The clinical significance of asymmetric dimethylarginine

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SUMMARY

It has been recognised that endogenous arginine analogues play a regulatory role in the arginine-nitric oxide (NO) pathway. NO is synthesised from the amino acid arginine by the action of NO synthases, a family of enzymes with endothelial, neuronal, and inducible isoforms. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all isoforms of NO synthase,[1] while symmetric dimethylarginine (SDMA) is not biologically active on NO synthase, but may interfere with NO synthesis by competing with arginine for transport across cell membranes.[2-5] In the cardiovascular system endothelium-derived NO causes vasodilation, prevents cellular adhesion to the vascular wall, inhibits platelet aggregation, and limits the proliferation of vascular smooth muscle cells. Furthermore, NO reduces superoxide radical generation and inhibits oxidation of low density lipoproteins. Thus, endothelial derived NO can be regarded as an anti-atherosclerotic molecule. In turn, inhibition of NO production by methylated arginines may result in impaired function of the vascular system, thereby playing a causative role in cardiovascular diseases.[6] The mechanism by which accumulation of ADMA is detrimental seems to be by non-selective inhibition of NO synthase, thereby interfering with important physiological functions that are dependent on constitutive NO production. Multiple studies using multivariate regression analyses, thereby taking all traditional risk factors into account, conclude that ADMA is a strong and novel cardiovascular risk factor. Determining ADMA levels in patients who are at risk for developing cardiovascular events may be helpful in the near future, but is still not executable because ADMA levels vary markedly within different clinical populations and between different analytical methods. **Chapter 1** discusses the origin and fate of dimethylarginines and particularly focuses on the organs involved in the elimination of ADMA and on the significance of ADMA in different clinical conditions.[7]

Several studies show that ADMA accumulates during renal failure and ADMA has been held responsible for the cardiovascular complications accompanying end-stage renal disease.[8-13] Besides elimination of ADMA via the kidney, another more important metabolic route for ADMA is degradation by the enzyme dimethylarginine

dimethylaminohydrolase (DDAH).[14] The recognition of DDAH as a potentially important regulator of plasma ADMA levels widens the field of research and points out that other organs may also be involved in the metabolism of dimethylarginines. Carnegie and coworkers[15] elucidated the potential role of the liver in the metabolism of dimethylarginines by reporting a decreased urinary excretion ratio of SDMA to ADMA in patients with chronic active hepatitis, owing to an increased output of ADMA. However, from this study no precise data on the hepatic metabolism of dimethylarginines can be derived, as only urine concentrations were measured. To study the exact contribution of the liver in the elimination of dimethylarginines from the portosystemic circulation, an organ balance study in rats has been performed in which arteriovenous concentration differences were determined, together with blood flow measurement using radiolabeled microspheres. The combination of arteriovenous concentration differences and blood flow determination allows calculation of net organ fluxes and fractional extraction rates for the liver and the kidney. The main finding of that study was the high uptake of ADMA by the liver.[16] The magnitude of hepatic uptake of ADMA was further clarified by estimating daily hepatic ADMA extraction. Accordingly, the liver extracted 4135 $\pm$ 480 nmol of ADMA per day, which is more than 700 times the amount of circulating ADMA in rat plasma. Importantly, and in contrast to ADMA, SDMA was hardly affected by the liver. Therefore, the probable explanation for the elimination of solely ADMA is an intense catabolism by the enzyme DDAH. Since the role of the human liver in the clearing of dimethylarginines has not been studied in detail, a study in patients undergoing liver surgery, which is described in **Chapter 2**, was designed.[17] During surgery, blood was drawn from an arterial line, the portal vein, hepatic vein, and renal vein. In addition, perioperative ultrasound Doppler measurements were perfomed to determine hepatic and renal blood flow. With these parameters, the exact contribution of the human liver and kidney in the elimination of both ADMA and SDMA was calculated. Results showed a significant net uptake (median (IQR)) of ADMA in both the liver (9.6 nmol/min (5.6-13.2)) and the kidney (12.1 nmol/min (1.3-17.1)). SDMA uptake was not only present in the kidney (12.7 nmol/min (3.5-25.4)), but also in the liver (7.7 nmol/min (2.8-16.4)). This study gives a detailed description of the hepatic and renal elimination of dimethylarginines and reveals that the clearing of SDMA is not only confined to the kidney, but the human liver also takes up substantial amounts of SDMA from the portosystemic circulation.

Considering the liver as a crucial organ in the clearing of ADMA, increased ADMA levels during hepatic failure and, consequently, a decline of ADMA concentrations after successful liver transplantation can be hypothesised. In **Chapter 3**, the role of the liver in the metabolism of ADMA in 42 patients with acute or chronic liver failure undergoing liver transplantation was investigated by studying the course of ADMA concentrations.[18] Results showed that preoperative ADMA concentrations were higher in patients with acute (1.26 μM, $p<0.001$) and in patients with chronic (0.69 μM, $p<0.001$) hepatic failure compared with healthy volunteers (0.41 μM). In addition, ADMA concentrations decreased from the preoperative day to the first postoperative day in both the acute (Δ_{ADMA}: -0.63 μM, $p=0.005$) and the chronic hepatic failure group (Δ_{ADMA}: -0.15 μM, $p<0.001$). Furthermore, in patients who experienced acute rejection, ADMA concentrations were higher during the whole first postoperative month compared with non-rejectors ($p=0.012$). Moreover, in 11 of 13 rejectors (85%) a clear increase in ADMA concentration preceded the onset of the first episode of rejection which was confirmed by liver biopsy. This study concluded that the transplanted liver graft is quickly capable of clearing ADMA, suggesting preservation of DDAH. In addition, this study showed that increased ADMA concentrations in the posttransplantation period reflect serious dysfunction of the liver graft during acute rejection. Future studies investigating the relation between histological (i.e. liver biopsy) and biochemical parameters of hepatic function must reveal whether ADMA may be used as a marker of acute liver graft rejection.

In patients suffering from liver cirrhosis, elevated ADMA and NO levels have been reported and it has been suggested that ADMA might oppose the peripheral vasodilation caused by excessive systemic NO production during liver cirrhosis. In **Chapter 4**, the role of dimethylarginines in cirrhotic patients is further elucidated by studying 25 cirrhotic patients with portal hypertension receiving transjugular intrahepatic portosystemic shunt (TIPS). 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.

This study concluded that 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 increase DDAH activity and confirms the major role of the liver as ADMA clearing organ. In the future, it would be of interest to determine hepatic and renal fractional extraction rates of both arginine and dimethylarginines before and 3 months after TIPS placement. In addition, retrieving histological data via liver and kidney biopsies would reveal additional information on DDAH activity and intracellular (dimethyl)arginine concentrations, thereby further unravelling the pathophysiological process during liver cirrhosis.

Besides the clinical signifiifcance of ADMA in conditions characterised by hepatic dysfunction, ADMA has been shown to be a strong and independent risk factor for ICU mortality in critically ill patients and a causal role for ADMA in the development of organ failure has been hypothesised.[19,20] By non-specific inhibition of NO synthase, ADMA may interfere with important physiological functions, eventually leading to the cascade of organ dysfunction and injury that may be fatal to the critically ill patient. Hypothetical consequences of non-selective inhibition of NO synthase, ultimately leading to organ failure are reduced perfusion of organs, reduced cardiac output, cardiac ischemia, capillary leakage, thrombocyte aggregation, reduced glomerular filtration rate, pulmonary hypertension, and increased adhesiveness of leucocytes. It is known that metabolic support is essential in critically ill patients. In these patients, intensive insulin therapy improves outcome and reduces morbidity.[21] The exact mechanism by which these beneficial effects are brought about were unknown. **Chapter 5** describes a study that investigates whether modulation of ADMA concentrations is involved in the beneficial effects on morbidity and mortality in critically ill patients receiving intensive insulin therapy.[22] ADMA levels were measured in 79 patients who were admitted to the ICU after complicated pulmonary and esophageal surgery, who required prolonged ($\geq$7 days) intensive care, and who were randomly assigned to receive either intensive or conventional insulin treatment. The ADMA plasma concentration significantly increased during the first 2 days after assignment of conventional insulin treatment, while the ADMA levels did not change between day 0 and day 2 in patients receiving intensive insulin treatment. Moreover, at the end of the ICU period ADMA levels were still significantly lower in the intensive treatment group compared with the conventional treatment group. These results,

together with the inverse association between mean daily insulin dose and ADMA concentration of all patients on the last day strongly suggest that ADMA is influenced by insulin therapy. Another important finding was the fact that ADMA levels were significantly higher in non-survivors compared with survivors, regardless the type of treatment received. Moreover, ADMA was related to several outcome parameters and the course of ADMA during the ICU period was independently associated with ICU mortality. Thus, this study concluded that insulin therapy modulates plasma concentration of ADMA. It seems most likely that this modulation is caused by a combination of factors, including preservation of DDAH, reduced protein breakdown and thus less ADMA release, and increased uptake of ADMA via transport systems in organs that eliminate ADMA. Future studies lowering ADMA levels with e.g. selective haemodialysis or upregulation of the ADMA degrading enzyme DDAH must confirm the finding that ADMA is associated with outcome parameters in critically ill patients.

Endothelial derived nitric oxide (NO) serves as an important regulator in the vascular physiological changes during normal pregnancy. Therefore, it seems very reasonable to assume that ADMA, as a natural occurring inhibitor of NO synthesis, is involved in the development of preeclampsia. However, results of studies investigating the role of ADMA during preeclampsia are contradictive. Although placental dysfunction of the ADMA degrading enzyme DDAH has been suggested as one of the initiating events in the development of preeclampsia, placental DDAH activity has never been measured and the contribution of the placenta in the metabolism of ADMA during pregnancy has hardly received attention. Therefore, in **Chapter 6**, we aimed to investigate the role of the placenta in the metabolism of ADMA during normal pregnancy and during preeclampsia.[23] We studied 27 nonpregnant healthy women, 15 normotensive pregnant females, 16 patients with preeclampsia, and 7 patients with the ‘hemolysis, elevated liver enzymes, and low platelets’ (HELLP) syndrome. The results of this study revealed that placental DDAH activity was not upregulated in patients with preeclampsia and showed that ADMA levels during preeclampsia are not elevated compared with nonpregnant and normotensive pregnant females. In addition, it became clear that during the HELLP syndrome, i.e. when organ dysfunction (especially liver and kidney) is present, ADMA levels were significantly increased compared with healthy pregnant females and preeclamptic women. In

order to prove causality between ADMA and the development of preeclampsia, a prospective study investigating the course of ADMA levels in a large group of pregnant patients must be executed.

CONCLUSIONS

NO became the molecule of the year 1992[24] and it was also in that year that ADMA was discovered to play a regulatory role in the arginine-NO pathway by inhibiting all isoforms of NO synthase.[1] From then, its role in regulating NO production has attracted increasing attention. Nowadays, ADMA is regarded as a novel cardiovascular risk factor. The role of the kidney and the liver in the metabolism of ADMA has been extensively studied and both organs have proven to play a key role in the elimination of ADMA. Although the liver removes ADMA exclusively via degradation by the enzyme DDAH, the kidney uses both metabolic degradation via DDAH and urinary excretion to eliminate ADMA. Modulating activity and/or expression of DDAH is still under research and may be a potential therapeutic approach to influence ADMA plasma levels. Other potential therapeutic options may be selective hemodialysis of ADMA alone or in combination with administration of arginine-enriched nutrition in order to increase the arginine/ADMA ratio and thereby restoring NO production. Interestingly, next to its association with cardiovascular disease, ADMA also seems to play a role in other clinical conditions such as hepatic failure, critical illness, and preeclampsia. In order to elucidate the clinical significance of ADMA in other important clinical conditions, the field of research on ADMA must be widened.

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