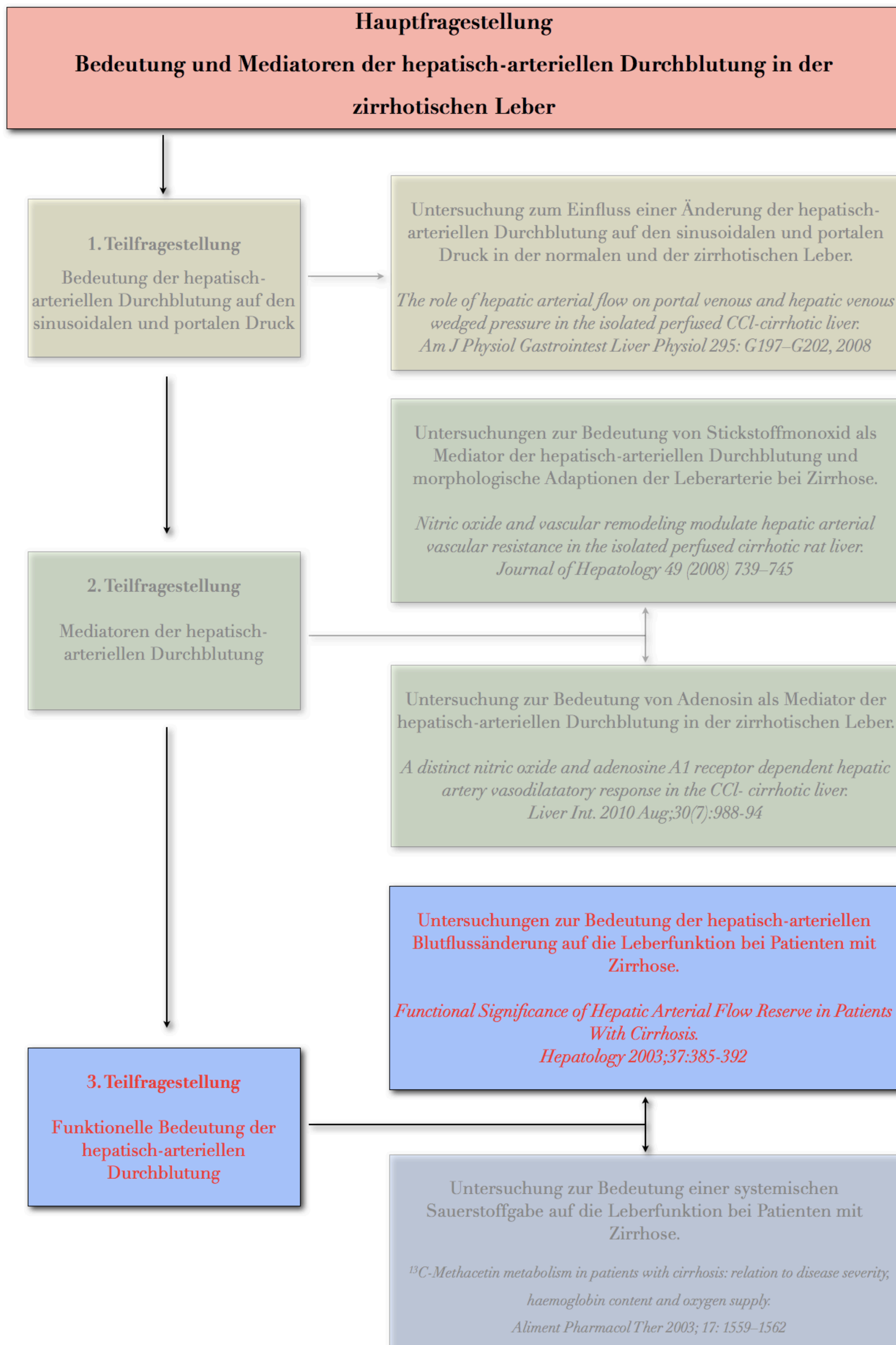
Hauptfragestellung
Bedeutung und Mediatoren der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber
1. Teilfragestellung
Bedeutung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck
Untersuchung zum Einfluss einer Änderung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck in der normalen und der zirrhotischen Leber.
The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl-cirrhotic liver.
Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008
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Untersuchung zur Bedeutung von Adenosin als Mediator der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber.
A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatatory response in the CCl- cirrhotic liver.
Liver Int. 2010 Aug;30(7):988-94
Untersuchungen zur Bedeutung der hepatisch-arteriellen Blutflussänderung auf die Leberfunktion bei Patienten mit Zirrhose.
Functional Significance of Hepatic Arterial Flow Reserve in Patients With Cirrhosis.
Hepatology 2003;37:385-392
3. Teilfragestellung
Funktionelle Bedeutung der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung einer systemischen Sauerstoffgabe auf die Leberfunktion bei Patienten mit Zirrhose.
13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply.
Aliment Pharmacol Ther 2003; 17: 1559–1562

4. Functional Significance of Hepatic Arterial Flow Reserve in Patients With Cirrhosis. Hepatology 2003;37:385-392

Diese Studie untersuchte den Effekt einer Adenosin-induzierten hepatisch-arteriellen Vasodilatation auf verschiedene quantitative Leberfunktionsteste bei Patienten mit Zirrhose.

Bei 21 Patienten mit Zirrhose wurde während gleichzeitiger Messung des hepatisch-arteriellen Blutflusses (mittels intravaskulärem Dopplerultraschall) der Blutfluss in der Leberarterie durch Adenosininfusion gesteigert und während dieser Dilatation verschiedene Leberfunktionsteste durchgeführt. Weiterhin wurden die Leberfunktionsteste bei den gleichen Patienten ohne hepatisch-arterielle Dilatation gemessen.

Die Adenosininfusion in die Leberarterie führte zu einer signifikanten Blutflusszunahme ($p<0,001$). Unter dieser hepatisch-arteriellen Blutflusszunahme kam es zu einer signifikanten Verbesserung der sauerstoff-abhängigen Leberfunktion, gemessen mit Hilfe des MEGX-Testes ($p=0,001$; Abbildung 8). Die absolute Änderung des MEGX-Testes korrelierte negativ mit der Blutflussänderung und war stärker ausgeprägt bei Patienten mit fortgeschrittenen Leberfunktionseinschränkungen (Child-Pugh-Klasse C). Die Steigerung des MEGX-Testes war somit nicht durch eine Blutflusszunahme bedingt. Die anderen Leberfunktionsteste (ICG und GEK) zeigten keine signifikanten Veränderungen unter hepatisch-arterieller Vasodilatation.

Schlussfolgerung: Die hepatisch-arterielle Vasodilatation kann zu einer Funktionssteigerung in der zirrhotischen Leber führen. Dieser Effekt ist wahrscheinlich verursacht durch die gesteigerte Sauerstoffversorgung und betrifft vor allem die fortgeschrittenen Stadien der Erkrankung.

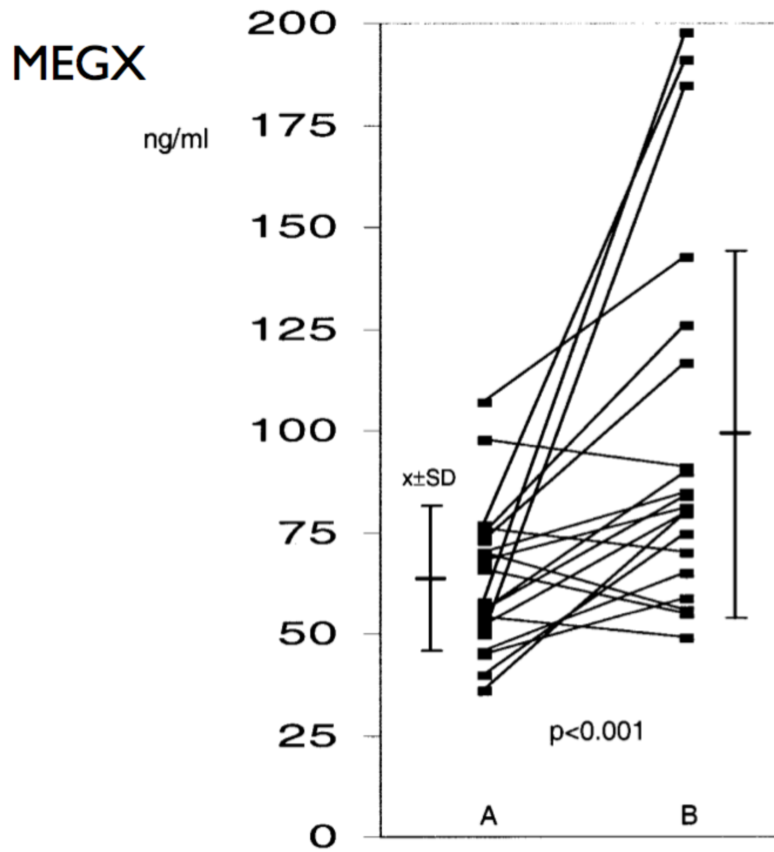

Abbildung 8: MEGX-Test (ng/ml) ohne (A) und mit (B) hepatisch-arterieller Dilatation durch intra-arterielle Infusion des Vasodilatators Adenosin in die Leberarterie [aus Referenz (69)]

Functional Significance of Hepatic Arterial Flow Reserve in Patients With Cirrhosis

Alexander Zipprich,[1] Norbert Steudel,[1] Curd Behrmann,[2] Frank Meiss,[1] Ursula Sziegoleit,[1] Wolfgang E. Fleig,[1] and Gerhard Kleber[1]

In cirrhosis, hepatic arterial vasodilatation occurs in response to reduced portal venous blood flow. However, although the hepatic arterial flow reserve is high in patients with cirrhosis, its impact on hepatic function is unknown. This study investigated the effect of adenosine-induced hepatic arterial vasodilatation on different markers of liver function. In 20 patients with cirrhosis (Child-Pugh class A/B/C: n = 2/7/11) adenosine (2-30 μg · min^{-1} · kg body wt^{-1}) was infused into the hepatic artery and hepatic arterial average peak flow velocities (APV), pulsatility indices (PI), and blood flow volumes (HABF) were measured using digital angiography and intravascular Doppler sonography. Indocyanine green (ICG), lidocaine, and galactose were administered intravenously in doses of 0.5, 1.0, and 500 mg/kg body weight in the presence of adenosine-induced hepatic arterial vasodilatation and, on a separate study day, without adenosine. ICG disappearance, galactose elimination capacity (GEC), and formation of the lidocaine metabolite monoethylglycinxylidide (MEGX) were assessed. Adenosine markedly increased APV and HABF and markedly decreased PI. Serum MEGX concentrations were 63.7 ± 18.2 (median, 62; range, 36-107) and 99.0 ± 46.3 (82.5; 49-198) ng/mL in the absence and presence of adenosine infusion, respectively (P = .001). Adenosine-induced changes in MEGX concentrations were correlated inversely to changes in APV ($r = -0.5$, P = .02) and PI ($r = -0.55$, P = .01) and were more marked in Child-Pugh class C compared with Child-Pugh class A patients (57.4 ± 49.9 [44; −14 to 140] vs. 8.4 ± 16.5 [13; −11 to 35] ng/mL, P < .01). In conclusion, hepatic arterial vasodilatation provides substantial functional benefit in patients with cirrhosis. The effect does not depend directly on hepatic arterial macroperfusion and is observed preferentially in patients with decompensated disease. (HEPATOLOGY 2003;37:385-392.)

In cirrhosis, reduced portal venous blood flow, development of intrahepatic shunts, sinusoidal capillarization, and derecruitment contribute to a reduced oxygen supply to hepatocytes (oxygen limitation theory).[1] Indeed, previous studies in animals and humans suggest improvement of hepatic function during oxygen supplementation.[2-4] As shown in normal animals[5] as well as in those with cirrhosis,[6] the reduction in portal venous blood flow may be compensated by increased hepatic arterial flow (*i.e.*, the hepatic arterial buffer response). Augmentation in hepatic arterial flow may improve hepatic oxygen supply and facilitate clearance of substrates metabolized via cytochrome P450 in the presence of molecular oxygen. Indeed, in the cirrhotic rat liver perfusion model, increasing hepatic arterial blood flow led to improvement in propranolol clearance, an effect related to increased oxygen supply.[7]

Both propranolol and lidocaine are metabolized in the liver by the cytochrome-P450 system. Cytochrome P450 3A4 catalyzes the oxidation of lidocaine to its metabolite monoethylglycinxylidide (MEGX), a process susceptible to hypoxia,[8] and the serum concentration of MEGX after intravenous injection of lidocaine (MEGX test) has been suggested as a marker of microsomal liver function.[9]

Adenosine is a potent vasodilatator of the hepatic artery and hepatic arterial flow reserve (adenosine-induced hepatic arterial vasodilatation) is related to Child-Pugh class in patients with cirrhosis.[10] However, little is known

Abbreviations: MEGX, monoethylglycinexylidide; APV, average peak flow velocities; PI, pulsatility index; HABF, hepatic arterial blood flow volume; GEC, galactose elimination capacity; ICG, indocyanine green.

From the [1]First Department of Medicine and [2]Institute of Diagnostic Radiology, Martin-Luther-University Halle-Wittenberg, Germany.

Received June 20, 2002; accepted November 18, 2002.

Supported by grant 3010A/0098H from the ministry of culture and education, Sachsen-Anhalt, Germany.

Address reprint requests to: Gerhard Kleber, M.D., First Department of Medicine, Martin-Luther-University Halle-Wittenberg, D 06097 Halle, Germany. E-mail: Kleber-Aalen@t-online.de; fax (49) 345-557-2253.

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0270-9139/03/3702-0021$35.00/0

doi:10.1053/jhep.2003.50065

Table 1. Characteristics of Patients

	Patients (n = 20)
Bilirubin (μmol·L^{-1})	52 ± 56 (27; 16-254)
Prothrombin time (%)	65 ± 16 (66; 39-94)
Albumin (g·L^{-1})	26 ± 8 (25; 13-42)
Ascites (n)	8
Encephalopathy (n)	5
Child-Pugh score	9.3 ± 2.3 (10; 5-12)
Child A/B/C (n)	2/7/11
MAP (mm Hg)	90 ± 12 (91; 66-110)
Heart rate (min^{-1})	81 ± 13 (80; 66-110)
Cardiac index (mL·min^{-1}·kg b.w.$^{-1}$)†	3.68 ± 0.84 (3.55; 2.4-5.2)
HVPG (mm Hg)†	18.1 ± 5.1 (19; 8-30)

NOTE. Values are given as mean ± SD (median; range) or as number of patients (n).

*No patient had encephalopathy > grade 1. When patients were analyzed separately according to allocation to study days 1 and 2, no significant differences were identified between groups.

†Obtained before study entry.

about the functional significance of hepatic arterial blood flow and its flow reserve in patients with cirrhosis. Experimental adenosine-induced hepatic arterial dilatation in patients with cirrhosis was described previously[10] and may be regarded as an ideal model to study the effects of augmentation both of arterial blood flow and oxygen availability in patients with cirrhosis.

The aim of the present study was to investigate the effect of adenosine-induced hepatic arterial vasodilatation (hepatic arterial flow reserve) on the cytochrome P450–dependent metabolization of lidocaine.

Patients and Methods

Between February 2000 and November 2001, 21 patients with clinically proven cirrhosis were referred to the First Department of Medicine, University of Halle-Wittenberg, for transjugular intrahepatic portosystemic shunt implantation. Angiography of the hepatic arterial tree was part of the diagnostic work-up in these patients. One patient was excluded because catheterization of the hepatic artery was unsuccessful. The remaining 20 patients (12 men, 8 women, age 47 ± 14 y, cirrhosis of alcohol, viral, primary biliary, and unknown etiology in 14, 3, 2, and 1 patients; cirrhosis proven by biopsy in 19) were included in the present study. The Child-Pugh score was assessed on the first study day. Patient characteristics are given in Table 1. The patients did not receive adrenergic antagonists and there was no evidence of inflammatory conditions such as peritonitis at the time of the study. All investigations were performed in fasted and supine patients. By using a transfemoral approach, a 5F guiding catheter was introduced selectively into the right (n = 13), left (n = 1), or main (n = 6) branch of the hepatic artery. A Doppler wire (Flowire; Cardiometrics, Mountain View, CA, diameter 0.46 mm, pulse repetition frequency 40 kHz, pulse duration 0.83 ms, sampling delay 6.5 ms) was used together with a Doppler instrument (Flomap; Cardiometrics) to measure hepatic arterial peak flow velocities as described previously.[10] The average peak flow velocities (APV) over 2 complete cardiac cycles, the pulsatility indices ([PI] *i.e.*, the differences between systolic and diastolic peak flow velocities divided by APV), and heart rates were computed continuously and systemic blood pressures were assessed every 2 minutes. After continuous recording of a stable Doppler signal over 5 minutes an intra-arterial adenosine infusion was started, increased during 10 minutes from 2 to 30 μg · min^{-1} · kg body wt^{-1}, and maintained for another 30 minutes. Thereafter, the infusion was stopped and the remaining adenosine was aspirated from the infusion catheter. The vessels' diameters were determined in 2 perpendicular planes (d1 and d2) as described[10] both before and at the end of the adenosine infusion at a point 5 mm downstream from the guidewire's tip by using high-resolution digital angiography with lumen edge detection by an observer (C.B.) unaware of the results of the Doppler measurements. The cross-sectional area and the hepatic artery blood flow (HABF) were calculated as described (cross-sectional area = 3.14 · d1 · d2 · 0.25; HABF = 0.6 · APV · cross-sectional area).

During the adenosine infusion 3 separate liver function tests were investigated: the lidocaine metabolism (MEGX test, n = 20), the galactose elimination capacity (GEC, n = 11), and the indocyanine green disappearance rate (ICG, n = 10). At 0, 9.5, and 10 minutes after start of the adenosine infusion galactose (500 mg · kg body wt^{-1}, given as a 40% solution over 5 min), indocyanine green (ICG Pulsion; Pulsion Medical Systems, Munich, Germany, 0.5 mg · kg body wt^{-1} over 0.5 min), and lidocaine (Xylocain; Jenapharm, Jena, Germany, 1 mg · kg body wt^{-1} over 2 min) were administered intravenously, respectively. For measurement of the serum concentration of the lidocaine metabolite MEGX via fluorescence polarization immunoassay (TDxFLx; Abbott, Wiesbaden, Germany),[9] a venous blood sample of 5 mL was taken 25 minutes after injection. For determination of serum galactose, serial venous blood samples were taken up to 65 minutes after galactose administration in 5-minute intervals. Plasma concentrations of galactose were measured and GEC was calculated as mg · min^{-1} as described.[11-13] For ICG determination, venous blood samples were obtained at 3-minute intervals for 21 minutes after injection. Serum concentrations of ICG were measured as described[14,15] and the rate constant of elimination was calculated from the extrapolated half-life as elimination constant $K_{EL} = \ln 2 * t_{1/2}^{-1}$.

Table 2. Hepatic Arterial Perfusion and Liver Function Test Results With and Without Selective Intra-arterial Adenosine Infusion (Wilcoxon's Matched Pairs Signed Rank Test)

	n	Adenosine	No Adenosine	Δ	*P*
APV (cm/s)	20	95 ± 44 91 (31-192)	33 ± 27 27 (11-115)	62 ± 40 50 (4-139)	<.001
PI	20	1.10 ± 0.42 1.04 (0.42-2.1)	1.73 ± 0.54 1.73 (0.67-3.1)	−0.62 ± 0.54 0.6 (−0.5-2.05)	<.001
HABF (mL/min)	20	947 ± 735 771 (119-2,778)	264 ± 296 109 (35-1,109)	683 ± 523 601 (45-1,944)	<.001
MEGX (ng/mL)	20	99.0 ± 46.3 83 (49-198)	63.7 ± 18.2 62 (36-107)	35.4 ± 45.3 28 (−14-140)	.001
K_{EL} ICG (min^{-1})	10	0.05 ± 0.03 0.05 (0.01-0.11)	0.04 ± 0.02 0.04 (0.01-0.07)	0.01 ± 0.02 0.0 (−0.01-0.07)	NS
GEC ($mg \cdot min^{-1}$)	11	264 ± 82 253 (178-487)	257 ± 54 252 (209-395)	7.3 ± 31.6 6 (−32-92)	NS

NOTE. Values are given as mean ± SD and median (range).
n, number of patients; ICG: elimination constant of indocyanine green.

All 3 liver function tests were performed both during adenosine infusion as well as on a separate study day without adenosine. This part of the study was performed in the same way after an overnight fast with the patients supine but without hepatic arterial catheterization and vasodilatation. The interval between both study days was less than 2 weeks. Special care was taken during this period to recognize a change in the patients' clinical condition or drug therapy. No patient received a transjugular intrahepatic portosystemic shunt during the study. Routine therapeutic paracentesis was necessary in 3 patients between the study days. The allocation of patients whether or not they received adenosine on the first (n = 13) or second study day (n = 7) was random and dependent on the availability of the laboratory facilities of our department.

Informed written consent was obtained from all participants. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of the University of Halle-Wittenberg.

Data are presented as means ± SD and medians (ranges). Wilcoxon's matched pairs signed rank test was used for intraindividual comparisons and the Mann-Witney-Wilcoxon test was used for intergroup comparisons. The relation between continuous variables was assessed by using Spearman rank correlation. Univariate ANOVA was used as appropriate. *P* values of .05 or less were considered significant.

Results

No side effects of arterial catheterization or of any of the drugs administered were observed except moderate epigastric pain in 2 patients during the highest dose of adenosine. This pain disappeared immediately after termination of the infusion. Mean arterial pressure and heart rate were constant throughout the whole procedure (90 ± 12 [81; 66-110] mm Hg, 81 ± 13 [80; 66-110] min^{-1} and 91 ± 11 [89; 72-118] mm Hg, 82 ± 12 [79; 67-111] min^{-1}, respectively, before and during adenosine infusion).

APV, HABF, and PI were of similar magnitude as reported previously[10] in a different group of patients (Table 2). Adenosine caused an increase in APV (271 ± 232 [225; 4 to 1,007]%, $P < .001$) and HABF (429 ± 397 [304; 62 to 1,675]%, $P < .001$), and a decrease in PI (−34 ± 24 [−38; −66 to 38]%, $P < .001$; Table 2). No significant differences were found between Child-Pugh classes, although there was a tendency toward blunted effects of adenosine in Child-Pugh class C patients (Table 3).

Serum MEGX concentrations were 63.7 ± 18.2 (62; 36-107) ng/mL^{-1} without adenosine compared with 99.0 ± 46.3 (82.5; 49-198) ng/mL^{-1} during adenosine infusion (Table 2), the difference being 62% ± 80% (46; −20 to 270) ($P < .001$). An increase was observed in 15 of the 20 patients (Fig. 1). In contrast, no significant changes in K_{EL} ICG and GEC were found during adenosine infusion when compared with the absence of adenosine (Table 2).

The adenosine-induced changes in serum MEGX concentrations as well as serum MEGX concentrations during adenosine infusion were correlated inversely with adenosine-induced changes both in PI ($r = -0.55$, $P = .013$ and $r = -0.63$, $P = .003$, respectively) and APV ($r = -0.5$, $P = .024$ and $r = -0.52$, $P = .02$, respectively; Fig. 2). Similar results were obtained when relative adenosine-induced changes in APV and PI were considered.

The adenosine-induced changes in serum MEGX concentrations were higher in Child-Pugh class C patients

Table 3. Hepatic Arterial Perfusion and Serum MEGX Concentrations With and Without Selective Adenosine Infusion Into the Hepatic Artery in Different Child-Pugh Classes

	Child-Pugh A/B (n = 9)			Child-Pugh C (n = 11)		
	No Adenosine	Adenosine	Δ	No Adenosine	Adenosine	Δ
APV (cm/s)	30 ± 26	99 ± 40	69 ± 37	35 ± 29	92 ± 49	57 ± 43
	23	105	82	28	73	45
	(11-96)	(36-154)	(5-109)	(14-115)	(31-192)	(4-139)
PI	1.75 ± 0.72	1.07 ± 0.44	0.68 ± 0.76	1.70 ± 0.36	1.13 ± 0.42	0.58 ± 0.29
	1.71	1.03	0.63	1.74	1.04	0.57
	(0.67-3.09)	(0.42-1.83)	(−0.50-2.05)	(1.32-2.28)	(0.69-2.08)	(0.12-1.17)
HABF (mL/min)	213 ± 342	774 ± 634	560 ± 433	305 ± 261	1,089 ± 809	784 ± 588
	86	443	407	186	803	617
	(35-1,108)	(119-1,887)	(45-1,391)	(80-834)	(278-2,778)	(198-1,944)
MEGX (ng/mL)	63.7 ± 17.5	72.1 ± 14.8*	8.4 ± 16.5†	63.6 ± 19.5	121.0 ± 52.1*	57.4 ± 49.9†
	66	75	13	58	117	44
	(40-98)	(49-91)	(−11-35)	(36-107)	(56-198)	(−14-140)

NOTE. Values are given as mean ± SD and median (range).
Child A/B vs. C: *$P<$ .04; †$P<$ .01 (Mann-Whitney-Wilcoxon test). n, number of patients; Δ, difference.

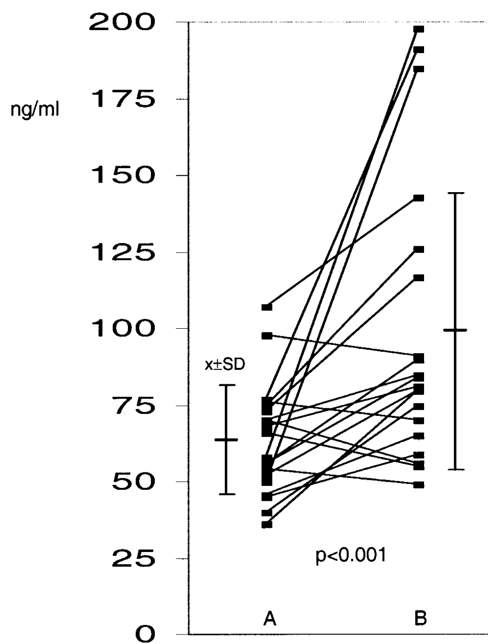

Fig. 1. Serum MEGX concentrations (ng/mL) after intravenous injection of lidocaine (1 mg · kg body wt^{-1}) in 20 patients with cirrhosis in the (A) absence and (B) presence of hepatic arterial vasodilatation induced by selective adenosine infusion into the hepatic artery (Wilcoxon's matched pairs signed rank test).

compared with Child-Pugh class A/B patients (57.4 ± 49.9 [44; −14 to 140] vs. 8.4 ± 16.5 [13; −11 to 35] ng/mL, $P = .006$; Table 3). Also, a weak correlation was found between adenosine-induced changes in serum MEGX concentrations and the Child-Pugh score ($r = 0.46$, $P = .043$; Fig. 2).

ANOVA of the changes observed in serum MEGX concentrations including the covariate Child-Pugh score and adenosine-induced changes in APV or PI revealed that only changes in APV and PI independently affected the changes in serum MEGX concentrations (Table 4).

GEC in the absence of adenosine was not related to basal hepatic arterial APV, PI, or HABF. However, it inversely correlated with the adenosine-induced absolute

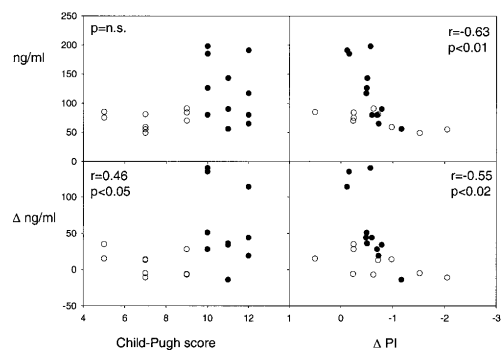

Fig. 2. Serum MEGX concentrations during adenosine infusion (upper panels) and their adenosine-induced changes (lower panels) in relation to Child-Pugh score and to adenosine-induced changes in PIs in 9 Child-Pugh class A/B patients and 11 Child-Pugh class C patients (○ and ●, respectively, Spearman's rank correlation test).

Table 4. Univariate Analysis of Adenosine-Induced Changes in Serum MEGX Concentrations and Serum MEGX Concentrations During Adenosine Using Pugh Score and Adenosine-Induced Changes in PI or APV as Covariates

Variable			Covariates			
	F	P	F	P	F	P
			Pugh score		Δ PI	
ΔMEGX	3.57	.05	2.87	NS	4.09	NS
MEGX Ade	4.7	<.03	4.03	NS	5.14	<.04
					Δ APV	
ΔMEGX	5.18	<.02	2.40	NS	6.90	<.02
MEGX Ade	5.54	<.02	3.34	NS	6.54	.02

Δ, difference; F, variance ratio according to Fisher and Yates' tests; ΔMEGX, MEGX concentrations; NS, not significant; MEGX Ade, MEGX concentrations with adenosine.

and relative decrease in PI ($r = -0.84$, $P = .001$ and $r = -0.88$, $P < .001$) and also correlated with PI during adenosine infusion ($r = 0.80$, $P < .01$). GEC during adenosine infusion inversely correlated with the adenosine-induced absolute and relative decrease in PI ($r = -0.79$, $P = .004$ and $r = -0.85$, $P = .001$) and also correlated with PI during adenosine ($r = 0.64$, $P = .03$). Likewise, GEC during adenosine infusion correlated with the adenosine-induced relative increase in APV ($r = -0.83$, $P = .002$). No relation between the observed GEC values and the Child-Pugh score was found.

When the earlier-described calculations were performed in patients without paracentesis and with intervals between study days no longer than 5 days, similar results were obtained (n = 15; data not shown).

Discussion

In patients with cirrhosis, we previously reported on a high hepatic arterial flow reserve as defined by adenosine-induced hepatic arterial vasodilatation.[10] Hepatic arterial vasodilatation (*i.e.*, the buffer response),[5] is a mechanism compensatory to reduced portal venous inflow both in normal and cirrhotic livers.[5,6] Among a number of theories that try to explain deterioration of liver function in cirrhosis,[1] the oxygen limitation theory describes a rate-limiting oxygen deficit confined to cirrhosis due to reduced portal venous blood flow, sinusoidal capillarization, and intrahepatic shunts.[1] Because with reduction in portal venous perfusion of sinusoids the necessary perfusion pressure may well decrease below the minimal pressure required to maintain sinusoids open, derecruitment (shut down) of hepatic sinusoids also may play a role. A compensatory increase in arterial inflow may increase perfusion pressure and thus enable reopening of closed sinusoids. In patients with cirrhosis, although disputed,[16] the clearance of substrates via conjugation is preserved relatively when compared with the clearance of those oxidated.[1] When portal venous perfusion decreases, oxygen supply to the liver mainly depends on hepatic arterial perfusion. Therefore, the hepatic arterial flow reserve should be of great functional importance in patients with cirrhosis and a reduced portal blood flow.

Indeed, in the presence of cirrhosis, hepatic arterial blood may be an ideal substitute for portal venous blood. Blood reaching the liver via the arterial route differs from portal blood in its higher oxygen content and, possibly, in the route of microvascular liver perfusion. In animals, markers of hepatocyte energy status are improved during oxygen supplementation,[17,18] a finding particularly important in view of the fact that hypoxemia is associated with more severe liver failure in patients with cirrhosis.[19] In normal human livers, hepatic arterial flow supplies a larger sinusoidal and extravascular volume than portal flow[20] and, in human cirrhosis, arterial perfusion of sinusoids is facilitated via opening of arteriolar-portal venular shunts.[21]

On the other hand, increased hepatic arterial perfusion may primarily open arterioportal or arteriovenous shunts and thus simply increase dead space perfusion of the liver without functional benefit. Indeed, the metabolism of lidocaine, meperidine, and phenacetine is less efficient when administered into the hepatic artery.[22,23] Also, unless during episodes of acute hypoxemia,[24] chronic hypoxia does not appear to be an important cause of liver damage in congestive heart failure[25] and liver test results are normal in patients with chronic hypoxia due to lung disease.[26]

In the present study we investigated a group of 20 patients with cirrhosis different from the one previously reported.[10] Adenosine infused into the hepatic artery led to similar changes in PI, APV, and HABF as we described,[10] although the reported difference between Child-Pugh classes was no longer significant. This may be due to the lower number of patients included.

We used marker xenobiotics with different metabolic targets. Lidocaine is metabolized rapidly to MEGX via cytochrome-P450 3A4–mediated oxidation. Therefore, its clearance depends on microsomal activity, which is impaired in liver disease,[27] and on the availability of molecular oxygen. It is cleared rapidly, which displays an advantage in view of the invasiveness of our study design. Nevertheless, in advanced liver disease, its clearance is independent of hepatic blood flow.[28] On the other hand, clearance of ICG depends on blood flow probably without interference with oxygen availability.[14] Finally, galactose elimination reflects cytosolic enzyme activity, which at the dose administered is less sensible to hepatic perfu-

sion and oxygen and mainly relates to functional liver cell mass.[12]

MEGX concentrations after lidocaine administration were markedly higher in the presence of adenosine-induced vasodilatation. These results point to a beneficial effect of hepatic arterial vasodilatation on microsomal liver function in patients with cirrhosis.

We found an inverse relationship between the effects of adenosine on the hepatic arterial perfusion and on the elimination of lidocaine. This suggests that adenosine improves elimination of lidocaine by a mechanism different from an increase in total or arterial hepatic blood flow. Indeed, we could not show an effect of adenosine on the ICG rate constant of elimination, keeping in mind that the latter is only a semiquantitative marker of hepatic blood flow and was studied in a lower number of patients. So far, a direct influence of adenosine on hepatic microsomal function has not been reported in the literature. Therefore, there may be differential effects of adenosine at the microcirculatory level. A large adenosine-induced increase in hepatic arterial perfusion observed in part of the patients may merely reflect preferential opening of intrahepatic arterioportal and arteriovenous shunts without beneficial effect on liver function (dead space perfusion), whereas increased arterioloportal venular perfusion[21] with consecutive hepatic functional improvement may prevail in other patients.

Likewise, due to augmented inflow, recruitment of previously closed sinusoids with a consecutive increase in sinusoidal volume and functional liver cell mass may facilitate the hepatic uptake of both oxygen and lidocaine. This may occur preferentially in the latter patient group. Indeed, the results in the small number of patients in whom GEC was assessed point to a higher functional liver cell mass in patients with low hepatic arterial resistance, a finding that appeared to be independent from whether or not adenosine was infused.

Of interest, MEGX concentrations during adenosine infusion were higher in Child-Pugh class C compared with Child-Pugh class A patients and the changes in concentrations induced by adenosine were weakly but significantly related to the Child-Pugh score. Therefore, the beneficial effect of hepatic arterial vasodilatation may preferentially occur in patients with decompensated liver disease. ANOVA revealed that this finding, however, was not independent from adenosine's effects on hepatic arterial perfusion.

Taken together, our results suggest that hepatic arterial vasodilatation is of benefit in patients with cirrhosis and impaired hepatic function, an effect that may be mediated by enhanced microcirculatory, but not total hepatic, perfusion, with consecutively increased uptake of substrate and oxygen by the diseased liver. Indeed, in the perfused cirrhotic rat liver model hepatic oxygen consumption increases with increasing hepatic arterial blood flow, an effect that though blunted is still present in cirrhosis.[29] We recently reported on an increased hepatic venous oxygen content during intra-arterial infusion of adenosine.[30]

Our results should nevertheless be interpreted with caution. First, instead of measuring intrinsic clearance of lidocaine we only assessed MEGX concentrations at a single time point after lidocaine administration. Although the MEGX test has been used frequently since its original description,[31] only the intrinsic clearance is an independent measure of enzyme activity and not to be expected to be affected by blood flow, changes in the volume of distribution of the test substance, or the pharmakinetics of its measured metabolite.[32] Furthermore, although less important in humans than in animals,[33] other primary and secondary metabolites of lidocaine may interfere with determination of MEGX by fluorescence polarization immunoassay and the serum MEGX concentration at a given time point results from both its formation and cleavage in hepatic microsomes,[34,35] a process not extensively clarified.

Second, lidocaine metabolism may be flow dependent in compensated cirrhosis[28] and oxygen dependent in decompensated disease. Thus, apart from the Child-Pugh class, both flow and oxygen availability may explain the variability in MEGX concentrations in our patients as well as that reported in the literature.[34] Our results confirm the limited discriminatory power of the MEGX test because MEGX concentrations in the absence of adenosine were similar in different Child-Pugh classes.

Smoking increases the clearance of lidocaine although it does not appear to affect MEGX formation at 15 minutes.[36,37] Some of our patients were smokers. However, we suggest that an impact on interday variability of test results in a given patient is unlikely. The cytochrome-P450 3A protein expression may be reduced to a larger extent in noncholestatic compared with cholestatic cirrhosis,[38] but we did not find any relation to the concentrations of bilirubin or alkaline phosphatase in our patients (data not shown).

Direct effects of adenosine on the systemic circulation, volume of distribution, or renal function are unlikely because, with the present study design, no systemic effects and only minor changes in portal pressure have been observed.[10] No invasive procedure with its potential to increase catecholamine concentrations and cardiac output was performed on the second study day and the protocol did not include a true placebo group. However, in view of the marked and selective[10] effect of adenosine on hepatic arterial perfusion a major impact by those systemic

changes seems unlikely. Finally, the hepatic arterial hemodynamic effects elicited by intra-arterial infusion of adenosine in our patients may have been artificially high, which implies that the functional significance of a physiologic buffer response would be more limited.

In summary, we hypothesize that the beneficial effect of hepatic arterial vasodilatation on liver function is explained by increased oxygen delivery to and/or substrate uptake by the diseased liver via the arterial route. The more diseased liver benefited most. The rapid oxidation of lidocaine rendered this substrate an ideal marker of liver function in the present study, but other oxygen-dependent metabolic functions probably will be optimized by increasing hepatic artery blood flow. In addition, because nonoxidative metabolic processes also are impaired in animal models during chronic hypoxia, the oxygen supplementation may even be beneficial for other hepatic functions as well.[39] Thus, drug-induced selective vasodilatation of the hepatic artery could be of substantial functional benefit in cirrhosis. Few treatment options exist to improve liver function in cirrhosis. Therefore, the results of the present study suggest that oral vasodilators with preferential action on the hepatic artery[40-42] or systemic oxygen supplementation[4] may be promising to further evaluate in clinical trials.

Acknowledgment: The assistance of Mrs. I. Rieth (study nurse) is gratefully acknowledged.

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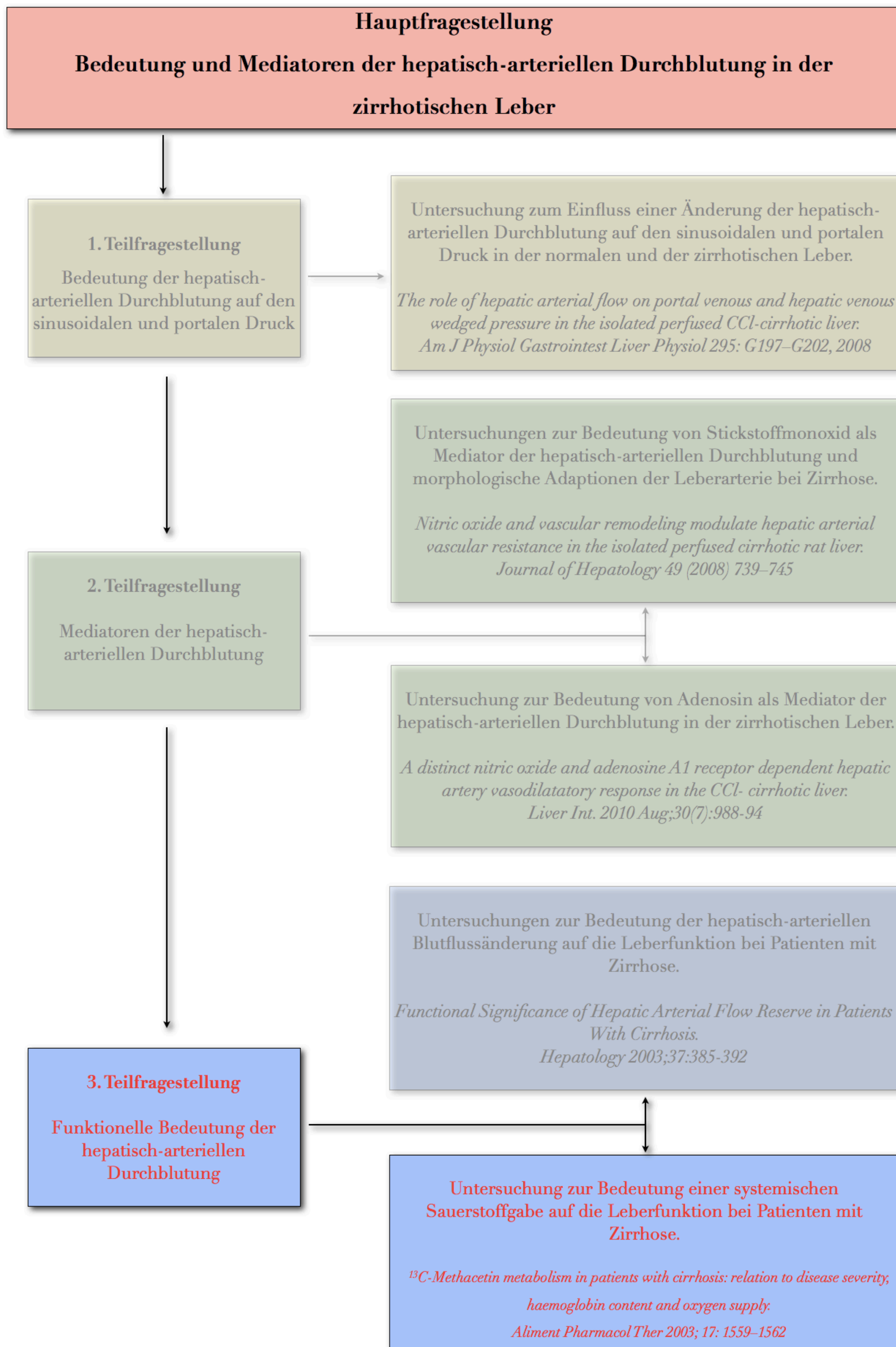
Hauptfragestellung
Bedeutung und Mediatoren der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber
1. Teilfragestellung
Bedeutung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck
Untersuchung zum Einfluss einer Änderung der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portalen Druck in der normalen und der zirrhotischen Leber.
The role of hepatic arterial flow on portal venous and hepatic venous wedged pressure in the isolated perfused CCl-cirrhotic liver.
Am J Physiol Gastrointest Liver Physiol 295: G197–G202, 2008
Untersuchungen zur Bedeutung von Stickstoffmonoxid als Mediator der hepatisch-arteriellen Durchblutung und morphologische Adaptionen der Leberarterie bei Zirrhose.
Nitric oxide and vascular remodeling modulate hepatic arterial vascular resistance in the isolated perfused cirrhotic rat liver.
Journal of Hepatology 49 (2008) 739–745
2. Teilfragestellung
Mediatoren der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung von Adenosin als Mediator der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber.
A distinct nitric oxide and adenosine A1 receptor dependent hepatic artery vasodilatatory response in the CCl- cirrhotic liver.
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Functional Significance of Hepatic Arterial Flow Reserve in Patients With Cirrhosis.
Hepatology 2003;37:385-392
3. Teilfragestellung
Funktionelle Bedeutung der hepatisch-arteriellen Durchblutung
Untersuchung zur Bedeutung einer systemischen Sauerstoffgabe auf die Leberfunktion bei Patienten mit Zirrhose.
13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply.
Aliment Pharmacol Ther 2003; 17: 1559–1562

5. ^{13}C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply. Aliment Pharmacol Ther 2003; 17: 1559–1562

Die Leberarterie versorgt die Leber vor allem mit sauerstoffreichem Blut. Eine systemische Sauerstoffgabe könnte daher die Leberfunktion steigern.

Bei 34 Patienten mit Zirrhose wurde ohne (Tag 1) und mit Sauerstoffgabe (4 l/min; Tag 2) die Leberfunktion mittels des ^{13}C-Methacetin-Atemtestes bestimmt. Die Sauerstoffsättigung und der Sauerstoffpartialdruck sowie die Hämoglobinkonzentration wurden an beiden Untersuchungstagen gemessen.

Das Ergebnis des Methacetin-Atemtests korrelierte negativ mit den Child-Pugh-Punkten ($r=-0,41$, $p<0,02$) und positiv mit den Hämoglobinkonzentrationen ($r=0,46$; $p=0,006$). Die Sauerstoffgabe verbesserte die Methacetin-Atemtestergebnisse signifikant in der Gesamtgruppe der Patienten ($p<0,001$; Abbildung 9), zeigte aber keine Unterschiede in den einzelnen Child-Pugh-Klassen.

Schlussfolgerung: Eine systemische Sauerstoffgabe verbessert die mikrosomale Leberfunktion. Die mikrosomale Leberfunktion wird demnach nicht nur durch die Schwere der Lebererkrankung sondern auch vom Sauerstoffangebot an die Leber beeinflusst.

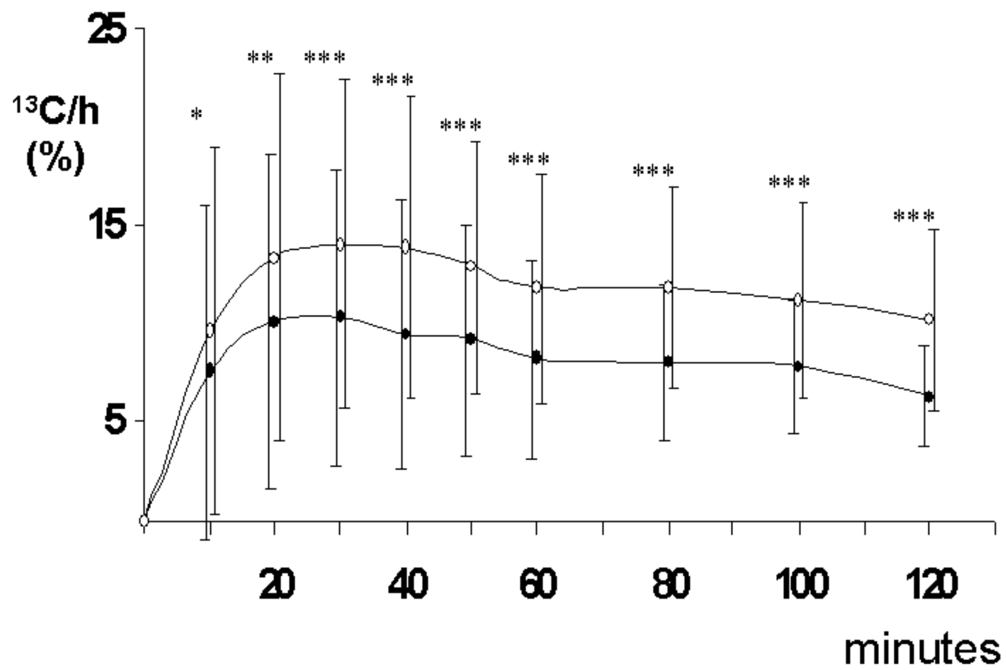

Abbildung 9: Relative Veränderungen der ausgeatmeten Methacetin-Konzentration (^{13}C/h) bei Patienten mit Zirrhose ohne (schwarze Punkte) oder mit (weiße Punkte) Sauerstoffgabe [aus Referenz (70)]

Aliment Pharmacol Ther 2003; 17: 1559–1562.
doi: 10.1046/j.0269-2813.2003.01604.x

^{13}C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply

A. ZIPPRICH, F. MEISS, N. STEUDEL, U. SZIEGOLEIT, W. E. FLEIG & G. KLEBER
First Department of Medicine, Martin-Luther-University Halle-Wittenberg, Germany

Accepted for publication 10 March 2003

SUMMARY

Background: Hypoxia may contribute to impairment of liver function and thus interfere with results of liver tests. In patients with cirrhosis, cytochrome P-450 mediated metabolism of substrates is facilitated in the presence of supplemental oxygen. It has not been studied how this relates to liver function and haemoglobin content.
Aim: We questioned how oxygen supplementation would influence the hepatic microsomal function as assessed by the ^{13}C-methacetin breath test in patients with cirrhosis of different severity and different degrees of anaemia.
Methods: $^{13}C/^{12}C$ ratios in exhaled breath assessed by non-dispersive infrared spectrometry were studied in 34 patients with cirrhosis (Child A/B/C 9/17/8) after administration of 75 mg ^{13}C-methacetin p.o. with and without oxygen inhalation (4 L/min).
Results: In patients breathing room air the total amount of ^{13}C exhaled weakly correlated both with the Child–Pugh score ($r = -0.41$, $P < 0.02$) and haemoglobin concentrations ($r = 0.46$; $p = 0.006$). Oxygen supplementation increased the total amount of ^{13}C exhaled by 68 ± 90% ($P < 0.001$). This effect was similar in Child–Pugh classes A (43 ± 55%), B (83 ± 80%) and C (66 ± 123%) and not related to the Child–Pugh score.
Conclusions: Our results suggest that tests of microsomal liver function are independently influenced both by oxygen delivery and the Child–Pugh score.

INTRODUCTION

According to the oxygen limitation hypothesis, systemic or local hepatic oxygen deficiency contributes to deterioration of liver function in patients with cirrhosis.[1] The cytochrome P-450 system may particularly be affected.[2] To date, it has not been studied whether this phenomenon is related to anaemia and to the degree of hepatic functional impairment or whether differences exist between Child–Pugh classes.

The ^{13}C-methacetin breath test is a non-invasive tool for differentiation of patients with cirrhosis from normal controls or those with non-cirrhotic liver disease.[3] We questioned how oxygen supplementation would influence this test and how any such influence would relate to liver function or to haemoglobin concentrations in patients with cirrhosis.

PATIENTS AND METHODS

In 34 patients with cirrhosis (23 male; age 54 ± 11 years; cirrhosis of alcoholic, viral, biliary and autoimmune aetiology in 24, 6, 2 and 2 patients, Child–Pugh class A/B/C in $n = 9/17/8$) a methacetin breath test was performed in duplicate on two separate days: After an overnight fast patients received 75 mg ^{13}C-methacetin p.o. (Wagner Analysentechnik, Bremen Germany, dissolved in 40 mL of fruit tea). All patients

*Correspondence to: Dr G. Kleber. M.D., First Department of Medicine, Martin-Luther-University Halle-Wittenberg, D 06097 Halle, Germany.
E-mail: Kleber-Aalen@t-online.de*

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remained in a quiet sitting position throughout the test. Breath samples were collected before and 10, 20, 30, 40, 50, 60, 80, 100 and 120 min after substrate administration. The total amount of CO_2 and its $^{13}C/^{12}C$ ratio were analysed in each breath sample using isotope-selective non-dispersive infrared spectrometry (IRIS, Wagner Analysen Technik, Bremen, Germany). Exhaled peak ^{13}C dose per hour ($^{13}C/h$) and cumulative percentage ^{13}C dose recovered over 120 min (cumulative ^{13}C) were calculated according to a standard formula and expressed as percentage of total ^{13}C dose administered.[4]

In 17 patients on day 1 transnasal oxygen inhalation (4 L/min) was started 15 min before methacetin administration and continued throughout the test procedure. On day 2 the methacetin breath test was repeated but no oxygen was administered. In the 17 remaining patients the same procedure was performed vice versa. The assignment of patients to these groups was random.

Oxygen saturation (SiO_2) and oxygen (pO_2) tension were determined from capillary blood samples 60 min after methacetin administration on each study day. Hemoglobin concentrations were obtained simultaneously. Oxygen content was calculated as described.[5]

Wilcoxon's matched-pairs signed-rank test was used for intra-individual comparison, the Mann–Whitney test for interindividual comparison and Spearman's rank test for constant variables. Analysis of variance was used as appropriate. Written informed consent was obtained from all patients, and the study was approved by the local ethics committee of the Medical Faculty, University of Halle-Wittenberg, in accordance with the Declaration of Helsinki. Results are expressed as $x \pm$ s.d.

RESULTS

An increase in breath $^{13}C/^{12}C$ ratios after administration of ^{13}C-methacetin was present both when breathing room air and during oxygen supplementation in all but one patient (Figure 1, Table 1).

Cumulative ^{13}C and peak $^{13}C/h$ correlated inversely with the Child–Pugh score both with and without

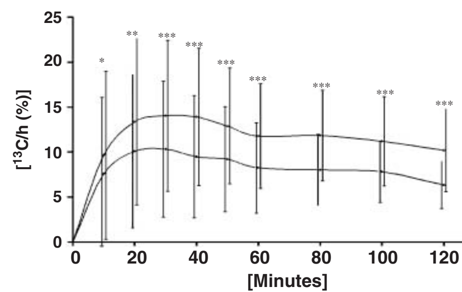

Figure 1. Rate of exhaled ^{13}C per hour ($^{13}C/h$) with (upper curve) and without (lower curve) oxygen inhalation over time. $^{*}P = 0.025$, $^{**}P = 0.002$, $^{***}P < 0.001$ (Wilcoxon's matched-pairs signed-rank test. Analysis of variance (ANOVA) for repated measurements: $F = 3.7$, $P = 0.004$. Bars denote $x \pm$ s.d.

oxygen supplementation (cumulative ^{13}C: $r = -0.32$, n.s. and $r = -0.41$, $P < 0.02$; peak $^{13}C/h$: $r = -0.42$, $P < 0.02$ and $r = -0.48$, $P < 0.01$; Table 2).

Capillary oxygen was weakly correlated to the Child-Pugh score both when breathing room air and during oxygen inhalation ($r = -0.37$, $P = 0.03$ and $r = -0.37$, $P = 0.03$; Table 2). In patients breathing room air, peripheral venous haemoglobin concentrations as well as capillary oxygen content were correlated with cumulative ^{13}C ($r = 0.46$, $P = 0.006$ and $r = 0.46$; $P = 0.006$) and peak $^{13}C/h$ ($r = 0.51$, $P = 0.002$ and $r = 0.52$; $P = 0.002$). However, analysis of variance of cumulative ^{13}C and peak $^{13}C/h$ using either Pugh score and haemoglobin concentrations or oxygen content as covariates revealed that only the Pugh score correlated with the variabilities found.

Seventy-five min after the start of oxygen inhalation capillary SiO_2, pO_2 and oxygen content improved and exhaled ^{13}C significantly increased regardless of whether being expressed as peak $^{13}C/h$ (72 ± 81%, $P < 0.001$) or cumulative ^{13}C (68 ± 90%, $P < 0.001$; Table 1, Figure 2). This effect was not related to liver function or to oxygen-induced changes in SiO_2, pO_2 or

Parameter	No oxygen	Oxygen	Δ	*P*
SiO_2 (%)	95.0 ± 1.9	98.0 ± 1.7	3.0 ± 2.0	< 0.001
pO_2 (kPa)	10.0 ± 1.5	16.3 ± 4.7	6.3 ± 4.3	< 0.001
O_2 (vol percentage)	9.1 ± 1.6	9.4 ± 1.6	0.3 ± 0.2	< 0.001
$^{13}C/h$ (%)	12.1 ± 8.5	17.4 ± 10.1	5.3 ± 5.9	< 0.001
Cumulative ^{13}C (%)	16.1 ± 9.8	23.4 ± 11.3	7.2 ± 7.0	< 0.001

Table 1. Effect of oxygen inhalation (4 L/min) on capillary blood gas parameters and exhaled ^{13}C

Table 2. Effect of oxygen inhalation (4 L/min) on capillary blood gas parameters and exhaled ^{13}C in different Child–Pugh classes

Parameter	Child–Pugh A	Child–Pugh B	Child–Pugh C
n	9	17	8
SiO_2 (%) without oxygen	94.8 ± 1.6	95.3 ± 2.0	94.9 ± 2.0
SiO_2 (%) with oxygen	99.0 ± 1.7^{b}	97.7 ± 1.8^{a}	98.6 ± 0.6^{b}
pO_2 (kPa) without oxygen	9.8 ± 1.1	10.2 ± 1.7	9.8 ± 1.5
pO_2 (kPa) with oxygen	17.2 ± 4.4$^{b,\#}$	15.8 ± 5.3^{a}	16.5 ± 3.1^{b}
Haemoglobin (mmol/L)	7.5 ± 1.1^{++}	7.3 ± 1.2^{+}	6.4 ± 1.1
O_2 (vol percentage) without oxygen	9.6 ± 1.5^{+++}	9.3 ± 1.7^{+++}	8.2 ± 1.4
O_2 (vol percentage) with oxygen	9.9 ± 1.4$^{c,++}$	9.6 ± 1.6$^{b,++}$	8.5 ± 1.5^{b}
^{13}C/h (%) without oxygen	18.3 ± 10.4^{++}	10.8 ± 5.5	7.8 ± 6.8
^{13}C/h (%) with oxygen	22.6 ± 12.3^{+}	17.0 ± 7.3^{a}	12.3 ± 9.2^{b}
cumulative ^{13}C (%) without oxygen	21.9 ± 10.2	15.4 ± 7.4	11.1 ± 9.9
cumulative ^{13}C (%) with oxygen	26.8 ± 8.8^{d}	24.6 ± 10.6^{a}	17.2 ± 12.0^{c}

^{a} $P = 0.001$; ^{b} $P < 0.01$; ^{c} $P < 0.02$; ^{d} $P < 0.05$ vs. without oxygen.
$P = 0.04$ vs. Child–Pugh B; $^{+}$ $P \leq 0.04$; ++ $P < 0.03$; +++ $P < 0.004$ vs. Child–Pugh C.

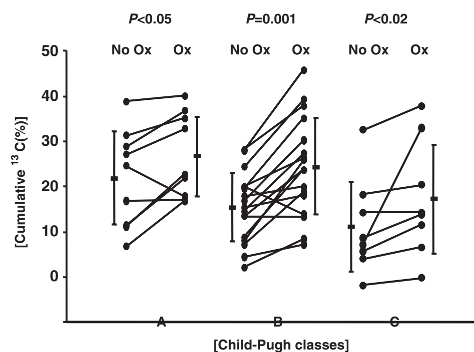

Figure 2. Individual values of exhaled cumulative ^{13}C expressed as percentage of total ^{13}C administered in 34 patients with cirrhosis both when breathing room air (No Ox) as well as during oxygen (Ox) inhalation. Patients are grouped according to Child–Pugh classes A, B and C. Bars denote x ± s.d.

oxygen content (Table 2, Figure 2). On the other hand, when oxygen-induced changes in cumulative ^{13}C and peak ^{13}C/h were expressed as a percentage of basal values they were inversely correlated with peripheral venous haemoglobin concentrations (cumulative ^{13}C: $r = -0.34$, $P < 0.05$; peak 13C/h: $r = -0.37$, $P = 0.03$).

DISCUSSION

In cirrhosis, the clearance of substrates metabolized via cytochrome P-450 enzymes is improved during oxygen supplementation.[2, 6–8] Up to now, the relation of this phenomenon to disease severity as well as to systemic haemoglobin and oxygen content has not been studied. The ^{13}C-methacetin breath test is a well established test of cytochrome P-450-dependent liver function which discriminates between healthy controls and patients with cirrhosis.[3, 9]

In the present study, we found similar ^{13}C breath kinetics as reported previously.[3, 9] The clearance of methacetin in patients breathing room air was related not only to the Child–Pugh score but also to peripheral venous haemoglobin concentrations and oxygen content. We also found an improvement of the clearance of methacetin during oxygen supplementation. Our data suggest that the clearance of methacetin is not only determined by liver function but also by anaemia and oxygen supply to the cirrhotic liver, a finding which may partly explain the overlapping test results observed in the literature in different Child–Pugh classes.[3] The effects of oxygen supplementation on methacetin clearance were present in most of the patients and were similar in the different Child–Pugh classes.

In conclusion, our data suggest an impact both of the Child–Pugh score as well as anaemia and systemic oxygen supply on microsomal liver function as assessed by the methacetin breath test. Oxygen supplementation exerts similar beneficial effects in the different Child–Pugh classes, implying that oxygen susceptibility of the cirrhotic liver is an early event in the course of the disease.

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IV. Diskussion

Über die funktionelle Bedeutung und die Blutflussveränderungen der Leberarterie in der zirrhotischen Leber ist bisher wenig bekannt. Ziel der vorliegenden Arbeit war es, einerseits die Mediatoren der Blutflussveränderungen in der Leberarterie und andererseits die Bedeutung der Leberarterie für den sinusoidalen Druck und die Leberfunktion in zirrhotischen Lebern zu untersuchen.

Dabei wurden die Fragestellungen in einzelnen Teilarbeiten in Tiermodellen und an Patienten untersucht. Zusammenfassend konnte gezeigt werden, dass die Leberarterie in der zirrhotischen Leber dilatiert ist. Dementsprechend war der Einfluss der Leberarterie auf den sinusoidalen Druck in der zirrhotischen Leber stärker ausgeprägt. Stickstoffmonoxid konnte als ein wichtiger Vasodilatator in der Leberarterie der zirrhotischen Leber identifiziert werden. Eine weiterer Mediator der Vasodilatation in der Leberarterie ist Adenosin. Die Wirkung von Adenosin in der zirrhotischen Leber ist vermehrt und führt zu einer erhöhten Dilatation in der zirrhotischen Leber. Die vermehrte Vasodilatation von Adenosin war vermittelt durch den Adenosin-A1-Rezeptor, ein Rezeptor der über eine Stickstoffmonoxiderhöhung zu einer Vasodilatation führt. Eine Adenosin-induzierte Vasodilatation führte bei Patienten mit Zirrhose zu einer Verbesserung der sauerstoffabhängigen mikrosomalen Leberfunktion. Auch eine systemische Sauerstoffgabe führte zu einer Verbesserung der mikrosomalen Leberfunktion bei Zirrhose. Diese Ergebnisse werden nachfolgend im Einzelnen diskutiert.

1. Einfluss der hepatisch-arteriellen Durchblutung auf den portal-venösen und den sinusoidalen Druck

Der Zusammenfluss der Leberarterien mit den Sinusoiden ist wahrscheinlich in der Zone 1 der Sinusoide lokalisiert. Allerdings versorgt die Leberarterie auch die

Gallenwege bzw. den peribiliären Plexus mit Blut. Darüber ist prinzipiell eine Kurzschlussverbindung des arteriellen Blutes um das sinusoidale Gefässsystem möglich. Wir untersuchten daher, inwieweit die Flussänderung der hepatisch-arteriellen Durchblutung zu Änderungen des sinusoidalen Widerstands führt und welchen Einfluss der hepatisch-arterielle Blutfluss auf den sinusoidalen Druck hat. Die Ergebnisse der Experimente zeigen, dass die hepatisch-arterielle Durchblutung zu einer Druckänderung im portal-venösen und sinusoidalen Gefäßsystem führt. Somit ist ein direkter Einfluss der hepatisch-arteriellen Durchblutung in der normalen und der zirrhotischen Leber auf den sinusoidalen Druck nachgewiesen. Dieses Ergebnis mit direktem und gleichsinnigem Einfluss der hepatisch-arteriellen Blutflussänderung auf den portal-venösen Druck wurde auch von anderen Untersuchern zuvor in der normalen Leber nachgewiesen (71, 72).

Ähnliche Ergebnisse wie in der isolierten Leberperfusion mit direkten Einfluss der Leberarterie auf den sinusoidalen Druck konnten auch in einer kleinen Gruppe von Patienten mit pharmakologisch induzierter Blutflusssteigerung in der Leberarterie beobachtet werden (14). Auch bei dieser Untersuchung führte eine Zunahme der hepatisch-arteriellen Durchblutung zu einer Zunahme des sinusoidalen Drucks (gemessen als Lebervenenverschlußdruck).

Normalerweise beträgt der Anteil der hepatisch-arteriellen Durchblutung an der Lebergesamtdurchblutung etwa 30 %. Dieser Anteil wird durch Abnahme der portalvenösen Durchblutung und gleichzeitiger Dilatation der Leberarterie bei Zirrhose größer und beträgt dann etwa 40 bis 50 % (73, 74). Somit kommt der hepatisch-arteriellen Blutzufuhr in Bezug auf die Oxygenierung als auch für die Versorgung mit Nährstoffen eine grössere Bedeutung zu. Die hier vorgestellten Ergebnisse unterstützen diese These und zeigten einen signifikanten größeren

Einfluss der hepatisch-arteriellen Durchblutung auf den sinusoidalen und portal-venösen Druck in der zirrhotischen Leber verglichen mit der gesunden Kontrollleber.

Zum möglichen Vergleich und zur Quantifizierung des Einflusses der portal-venösen und der hepatisch-arteriellen Durchblutung auf den sinusoidalen Druck, wurden in der Studie sowohl die portal-venösen als auch die hepatisch-arteriellen Perfusionsgeschwindigkeiten geändert. Da der portal-venöse Blutfluss zu einer direkten Perfusion der Sinusoide führt und in den Sinusoiden der Gefäßwiderstand der portal-venösen Durchblutung determiniert ist, muss eine Änderung der portal-venösen Perfusionsgeschwindigkeit zu einer Änderung des sinusoidalen Widerstandes führen. Die von uns erhaltenen Ergebnisse zeigen weiterhin, dass diese Widerstandsänderungen zwischen normalen und zirrhotischen Versuchstieren nicht unterschiedlich waren.

Interessanterweise zeigte sich aber bei einem Vergleich der gleichen Flussänderungen in der Portalvene und der Leberarterie eine vergleichbare Änderung des sinusoidalen und des portalen Widerstandes, d.h. eine Erhöhung der Flussgeschwindigkeit in der Portalvene um 3 ml/min verglichen mit einer Änderung von 3 ml/min in der Leberarterie hatten die selben Änderungen des sinusoidalen Drucks zur Folge. Der geringere Einfluss der Leberarterie auf den sinusoidalen bzw. portal-venösen Widerstand in der gesunden Leber ist somit durch den geringeren Anteil an der prozentualen Gesamtdurchblutung (70 % Portalvene vs. 30% Leberarterie) und nicht durch eine geringere Anzahl der perfundierten Sinusoide zurückzuführen. In der zirrhotischen Leber steigt der prozentuale Anteil der Leberarterie an der Lebergesamtdurchblutung und somit auch der Einfluss der Leberarterie auf den sinusoidalen Druck. Ein weiterer Beleg dafür war, dass die Änderungen des hepatisch-arteriellen Widerstandes in den

zirrhotischen Lebern trotz gleicher Änderungen der hepatisch-arteriellen Perfusionsgeschwindigkeit geringer ausfielen verglichen mit gesunden Lebern, der Einfluss auf den sinusoidalen Widerstand aber vergleichbar war. Dies weist auf einen größeren Anteil der hepatisch-arteriellen Durchblutung an der sinusoidalen Durchblutung in der zirrhotischen Ratte hin.

Umgekehrt zeigte die Änderung der portal-venösen Perfusionsgeschwindigkeit keine Änderung der hepatisch-arteriellen Widerstände. Der Nachweis einer hepatisch-arteriellen buffer response (HABR) gelang somit mit diesen Experimenten nicht. Dies ist in Übereinstimmung mit früheren Studien, welche ebenfalls keine Änderung der hepatisch-arteriellen Widerstände bei Veränderung des Portalflusses dokumentieren konnten (71). Tatsächlich scheint aber die Reduktion (minimaler Fluß 57% des Ausgangwertes) des portal-venösen Flusses nicht ausreichend gewesen zu sein, um eine HABR zu induzieren. Eigene unveröffentlichte Daten zeigten, dass bei einer Reduktion des portal-venösen Flusses auf 14% des Ausgangswertes, es zu einer Abnahme des hepatisch-arteriellen Widerstandes kam. Die Ursache für die geringere Reduktion in den vorliegenden Experimenten war die Verhinderung einer hypoxischen Stoffwechsellage innerhalb der Leber, welche wiederum die Ergebnisse der Fluss-Druck-Kurven beeinflusst hätten.

2. Stickstoffmonoxid als Mediator der hepatisch-arteriellen Durchblutung in der zirrhotischen Leber

Über die Mediatoren der hepatisch-arteriellen Durchblutung ist im Gegensatz zur portal-venösen Durchblutung nur wenig bekannt (53). Arbeiten an Normaltieren und Ergebnisse einer Arbeit an einem Zirrhosemodell mit initialer prä-sinusoidaler Veränderung der Zirrhose (gallengangsligiertes Zirrhosemodell) lassen

vermuten, dass Stickstoffmonoxid und Adenosin Mediatoren sein könnten (55, 59). Inwieweit auch andere Zirrhosemodelle diese Veränderungen aufweisen und ob es einen Zusammenhang zwischen beiden Signalwegen gibt, war bisher unklar und wurde hier untersucht.

Die Ergebnisse zeigen, dass in beiden verwendeten Zirrhosemodellen (gallengangsligierte [BDL] und CCl_4-induzierte Zirrhose) Stickstoffmonoxid vermehrt gebildet wird. Beide Zirrhosemodelle sind von der Entstehung der Zirrhose unterschiedlich und repräsentieren somit verschiedene Zirrhoseätiologien. Die Zirrhose im BDL-Modell entwickelt sich primär prä-sinusoidal durch Inflammation der Gallenwege, d.h. in den Abschnitten der Leber die vor den Sinusoiden lokalisiert sind. Für die Untersuchung der Leberarterie ist dies von entscheidender Bedeutung, da der Gefässwiderstand der Leberarterie in den prä-sinusoidalen Abschnitten determiniert ist. Demgegenüber ist der initiale Schaden bei den CCl_4-induzierten Zirrhosen in den sinusoidalen und post-sinusoidalen Abschnitte lokalisiert. Dieses Modell repräsentiert dadurch die Veränderungen der häufiger vorkommenden alkoholischen Leberzirrhose. Arbeiten in den beiden Zirrhosemodellen zeigten einen mehr als zweifach höheren Blutfluss in der Leberarterie der BDL-Zirrhose verglichen mit der CCl_4-Zirrhose (66, 75). Die hier vorgestellten Ergebnisse zeigen eine vermehrte Produktion von Stickstoffmonoxid in den Leberarterien beider Zirrhosemodelle verglichen mit den entsprechenden Kontrollgruppen. Stickstoffmonoxid konnte als ein wichtiger Mediator der hepatisch-arteriellen Vasodilatation in der zirrhotischen Leber, unabhängig von dem verwendeten Tiermodell, identifiziert werden. Allerdings zeigen BDL-Ratten entsprechend dem Vorhandensein eines prä-sinusoidalen Zirrhosemodells eine stärkere hepatisch-arterielle Vasodilatation. Da der initiale Schaden in dem CCl_4-induzierten Zirrhosemodell sinusoidal und post-

sinusoidal lokalisiert ist, zeigten die Experimente hier eine vergleichsweise geringere hepatisch-arterielle Dilatation. Inwieweit in diesem Zusammenhang eine vermehrte Produktion von Stickstoffmonoxid aufgrund der inflammatorischen Aktivität durch die induzierbare Stickstoffmonoxidsynthese (iNOS) im BDL-Zirrhosemeodell hervorgerufen wurde, ist in der Arbeit nicht weiter untersucht worden, lässt sich aber vermuten.

Die vermehrte hepatisch-arterielle Vasodilatation war aber nicht ausschliesslich ein Stickstoffmonoxid-vermittelter Prozess. Inhibition der Stickstoffmonoxidsynthese führte in beiden Zirrhosemodellen zu einer Zunahme der Kontraktilität, korrigierte die Dosis-Wirkungs-Kurven aber nur in der CCl_4-zirrhotischen Ratte zu vergleichbaren Werten der Normalratten. Die BDL-induzierte Zirrhoseratte hatte auch nach Blockade der Stickstoffmonoxidsynthese eine signifikant niedrigere Kontraktion in der Leberarterie verglichen mit Kontrolltieren. Als mögliche Ursachen kommen einerseits weitere Mediatoren oder andererseits strukturelle Veränderungen der Gefäßwand in Betracht. Tatsächlich konnten vor allem in der BDL-zirrhotischen Leber Veränderungen der Gefässwand mit Abnahme der Gefässmuskulatur nachgewiesen werden.

Dagegen konnte durch Hemmung der Stickstoffmonoxidproduktion in der CCl_4-zirrhotischen Leber eine nahezu vollständige Korrektur der Hypokontraktilität erreicht werden. Daraus lässt sich schlussfolgern, dass Stickstoffmonoxid der Hauptfaktor der hepatisch-arteriellen Vasodilatation in diesem Zirrhosemodell zu sein scheint. Trotzdem konnten keine signifikanten Unterschiede in der absoluten Zunahme der Kontraktilität zwischen beiden Zirrhosemodellen gefunden werden, sodass auch im CCl_4-Modell von weiteren Mediatoren oder strukturellen Veränderungen ausgegangen werden muss. Als entscheidendes Syntheseenzym konnte in der Leberarterie von CCl_4-zirrhotischen Lebern die endotheliale

Stickstoffmonoxidsynthetase gefunden werden. Dieses Ergebnis ist somit vergleichbar mit anderen Gefäßgebieten bei Leberzirrhose (2, 76, 77).

Zahlreiche Studien belegen, dass die Stickstoffmonoxidproduktion in der zirrhotischen Leber vermindert ist (19, 39, 42, 43, 49, 78). Es konnte gezeigt werden, dass nicht nur die Produktion von Stickstoffmonoxid vermindert ist, sondern auch die Dilatation der portalvenösen Gefäße durch Stickstoffmonoxid geringer ist (50). Unsere Ergebnisse zur hepatisch-arteriellen Durchblutung scheinen zunächst entgegengesetzt zu dieser intrahepatischen Verminderung der Stickstoffmonoxidkonzentration und intrahepatischen Vasokonstriktion zu sein. Tatsächlich ist aber davon auszugehen, dass innerhalb der intrahepatischen Zirkulation regionale Unterschiede zwischen verschiedenen Gefässabschnitten in Bezug auf die Stickstoffmonoxidkonzentration bzw. -produktion bestehen (62). Es konnte gezeigt werden, dass das Stickstoffmonoxiddefizit in der CCl_4-zirrhotischen Leber in den sinusoidalen und post-sinusoidalen Abschnitten lokalisiert ist, während die prä-sinusoidalen Gefässabschnitte des portal-venösen Gefässgebietes eine höhere Stickstoffmonoxidkonzentration aufweisen (62). Tatsächlich würden die hier vorgestellten Daten diese These unterstützen. Die gefundene erhöhte Stickstoffmonoxidkonzentration in den prä-sinusoidalen Gefässabschnitten der Leberarterie führt zu einer hepatisch-arteriellen Vasodilatation (Abbildung 10).

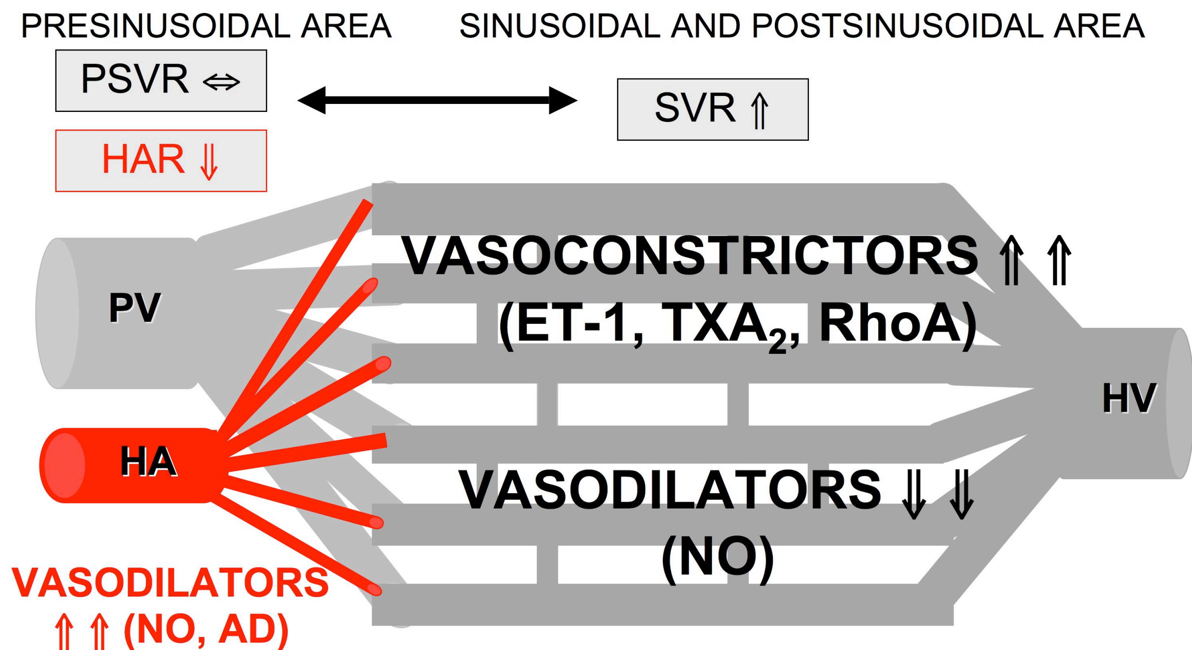

Abbildung 10: Schematische Darstellung der verschiedenen intrahepatischen Gefässabschnitte mit den wichtigsten Mediatoren der Blutflussregulierung. Der Gefässwiderstand der portal-venösen Durchblutung ist in den sinusoidalen und post-sinusoidalen Abschnitten lokalisiert. Der Gefässwiderstand der Leberarterie ist in den prä-sinusoidalen Abschnitten determiniert. Die Pfeile hinter den vaskulären Widerständen (PSVR, HAR, SVR) zeigen die Veränderungen im Vergleich zur gesunden Leber. Bei der Abbildung handelt es sich um eine Erweiterung der Abbildung 4 mit Illustration der hier vorgestellten Ergebnisse.

Zeichenerklärung: AD=Adenosin; ET-1=Endothelin-1; HA=Leberarterie; HAR=Leberarteriengefäßwiderstand; HV=Lebervene; NO=Stickstoffmonoxid; PSVR=prä-sinusoidaler Gefäßwiderstand; PV=Portalvene; RhoA=Isoform von Rho; SVR=sinusoidaler Gefäßwiderstand; TXA2 =Thromboxan A2

3. Adenosin als Mediator der hepatisch-arteriellen Vasodilatation in der zirrhotischen Leber

Adenosin ist als Mediator der hepatisch-arteriellen buffer response (HABR) in zahlreichen Studien sowohl in der Leber gesunder Tiere als auch in der zirrhotischen Leber nachgewiesen worden (54-56, 58). Insgesamt sind vier verschiedene Adenosin-Rezeptoren bekannt, wobei für die Vasodilatation die Adenosin-Rezeptoren A1 und A2 verantwortlich sind (79, 80). In der Leberarterie der normalen Leber konnte als entscheidender Rezeptor der Adenosin-A2-Rezeptor für den vasodilatativen Effekt gefunden werden (58). Unsere Daten bestätigen, dass dieser Rezeptor der entscheidende Rezeptor in der Normalratte ist. Dies konnte sowohl in der Leberperfusion mit verschiedenen Adenosinrezeptorhemmern als auch durch Rezeptornachweis im Western Blot gezeigt werden.

In der zirrhotischen Leber zeigte sich eine stärkere hepatisch-arterielle Vasodilatation nach Adenosingabe. Der Mehreffekt auf Adenosingabe war bedingt durch eine vermehrte Expression des Adenosin-A1-Rezeptors. Tatsächlich konnte durch Inhibition des Adenosine-A1-Rezeptors die Dilatation in der Leberarterie der zirrhotischen Leber auf Werte der Kontrolltiere verringert werden. Da der Adenosin-A1-Rezeptor ein Stickstoffmonoxid-abhängiger Rezeptor ist, wird der vasodilatatorische Effekt über eine Erhöhung der Stickstoffmonoxidkonzentration vermittelt (81). Tatsächlich zeigten unsere Experimente, dass eine Inhibition der Stickstoffmonoxidproduktion die Adenosin-vermittelte Vasodilatation nur in der zirrhotischen Leber verändert. Nach Inhibition des Adenosin-A1-Rezeptors zeigte sich eine Verringerung der absoluten und relativen Werte auf ein Niveau der Kontrolltiere. Dementsprechend war nach Gabe eines Adenosin-A1-Rezeptorhemmers die vermehrte Dilatation in der zirrhotischen Leberarterie

aufgehoben, so dass aus den Perfusionsergebnissen geschlussfolgert werden kann, dass der Adenosin-A1-Rezeptor für die vermehrte Dilatation der zirrhotischen Leberarterie durch Adenosin verantwortlich ist. Die weiterhin durchgeführten Untersuchungen zur Rezeptorenexpression im Western Blot bestätigten diese Schlussfolgerung.

Der Adenosin-A1-Rezeptor ist ein Rezeptor der auf Endothelzellen lokalisiert ist. Das vermehrte Stickstoffmonoxid wird durch die endotheliale Stickstoffmonoxidsynthetase (eNOS) produziert, welche in erhöhter Expression in der zirrhotischen Leberarterie nachgewiesen werden konnte. Interessanterweise konnte in anderen Gefässgebieten eine vermehrte Expression des Adenosin-A1-Rezeptor bei Hypoxie gezeigt werden (82-84). Es kann somit spekuliert werden, dass die vermehrte Expression des Rezeptors durch die bei der Entstehung der Zirrhose vorhandenen Hypoxie verursacht ist (85). Inwieweit diese vermehrte Adenosin-A1-Rezeptorexpression ein Phänomen der Zirrhoseentstehung oder ein Phänomen der fortgeschritten Zirrhose ist bleibt unklar. Interessanterweise können die vasodilatatorischen Effekte von Adenosin auch bei Patienten mit Leberzirrhose nachgewiesen werden (14). In einer früheren Arbeit unserer Arbeitsgruppe zeigten Patienten mit kompensierter Zirrhose eine geringere hepatisch-arterielle Dilatation unter Ruhebedingungen, aber einen größeren vasodilatatorischen Effekt auf eine intra-arterielle Adenosininfusion. Dies wäre möglicherweise vereinbar mit einer bereits in den frühen Stadien der Zirrhose vermehrten Expression des Adenosin-A1-Rezeptors.

4. Strukturelle Veränderungen der Leberarterie in der zirrhotischen Leber

Die Vasodilatation in der zirrhotischen Leber ist ein multifaktorieller Prozess, bei dem neben den oben beschriebenen Mediatoren (Stickstoffmonoxid und

Adenosin) auch strukturelle Veränderungen beteiligt sind. In den hier durchgeführten Untersuchungen konnten zwei verschiedene strukturelle Veränderungen gefunden werden.

Zum Einen konnte durch Untersuchungen der Gefässwand eine geringere Gefässwanddicke gefunden werden. Diese Abnahme der Gefässwanddicke war Ausdruck einer Abnahme der Gefässmuskulatur. Dieser Prozess der Verringerung der Gefässwanddicke ist aus anderen Gefässgebieten bekannt und wird Remodeling genannt (86, 87). Stickstoffmonoxid ist ein essentieller Faktor für Remodeling, d.h. diese Veränderungen treten nur im Zusammenhang mit Stickstoffmonoxid auf (88, 89). Da in der Leberarterie die Stickstoffmonoxidproduktion gesteigert ist, ist somit die Voraussetzung zum Remodeling in diesem Gefässgebiet vorhanden. Tatsächlich konnten wir in beiden zirrhotischen Tiermodellen Remodeling nachweisen. Leberarterien der BDL-zirrhotischen Leber hatten ferner ein geringere Gefässwanddicke verglichen mit CCl_4-zirrhotischen Leberarterien, welches auf ein ausgeprägteres Remodeling bei BDL-Zirrhose hinweist. Eine möglich Ursache für diesen Unterschied könnte die prä-sinusoidale Zirrhoseentstehung in diesem Tiermodell sein, da davon auszugehen ist, dass dies zu frühzeitigen Erhöhungen der Stickstoffmonoxidkonzentrationen in den prä-sinusoidalen Gefässabschnitten führt.

Der zweite strukturelle Prozess, der in den durchgeführten Experimenten beobachtet werden konnte, ist Neoangiogenese. Die Untersuchungen waren nicht darauf ausgerichtet, Angiogenese oder Neoangiogenese in dieser Experimentenanordnung zu untersuchen, die Aufarbeitung der histologischen Schnitte lässt jedoch diese Veränderung eindeutig durch Bestimmung der Arterienanzahl erkennen. Tatsächlich sind in anderen Gefässgebieten bei

Vorhandensein einer Zirrhose, z. B. dem splanchnischen Gefässgebiet, Neoangiogenenese und zahlreiche damit verbundene Mediatoren und Rezeptoren (z.B. PDGF und VEGF) nachgewiesen worden (90-92). Inwieweit diese Mechanismen auch in der Leberarterie vorhanden sind, ist bisher nicht untersucht.

5. Funktionelle Bedeutung einer gesteigerten hepatisch-arteriellen Durchblutung auf die sauerstoffabhängige Leberfunktion

Bei der Entstehung der Zirrhose kommt es zu Veränderungen der Sinusoide (19, 93). Dabei wird fibrotisches Material um die Sinusoide angereichert und die Fenestrae somit verschlossen. Dieser Prozess wird als Kapillarisierung der Sinusoide bezeichnet (94). Dadurch kommt es nicht nur zum Anstieg des intrahepatischen Gefässwiderstands, sondern auch zur gestörten Diffusion von Substraten (impaired drug uptake theory) und Sauerstoff (oxygen limitation theory) in die zirrhotische Leber (95-98). Weiterhin führt die Abnahme des portal-venösen Blutflusses und die Entwicklung von intrahepatischen Shunts zu einer Abnahme der Sauerstoffversorgung. Es entsteht in Folge aller dieser Faktoren eine Hypoxie innerhalb der Leber. Diese Hypoxie ist einer der initialen Faktoren, die zur Entstehung einer Zirrhose führen (9, 10). Tatsächlich konnte in den hier vorgestellten Ergebnissen ein entsprechend bei hypoxischer Stoffwechsellage vermehrt exponierter vasodilatatorischer Rezeptor, der Adenosin-A1-Rezeptor, in der Leberarterie von zirrhotischen Tieren nachgewiesen werden (83, 84, 99). Auf der anderen Seite beschäftigte uns die Frage, inwieweit eine Verbesserung des Sauerstoffangebots an die zirrhotische Leber die Leberfunktion steigern könnte. In den von uns durchgeführten Untersuchungen konnte eine Zunahme der sauerstoffabhängigen Leberfunktion durch Steigerung der hepatisch-arteriellen Durchblutung nachgewiesen werden. Interessanterweise profitierten Patienten mit

einer schlechteren Leberfunktion stärker von der hepatisch-arteriellen Flusszunahme. Es ist also davon auszugehen, dass nicht nur in der Entstehung der Zirrhose, sondern auch bei etablierter Zirrhose eine manifeste Hypoxie vorhanden ist (97, 100). Dass die beobachtete Zunahme der Leberfunktion tatsächlich eher durch eine bessere Versorgung mit Sauerstoff und nicht durch eine gesteigerte Durchblutung verursacht ist, kann aus einer negativen Korrelation der Flusszunahme und der Leberfunktionssteigerung geschlussfolgert werden.

In diesem Zusammenhang interessierte uns zusätzlich die Fragestellung, ob eine systemische Sauerstoffgabe die Leberfunktion ebenfalls steigern kann. Die Ergebnisse des mit und ohne Sauerstoffgabe durchgeführten Leberfunktionstests (Methacetin-Atemtest) zeigen, dass auch eine systemische Sauerstoffgabe die mikrosomale Leberfunktion steigern kann. Dieses Ergebnis korreliert gut mit früheren Untersuchungen im Tiermodell, in denen gezeigt werden konnte, dass eine Sauerstoffgabe die Metabolisierung von Propranolol als Ausdruck der Leberfunktion verbesserte (97, 101). Interessanterweise korrelierten die Ergebnisse des Methacetin-Atemtests nicht nur mit dem Sauerstoffpartialdruck (ein Maß für die Oxygenierung des Körpers) sondern auch mit der Hämoglobinkonzentration. Da Sauerstoff im Blut an Hämoglobin gebunden transportiert wird, sollte zur Beurteilung der sauerstoffabhängigen Leberfunktion nicht nur der Sauerstoffpartialdruck sondern auch der Hämoglobinwert berücksichtigt werden. Die Steigerung der Leberfunktion durch Sauerstoffgabe zeigte keine Unterschiede zwischen den verschiedenen Child-Pugh-Klassen. Dies scheint zunächst gegensätzlich zu dem Ergebnis der vorherigen Studie, in der eine grössere Steigerung der mikrosomalen Leberfunktion bei fortgeschrittener Zirrhose beobachtet wurde. Erklärt werden kann dies durch die hier ebenfalls gezeigte Abhängigkeit des Atemtestes von der Hämoglobinkonzentration. Diese hat einen

grösseren Einfluss bei der Durchführung des Methacetin-Atemtestes und systemischer Sauerstoffgabe und sollte nur einen geringeren Einfluss auf die Leberfunktionssteigerung durch Blutflusszunahme der Leberarterie haben.

V. Zusammenfassung

In den hier zusammengefassten Ergebnissen aus Untersuchungen verschiedener Tierexperimente und Daten von Untersuchungen bei Patienten mit Leberzirrhose wurden einerseits Mediatoren der hepatisch-arteriellen Durchblutung und andererseits die funktionelle Bedeutung des hepatisch-arteriellen Blutflusses untersucht. Der hepatisch-arterielle Blutfluss ist bei Vorliegen einer Zirrhose erhöht, gleichbedeutend mit einer hepatisch-arteriellen Vasodilatation. Diese vermehrte arterielle Durchblutung führt zu einem grösseren Anteil der hepatisch-arteriellen Durchblutung an der Lebergesamtdurchblutung bei Zirrhose. Weiterhin konnte ein direkter Einfluss der hepatisch-arteriellen Durchblutung auf den sinusoidalen als auch portalen Widerstand nachgewiesen werden. Diese hepatisch-arterielle Vasodilatation ist ein multifaktorieller Prozess, wobei mehrere Mediatoren und verschiedene strukturelle Veränderungen beteiligt sind. Sowohl Stickstoffmonoxid als auch Adenosin (über einen stickstoffmonoxid-abhängigen Rezeptor) konnten als Mediatoren in der zirrhotischen Leber nachgewiesen werden. Weiterhin führen strukturelle Veränderungen mit Abnahme der Gefässwanddicke und der Gefässwandmuskulatur zu einer Vasodilatation und verminderten Kontraktionsfähigkeit. Ursache der Vasodilation ist wahrscheinlich die in der zirrhotischen Leber vorhandene Hypoxie. Das dabei gebildete Adenosin könnte über eine hepatisch-arterielle Vasodilatation zu einer Funktionsverbesserung der Leber führen. Tatsächlich konnte durch einer Vasodilatation mittels Adenosin bei Patienten mit Zirrhose eine Funktionsverbesserung der sauerstoffabhänigen Leberfunktion nachgewiesen werden. Die sauerstoffabhängige Leberfunktion kann zusätzlich auch durch eine systemische Gabe von Sauerstoff verbessert werden, allerdings sind hier weitere Faktoren, z. B. die Hämoglobinkonzentration zu berücksichtigen.

Als Konsequenz der Untersuchungen sollten zukünftige medikamentöse Therapien zur Senkung des portalen Drucks neben der direkten Wirkung auf die portal-venöse Durchblutung auch die hepatisch-arterielle Durchblutung beeinflussen und somit die geänderte Lebergesamtdurchblutung bei Zirrhose berücksichtigen. Weiterhin könnte eine gezielte Verbesserung des Sauerstoffangebots an die Leber der abnehmenden Leberfunktion bei Zirrhose entgegenwirken und sich möglicherweise günstig auf den Verlauf auswirken.

VI. Literatur

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VII. Thesen

1. Leberzirrhose geht mit Änderungen der intrahepatischen Zirkulation einher. Es kommt zur Abnahme der portal-venösen Durchblutung und Vasodilatation der Leberarterie mit Zunahme der hepatisch-arteriellen Durchblutung. Dadurch steigt der Anteil der hepatisch-arteriellen Durchblutung an der Lebergesamtdurchblutung an. Der Einfluss der hepatisch-arteriellen Durchblutung auf den sinusoidalen und den portalen Druck nimmt zu.

2. Stickstoffmonoxid konnte als ein wichtiger Mediator der hepatisch-arteriellen Vasodilatation in der zirrhotischen Leber bei zwei unterschiedlichen zirrhotischen Tiermodellen identifiziert werden.

3. Adenosin ist ein Vasodilatator der Leberarterie, sowohl in der gesunden als auch in der zirrhotischen Leber. Der wichtigste Adenosinrezeptor für die hepatisch-arterielle Dilatation in der gesunden Leber ist der Adenosin-A2 Rezeptor. In der zirrhotischen Leber kommt es zur vermehrten Expression des Adenosin-A1 Rezeptors. Die vermehrte Expression des Rezeptors in der zirrhotischen Leberarterie führt zu einer gesteigerten Vasodilatation von Adenosin bei Zirrhose.

4. Der Adenosin-A1 Rezeptor ist ein endothelialer Rezeptor, der die vasodilatatorische Wirkung über eine Erhöhung der Stickstoffmonoxidkonzentration vermittelt.

5. Strukturelle Veränderungen der Gefässwand (Remodeling) in der zirrhotischen Leber tragen ebenfalls zur verminderten Kontraktionsfähigkeit der Leberarterie

in der zirrhotischen Leber bei. Die strukturelle Veränderungen der Gefässwand sind charakterisiert durch eine Abnahme der Gefässwandmuskulatur und der Gefässwanddicke in den intrahepatischen Gefässabschnitten der Leberarterie.

6. Bei Patienten mit Leberzirrhose führt eine Adenosin-induzierte hepatisch-arterielle Vasodilatation zu einer Verbesserung der sauerstoffabhängigen Leberfunktion. Die Funktionsverbesserung war besonders ausgeprägt bei Patienten mit fortgeschrittener Leberzirrhose.

7. Eine systemische Sauerstoffgabe führt zur Steigerung der mikrosomalen Leberfunktion bei Patienten mit Zirrhose. Die Steigerung der mikrosomalen Leberfunktion durch systemische Sauerstoffgabe konnte bei Patienten mit kompensierter und dekompensierter Leberzirrhose nachgewiesen werden.

Tabellarischer Lebenslauf

- Geboren in Halle (Saale) am 23.12.1972
- 1979-1982 Zehnklassige POS in Greifswald
- 1982-1989 Zehnklassige POS Kröllwitz in Halle (Saale)
- 1989-1991Oberschule Thomas-Müntzer in Halle (Saale), Abschluß Abitur
- 1991-1992 Zivildienst an der MLU Halle-Wittenberg, Universitätsklinik und Poliklinik für Urologie
- 1992-1999 Studium der Humanmedizin an der MLU Halle-Wittenberg
- 1/2000-7/2001 Arzt im Praktikum an der Klinik und Poliklinik für Innere Medizin I der MLU Halle-Wittenberg
- 7/2001-2008 Assistenzarzt an der Klinik und Poliklinik für Innere Medizin I der MLU Halle-Wittenberg
- 2002 Promotion an der Klinik und Poliklinik für Innere Medizin I der MLU Halle-Wittenberg, Thema: Hepatisch-arterielle Durchblutung und Flußreserve bei Patienten mit Zirrhose: Messung mittels intravaskulärer Dopplersonographie. Einflußgrößen und prognostische Bewertung.
- 2005-2006 Wilhelm-Roux-Stipendium der Martin-Luther-Universität, Hepatic Hemodynamic Laboratory, School of Medicine, Yale University, Prof. R. J. Groszmann
- 2008 Facharzt für Innere Medizin

- 2010 Facharzt für Innere Medizin und Gastroenterologie
- 10/2010-12/2010 Forschungsaufenthalt Universität Barcelona, Hepatic Hemodynamic Laboratory, Prof. J. Bosch
- seit 2008 angestellt als Facharzt für Innere Medizin an der Universitätsklinik und Poliklinik für Innere Medizin I der Martin-Luther-Universität Halle

Danksagung

Für die Möglichkeit, die Arbeit an der Universitätsklinik und Poliklinik für Innere Medizin I der Martin-Luther-Universität durchführen zu können und für die stetige Unterstützung und Beratung bei der Ausarbeitung der Arbeit danke ich dem Direktor der Klinik, Herrn Prof. Dr. med. Th. Seufferlein, ganz herzlich.

Bedanken möchte ich mich besonders bei Herrn Prof. Dr. Roberto J. Groszmann von der Yale University in New Haven. Wesentliche Teile der Arbeit wurden unter seiner Leitung im Hepatic Hemodynamic Laboratory erstellt. Für die herzliche Betreuung und die zahlreichen hilfreichen Diskussionen und Anregungen bei der Erarbeitung der Daten gilt mein besonderer Dank.

Mein Dank gilt weiterhin Prof. Dr. med. W. E. Fleig für die stetige Unterstützung und Betreuung während seiner Zeit als Direktor der Universitätsklinik und Poliklinik für Innere Medizin I. Insbesondere für die Unterstützung bei der Realisierung des Forschungsaufenthalt an der Yale University gilt mein Dank.

Mein Dank gilt weiterhin Prof. Dr. med. G. Kleber, ehemaliger leitender Oberarzt der Universitätsklinik und Poliklinik für Innere Medizin I, und Herrn Dr. med. M. M. Dollinger, leitender Oberarzt der Universitätsklinik und Poliklinik für Innere Medizin I, für die Unterstützung und Beratung während der Durchführung und Erarbeitung der Ergebnisse der Patientenstudien.

Hiermit erkläre ich, nur diesen

Antrag auf Eröffnung eines

Habilitationsverfahrens eingereicht zu haben.

Frühere Habilitationsverfahren

wurden von mir nicht unternommen.

Hiermit erkläre ich, dass ich die vorliegende Habilitation selbstständig und nur unter Zuhilfenahme der darin angegeben Mittel angefertigt habe.