The clinical significance of asymmetric dimethylarginine

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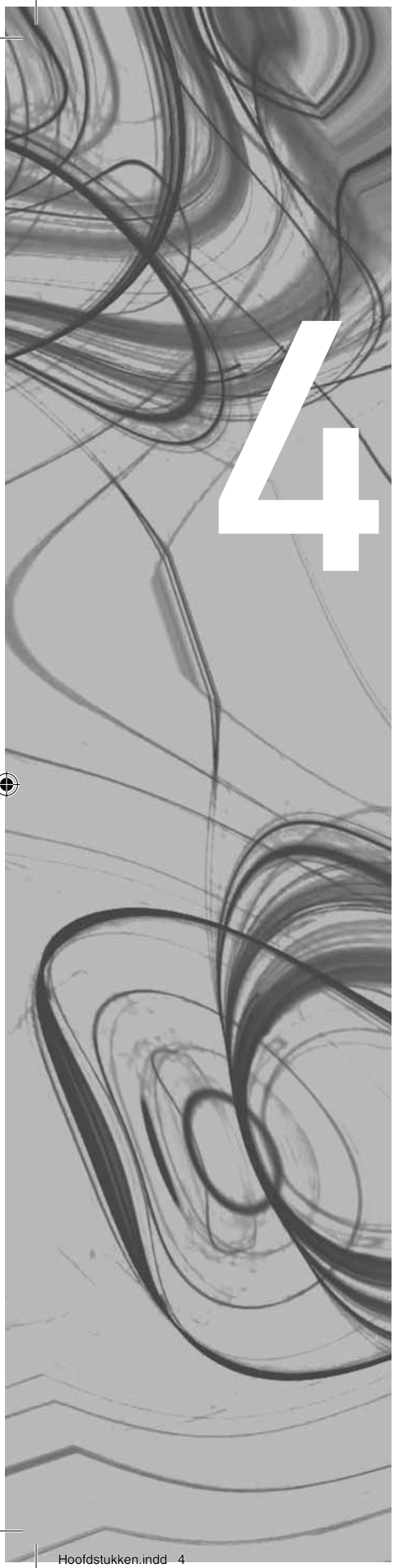

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TIPS placement increases arginine/ADMA ratio in patients suffering from liver cirrhosis

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ABSTRACT

The liver plays a key role in the metabolism of dimethylarginines which may be of relevance in the pathophysiology of portal hypertension in liver cirrhosis. Both asymmetric (ADMA) and symmetric dimethylarginine (SDMA) are excreted by the kidney, but for ADMA the main metabolic route is degradation via the hepatic enzyme dimethylarginine dimethylaminohydrolase (DDAH). Since liver cirrhosis is characterised by endothelial dysfunction and NO deficiency in the intrahepatic circulation, dimethylarginines may be involved in its pathophysiology. We therefore studied 25 cirrhotic patients with portal hypertension receiving transjugular intrahepatic portosystemic shunt (TIPS). To determine (dimethyl)arginine and nitric oxide (NO) plasma levels, blood samples were collected from the superior caval, hepatic, and portal vein during and 3 months after TIPS placement. Results showed a significant increase in the arginine/ADMA ratio after TIPS placement. Moreover, TIPS placement enhanced renal function and thereby decreased systemic SDMA levels. Hepatic function did not change significantly after TIPS placement and no significant decline in ADMA plasma levels was measured. In conclusion, the increase in the arginine/ADMA ratio after TIPS placement suggests an increase in intracellular NO bioavailability. In addition, this study suggests that TIPS placement does not alter DDAH activity and confirms the major role of the liver as ADMA clearing organ.

INTRODUCTION

In 1977, Carnegie and coworkers[1] pointed out the potential role of the liver in the metabolism of ADMA by reporting an increased urinary excretion of ADMA in patients with liver disease. Later, it was shown in an organ balance study in rats that the liver takes up substantial amounts of ADMA, thereby suggesting a crucial role for the liver in regulating systemic ADMA concentrations.[2] These results were confirmed in patients undergoing hepatic surgery in whom it was also shown that the clearing of SDMA was not only confined to the kidney, but the human liver also took up small amounts of SDMA from the portosystemic circulation.[3] Elevated ADMA levels have been reported in patients eligible for liver transplantation,[4,5] in postoperative patients undergoing major liver resection,[6] in patients suffering from decompensated alcoholic cirrhosis,[7] and in critically ill patients with hepatic dysfunction.[8] ADMA plays a regulatory role in the arginine-nitric oxide (NO) pathway by inhibiting the enzyme NO synthase[9] and by competing with arginine and symmetric dimethylarginine (SDMA) for cellular transport across cationic amino acid transporters (CAT) of system y^+.[10] Both ADMA and SDMA are removed from the body by urinary excretion. However, the main eliminatory route for ADMA is degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) which is highly expressed in the liver, but also present in the kidney, and in endothelial cells.[11,12]

Dimethylarginines may play an important pathophysiological role in liver cirrhosis because this disease is characterised by endothelial dysfunction and NO deficiency in the intrahepatic circulation.[13] In cirrhotic patients, elevated ADMA and NO levels have been reported[7,14] and it has been suggested that ADMA might oppose the peripheral vasodilation caused by excessive systemic NO production during liver cirrhosis.[7] To further elucidate the role of dimethylarginines in cirrhotic humans, we studied patients receiving transjugular intrahepatic portosystemic shunt (TIPS).

PATIENTS and METHODS

The study was approved by the institutional review board and medical ethical review committee of the University Hospital Regensburg in Germany. Before study entry,

patients were informed on the purpose of the study and informed consent was obtained from all patients.

Patients

The study population consisted of 25 patients suffering from liver cirrhosis and undergoing TIPS placement mainly because of refractory ascites or recurrent esophageal variceal bleeding. All patients had severe portal hypertension (portal pressure >12 mmHg) which was determined during TIPS placement. The diagnosis of liver cirrhosis was based on clinical, biochemical, and ultrasonographic data. Severity of hepatic failure was scored according to the Child-Pugh Classification.[15]

Blood sampling and analysis

Blood samples were collected during TIPS placement from the superior caval vein, hepatic vein, and portal vein. Thirty minutes and 3 months after placement of the stent, blood was drawn again from the superior caval vein, portal vein and from another hepatic vein to prevent sampling from the extended portal venous tract.

ADMA, SDMA, and arginine plasma concentrations were measured by high-performance liquid chromatography with fluorescence detection using monomethylarginine as internal standard, as previously described.[16] After sample cleanup by solid-phase extraction, the analytes were derivatised with ortho-phthaldialdehyde reagent containing 3-mercaptopropionic acid. Chromatographic separation of the fluorescent derivatives was performed on a monolithic column as recently described.[17] Intra-assay coefficients of variation (CVs) were <1.2% for all analytes. Inter-assay CVs were <3.0% for ADMA and arginine and <4% for SDMA.

NO_x concentrations were measured using the Nitric Oxide Analyzer from Sievers Instruments (Boulder, Colorado, USA), as described previously.[18,19] Briefly, this assay is based upon spectrophotometric analysis after chemiluminescent reaction between NO and ozone (detection limit <1 µmol/L). Creatinine clearance was calculated from plasma and urinary concentrations in 24 hour collected urine. Other biochemical parameters were determined by standard laboratory methods.

Statistical analyses

Differences between timepoints were tested with Wilcoxon signed ranks test. For each comparison, the overall α-level was set at 0.05. Relations between variables

were investigated by Spearman's rho. Data are presented as medians and interquartile ranges (IQR). Statistical analyses were performed using SPSS (SPSS 11.0 for Windows).

RESULTS

Patients

Patient characteristics are presented in **Table 1**. Hepatic synthetic and clearing functions were slightly impaired as reflected by decreased factor V, antithrombin III, albumin, and cholinesterase concentrations and slightly increased bilirubin levels. Three months after TIPS placement, no significant improvement was seen in either laboratory parameters of hepatic function, hepatic enzyme concentrations, or Child-Pugh score.

Table 1: Demographic data and parameters of hepatic and renal function

Number of patients	25		
Gender: male / female	18 / 7		
Age: median (range)	55 (40-81)		
TIPS indication			
ascites	20		
esophagial varices	2		
others	3		
Child-Pugh classification			
A	9		
B	10		
C	6		
Biochemical markers of hepatic function	median	IQR	reference range
bilirubin (μmol/L)	20	13-35	<17
aspartate aminotransferase (U/L)	19	13-34	<50
alanine aminotransferase (U/L)	15	7-27	<50
alkaline phosphatase (U/L)	135	99-185	<124
prothrombin time (%)	72	59-81	>70
factor V (%)	52	40-80	>75
antithrombin III (%)	67	48-79	>75
fibrinogen (mg/dL)	299	232-408	180-350
albumin (g/L)	35	31-41	37-53
cholinesterase (U/L)	2049	1461-2800	5320-12920
alpha-fetoprotein (ng/mL)	3.1	2.5-4.0	<8.1
Biochemical markers of renal function			
urea (mmol/L)	13	8-18	4-18
creatinine (μmol/L)	77	59-113	<97
creatinine clearance (mL/min)	80	38-115	97-160

TIPS: transjugular intrahepatic portosystemic shunt.

Although creatinine and urea levels were within the normal range, creatinine clearance was decreased at baseline. Placement of TIPS enhanced renal function as illustrated in **Table 2**.

Table 2: Biochemical markers of renal function

	just before TIPS		directly after TIPS		3 months after TIPS	
	median	IQR	median	IQR	median	IQR
urea (mmol/L)	13	8-18	12	5-33	11†	5-16
creatinine (μmol/L)	77	59-113	63	54-107	70†	57-89
creatinine clearance (mL/min)	80	38-115	110	75-138	128†	97-161

† P<0.05 vs just before TIPS placement. TIPS: transjugular intrahepatic portosystemic shunt.

Portal and systemic pressures

Portal hypertension was present in all patients. TIPS placement caused an immediate decrease in both portal pressure and in portosystemic pressure gradient (**Table 3**). After 3 months, these pressures were still decreased in comparison to baseline values.

Table 3: Pressure (mmHg) in portal vein and right atrium

	just before TIPS		directly after TIPS		3 months after TIPS	
	median	IQR	median	IQR	median	IQR
portal vein	30	20-35	23†	16-27	17†	10-23
right atrium	9	1-12	13†	5-17	6	2-15
gradient (pv-ra)	21	19-24	10†	9-11	11†	7-15

pv = portal vein; ra = right atrium. † P<0.05 vs just before TIPS placement.
TIPS: transjugular intrahepatic portosystemic shunt.

Concentrations of ADMA, SDMA, and arginine

The changes in ADMA, SDMA, and arginine concentrations in the systemic, portal, and hepatic vein are shown in **Table 4**. At all 3 three timepoints, median systemic ADMA and SDMA plasma levels were higher in cirrhotic patients compared to healthy volunteers[16] (ADMA: 0.42 μM (0.37-0.47); p<0.05, SDMA: 0.46 μM (0.42-0.52); p<0.05, respectively). In contrast, arginine concentrations were lower at baseline compared to healthy individuals (arginine: 88 μM (76-113); p<0.05), but did not differ anymore after TIPS placement.

Although ADMA levels did not show a significant change due to TIPS placement, SDMA concentrations were significantly lower 3 months after TIPS placement in

comparison to baseline values. Placement of TIPS caused a significant increment of both arginine levels and arginine/ADMA ratios.

Table 4: Plasma concentrations (µmol/L) of ADMA, SDMA, and arginine

	just before TIPS		directly after TIPS		3 months after TIPS	
	median	IQR	median	IQR	median	IQR
ADMA						
caval vein	0.64	0.58-0.72	0.69	0.61-0.78	0.59	0.53-0.74
portal vein	0.67	0.60-0.79	0.70	0.62-0.80	0.58	0.52-0.77
hepatic vein	0.62	0.59-0.71	0.66	0.57-0.84	0.57	0.52-0.74
SDMA						
caval vein	0.74	0.60-1.16	0.81	0.64-1.11	0.61†	0.46-0.86
portal vein	0.79	0.62-1.13	0.72	0.64-1.06	0.62†	0.46-0.89
hepatic vein	0.79	0.61-1.09	0.77	0.64-1.09	0.60†	0.44-0.89
arginine						
caval vein	64	56-85	76	68-102	77†	65-104
portal vein	69	62-93	79	64-102	90†	70-106
hepatic vein	54	46-75	67	59-88	72	57-101
arginine/ADMA						
caval vein	95	83-132	117	89-141	128†	110-175
portal vein	83	68-123	98	80-130	123†	92-152
hepatic vein	108	88-138	114	88-137	141†	112-185

† P<0.05 vs just before TIPS placement.
TIPS: transjugular intrahepatic portosystemic shunt.

NO_x plasma concentrations

Although NO_x plasma levels showed a decreasing tendency after TIPS placement, changes did not reach statistical significance (**Table 5**).

SDMA concentrations were positively related to NO_x before and 3 months after TIPS placement (r=0.53; p=0.027 and r=0.60; p=0.025, respectively). ADMA was also related to NO_x plasma levels 3 months after TIPS placement (r=0.67; p=0.009). Neither significant relations were present between arginine/ADMA ratios and NO_x plasma levels nor between NO_x concentrations and portosytemic pressure gradient.

NO_x concentrations were also related to the severity of hepatic dysfunction according to the Child-Pugh score before and 3 months after placement of TIPS (r=0.56; p=0.017 and r=0.54; p=0.047, respectively).

Table 5: Plasma concentrations of NO_x (µmol/L)

NO_x	just before TIPS		directly after TIPS		3 months after TIPS	
	median	IQR	median	IQR	median	IQR
caval vein	112	37-243	107	33-212	44	26-103
portal vein	83	35-241	85	45-223	57	21-96
hepatic vein	85	35-304	86	32-220	39	21-76

Relations between dimethylarginines and hepatic and renal function

Both ADMA and SDMA were positively related to Child-Pugh score before and 3 months after TIPS placement (ADMA: r=0.42; p=0.047 and r=0.59; p=0.028, respectively. SDMA: r=0.56; p=0.029 and r=0.66; p=0.011, respectively).

SDMA was related to creatinine clearance before and 3 months after TIPS placement (r=-0.70; p<0.001 and r=-0.85; p<0.001, respectively). ADMA was also related to creatinine clearance 3 months after TIPS placement (r=-0.70; p=0.007).

Neither ADMA nor SDMA was related to portosystemic pressure gradient.

DISCUSSION

The main finding in the present study was an increase of the arginine/ADMA ratio three months after placement of TIPS in cirrhotic patients. In addition, TIPS placement caused a decrease in SDMA levels which may be partially explained by an advantageous effect on renal function as reflected by an increase in creatinine clearance rate due to TIPS placement. This is also reflected by the strong and significant correlation between SDMA plasma levels and creatinine clearance before and particularly after TIPS placement. Interestingly, the clearing of SDMA is not only confined to the kidney, but the human liver also takes up substantial amounts of SDMA.[3] Indeed, we also found a relation between SDMA and Child-Pugh score, thereby underlining the reported SDMA clearing capacity of the liver. Thus, increased SDMA levels in our studied cirrhotic patients may be caused by a combination of renal and hepatic dysfunction. Also for ADMA, we observed increased plasma concentrations at baseline being also closely related to the severity of liver dysfunction. This is in accordance with the findings of Lluch and coworkers[7] who likewise reported a direct relationship with the Child-Pugh score of patients being evaluated.

The main metabolic route for ADMA is degradation via DDAH[20] and the liver, which has a high DDAH activity, has been shown to be an important regulator of plasma ADMA levels in both animals and humans.[2,3] In portal hypertensive conditions, a recent study of Mookerjee and coworkers[21] showed reduced DDAH expression and increased ADMA levels in liver tissue of patients with severe alcoholic hepatitis. In addition, they suggested that elevated dimethylarginines may serve as a marker of deleterious outcome in patients with alcoholic hepatitis. Also in patients undergoing liver transplantation, ADMA has been shown to be a potential marker of acute allograft rejection.[4] Moreover, in cirrhotic animals, significantly decreased hepatic clearance of ADMA has been demonstrated.[22]

In our study population, systemic ADMA concentrations did not decrease after TIPS placement. This is not surprising considering the well-known TIPS-induced decrease in hepatic extraction capacity. In other words, we assumed ADMA levels to increase after TIPS placement due to shunting and thus less degradation by hepatic DDAH. However, this increasing effect on ADMA serum levels induced by the TIPS implantation may be offset by the observed increase in renal function and the most likely improvement in renal ADMA clearance. In fact, ADMA plasma levels strongly correlated with creatinine clearance 3 months after TIPS placement. Moreover, these data are in accordance with the observation of unaltered liver function represented by the lack of significant changes in biochemical laboratory parameters indicating hepatic function nor in Child-Pugh score after TIPS placement. Because we did neither measure arterial dimethylarginine concentrations nor organ blood flow of the liver and kidney no definite conclusions can be drawn about hepatic and renal elimination of dimethylarginines. Nonetheless, renal dysfunction often develops in patients with severe liver disease and a causal role for ADMA has been proposed in the development of the hepatorenal syndrome.[23] Recently, Lluch and coworkers[14] studied dimethylarginine concentrations in cirrhotic patients with hepatorenal syndrome and confirmed this hypothesis. Moreover, they suggested that SDMA may be a marker of renal dysfunction in cirrhotic patients. Therefore, the lack of increase in ADMA and actual decrease in SDMA levels may well contribute to the well-known beneficial effects of TIPS with respect to neurohormonal status and kidney function in liver cirrhosis.

In liver cirrhosis, Laleman and coworkers[22] substantiated the potential role of ADMA in the pathogenesis of impaired intrahepatic NO production. The known decrease in

intrahepatic NO synthase activity in rats with biliary cirrhosis was found to be associated with an increase in circulating ADMA concentrations. In addition, endothelium-dependent vasorelaxation, measured in a liver perfusion model, was reduced in bile-duct ligated rats and addition of ADMA to the perfusate further blunted this vasodilatory response. However, in our study no association between ADMA levels and the severity of portal hypertension could be detected. A potential explanation may be the mode of action by which TIPS lowers portal pressure being independent from ADMA. Moreover, also SDMA has been reported to interfere with NO synthesis by competing with arginine for transport across cell membranes.[10] Especially high levels of SDMA in combination with low arginine concentrations may decrease NO synthesis significantly and hemodynamical consequences may be the same as reported for ADMA.[20,24]

The gut produces citrulline which is used by the kidneys to synthesise arginine. It can be hypothesised that a decrease in portal pressure will have an advantageous effect on blood flow and function of the intestines, thereby increasing citrulline production by the gut and possibly enhancing renal arginine synthesis. Arginine is degraded in the liver that contains large amounts of arginase which breaks down arginine into urea and ornithine. TIPS placement may lead to a decreased eliminatory capacity of arginine in the liver because blood does not enter the hepatocyte but shunts directly from a portal branch into the hepatic vein. This may explain the increase in arginine plasma levels after TIPS placement. As a precursor of NO, this increase in arginine concentrations may enhance renal blood flow, thereby stimulating glomerular filtration rate and clearing a larger amount of dimethylarginines from the systemic circulation. This compensatory increase in renal excretion of dimethylarginines in fact will prevent a rise in ADMA levels induced by TIPS-induced portal decompression.

Liver cirrhosis is characterised by excessive systemic and particularly splanchnic NO production representing the pathophysiological hallmark in the development of the hyperdynamic circulatory syndrome. This vascular NO overproduction is stimulated by an in increase in portal pressure[25] and is an attempt to open the portal circulation and to enhance collateral blood flow in the systemic circulation bypassing the hepatic circulation. Besides a rise in arginine levels, the arginine/ADMA ratio increased significantly after TIPS placement. Theoretically, an increase in the arginine/ADMA ratio leads to an elevation in NO bioavailability. In our study, nonetheless, NO_x plasma levels showed a decreasing tendency after TIPS placement. While no

relationship was found between NO_x and the arginine/ADMA ratio, both ADMA and SDMA were positively related to NO_x levels. This finding substantiates the hypothesis that dimethylarginines might oppose the peripheral vasodilation caused by excessive systemic NO production during liver cirrhosis.[7] It can be hypothesised that after TIPS placement, portal pressure drops and thus the main stimulus for splanchnic NO overproduction is greatly attenuated. In addition, renal and probably also gut function improve, thereby causing arginine synthesis (via gut-derived citrulline) by the kidney to increase. Furthermore, ADMA slightly decreases due to enhanced function of the kidney causing the arginine/ADMA ratio to increase. This is advantageous for the y^+ pump that is now able to transport more arginine into the cell, where it is converted to NO. The increased arginine/ADMA ratio after TIPS may result in a better NO-availability on tissue level, while systemic (plasma) NO is decreased due to less splanchnic NO release.

In conclusion, the main finding of the present study was a significant increase in the arginine/ADMA ratio in cirrhotic patients 3 months after TIPS placement. In addition, TIPS enhanced renal function and concomitantly significantly lowered systemic SDMA levels but did not change hepatic function. In line with this unaltered liver function, no significant decline in ADMA plasma levels could be detected, thereby confirming the major role of the liver as ADMA clearing organ.

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