The importance of the arginine/ADMA ratio on the bioavailability of nitric oxide and organ function in critically ill patients

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SUMMARY

The arginine-nitric oxide (NO) pathway plays an important role in regulating vascular tone, which is of significant importance for regulating the perfusion of major organs, such as the kidney, liver, and heart, and especially in times of stress (1). Other important functions of NO are inhibition of platelet aggregation, inhibition of adhesion of leukocytes to endothelial cells, transplant rejection, organ injury, and cytotoxicity against microorganisms (2-4). Arginine is converted into NO and citrulline by the action of NO synthase (NOS), a family of enzymes with endothelial, neuronal, and inducible isoforms (5). Recent insights into NO metabolism have shown an important role of endogenously produced inhibitors of the enzyme NOS, in particular ADMA (6). ADMA is an endogenous derivate of arginine, and inhibits all isoforms of NOS. Because of this, ADMA plays an essential role in the availability of NO (6). ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) into citrulline and dimethylamine (7-9). DDAH is widely expressed in human tissues, but is mainly found in the liver and kidneys. Raised plasma ADMA concentrations are associated with conditions such as coronary and peripheral artery disease, diabetes mellitus, hypercholesterolemia, and hyperhomocysteinemia (10). Moreover, concentrations of ADMA are elevated in critically ill postoperative surgical ICU patients, and this elevation is associated with an increase in morbidity and mortality (11). The effects of ADMA on different organ systems were investigated in the studies described in this thesis, entitled **'The importance of the arginine/ADMA ratio on the bioavailability of nitric oxide and organ function in critically ill patients'**.

Chapter 1 of this thesis provides an overview of the synthesis and metabolism of ADMA, and the relationship between ADMA and different clinical conditions. Over past decades, several studies have investigated NO metabolism and the role of ADMA in adult patients. It would also appear that plasma ADMA concentrations are increased in preterm infants (12-14), but the clinical consequences of this increase are not yet known. NO metabolism is thought to play a role in necrotizing enterocolitis (NEC), a serious disease of the gastrointestinal tract that occurs almost exclusively in preterm infants (15). Damage to the intestinal mucosa renders it susceptible to bacterial invasion, which can rapidly deteriorate into sepsis (16). That NO metabolism might have a role in the multifactorial disease NEC is supported by the finding that plasma levels of arginine are decreased in premature infants at the time of diagnosis of NEC (17;18). Given the similarity between NEC and sepsis, it is plausible to assume that plasma concentrations of ADMA are raised in preterm infants with NEC. In the prospective case–control study described in **chapter 2,** we measured plasma concentrations of arginine and ADMA and the arginine/ADMA

ratio in preterm infants with and without NEC. We found that arginine and ADMA concentrations, as well as the arginine/ADMA ratio, were significantly lower in preterm infants with NEC than in preterm infants without NEC. Furthermore, in the NEC group, arginine levels and the arginine/ADMA ratio were significantly lower in non-surviving infants than in surviving infants. The results of this study suggest that a diminished NO production is involved in the pathophysiology of NEC. When these data were published, the article was accompanied by an editorial comment stating that it would be desirable to design a clinical trial to measure the effect of arginine supplementation in premature infants (19).

In addition to the importance of NO metabolism for the regulation of mucosal blood flow and the barrier function of the gut, recent studies have shown that NO is also important for the normal development and maturation of the lungs of preterm infants (20-22). NO produced in the lung is an important mediator of vascular smooth muscle relaxation, ventilation perfusion matching, neurotransmission, host defense and bacteriostasis, mucociliary clearance, and airway mucus secretion (23-27).

In the study presented in **chapter 3,** we compared ADMA and arginine levels in preterm infants requiring or not requiring mechanical ventilation. Plasma ADMA concentrations were significantly higher in infants who required mechanical ventilation than in infants who did not require mechanical ventilation. In addition, ADMA concentrations were significantly correlated with the duration of mechanical ventilation, even after adjustment for gestational age. The results of this study suggest that an increased ADMA concentration could reduce NO synthesis, which could lead to insufficient gas exchange and, consequently, a longer duration of mechanical ventilation.

In addition to its importance in critically ill preterm infants, ADMA has also been shown to be a strong and independent risk factor for ICU mortality in critically ill adult patients (11). Nijveldt and coworkers (28) hypothesized that, based on the non-specific inhibition of NOS, ADMA may interfere with important physiological functions, which could eventually lead to the cascade of multiple organ failure. In critically ill patients, not only are ADMA levels elevated, but arginine concentrations are also reduced. Arginine levels are also decreased after major surgery, such as thoracoabdominal aortic surgery (29), after trauma (30) and during sepsis (31). Many studies have been performed to investigate the clinical effects of reduced arginine concentrations or elevated ADMA concentrations; however, to date little is known about the clinical effects of the combination of low arginine levels and high ADMA levels. Therefore, in the randomized, placebo-controlled animal study described in **chapter 4,** we investigated the effects of low arginine plasma concentrations in combination with high ADMA plasma concentrations on systemic hemodynamics and organ blood flow. Male Wistar rats were randomly assigned to three groups: a control group, a group with elevated ADMA plasma concentrations, and a group with low arginine levels and elevated ADMA levels. Compared with control values, mean arterial pressure

and systemic vascular resistance were increased after infusion of ADMA, whereas systemic hemodynamics (mean arterial pressure, cardiac output, stroke volume, systemic vascular resistance) and organ blood flow through the kidney and spleen deteriorated after arginine levels were lowered. These data support the hypothesis that a diminished NO production through a combination of low arginine and high ADMA concentrations may be involved in the onset of organ failure.

Critically ill surgical patients on the ICU have an increased risk of developing multiorgan failure, which is associated with morbidity and mortality. As shown by the Leuven study (32), strict glucose regulation by intensive insulin therapy in this patient population leads to fewer complications and even to an increased survival. However, the mechanism underlying this clinical benefit is not completely understood, but of potential mechanisms (33), the modulation of NO metabolism emerges as a potentially important mechanism contributing to the clinical benefit of tight glycemic control. Recently, Siroen and colleagues (34) demonstrated that strict glucose regulation by intensive insulin therapy lowers the plasma concentrations of ADMA in critically ill surgical patients. Furthermore, ADMA levels in patients who died were significantly higher than those in survivors. In the studies reported in **chapter 5 and 6,** we endeavored to gain insight into the effects of strict glucose regulation on NO metabolism. Using a "2x2 factorial design" and a rabbit model of prolonged critical illness, we investigated the influence of insulin and glucose on the availability of NO, the activity and expression of NOS, the activity of DDAH, and the concentrations of arginine and ADMA in different organs. Compared with normoglycemic groups, both hyperglycemic groups had significantly higher plasma NO levels on day 3, but this difference was no longer seen on day 7. Furthermore, in skeletal muscle biopsies and aortic endothelium NOS activity was significantly lower in the hyperglycemic groups than in the normoglycemic groups. The expression of iNOS in muscle and eNOS in aorta was higher in the hyperglycemic groups than in the normoglycemic groups. The expression of eNOS was particularly elevated in the aorta of the hyperglycemic groups. ADMA concentrations were raised and DDAH activity was reduced in the hyperglycemic groups compared with the normoglycemic groups. The conclusion of these studies was that inadequate regulation of glucose levels could lead to a decreased activity of DDAH, leading to an increase in ADMA levels. The increased levels of ADMA could, in turn, lead to diminished NOS activity, mainly in endothelium and muscle. Subsequently, there would be a compensatory increase in the expression of endothelial eNOS and iNOS in muscle.

In this animal model of prolonged critical illness, maintenance of normoglycemia, rather than glycemia-independent actions of insulin, had a beneficial effect on ADMA concentrations, probably by protecting DDAH against inactivation by glucose-induced oxidative stress. This in turn protects NOS activity at an organ level, which could have a positive effect on the

microcirculation and the function of important organs. So, maintaining normoglycemia in critically ill patients would seem to be an important aspect of treatment to prevent an increase in plasma ADMA levels. Considerable research has been done in the last 10 years to identify agents that can decrease ADMA levels. Stühlinger (35) and Wang (36) demonstrated the ADMA-lowering effect of the peroxisome proliferator-activated receptor (PPAR) agonist rosiglitazone. On the basis of these findings, we investigated the effect of rosiglitazone on ADMA plasma levels in critically ill patients in the randomized controlled pilot study described in **chapter 7**. ADMA, arginine, and symmetric dimethylarginine (SDMA) were measured in 21 critically ill patients on the ICU, 12 of whom received rosiglitazone (4 mg) once a day for a maximum of 6 weeks or until discharge or death; the remaining 9 patients served as controls. ADMA levels were significantly higher in critically ill patients than in healthy individuals; ADMA levels were independently related to SOFA scores. However, overall, rosiglitazone treatment had no effect on ADMA levels. In conclusion, ADMA levels were significantly elevated in critically ill patients, and this increase was associated with the extent of multiple organ failure. However, rosiglitazone did not significantly lower ADMA levels.

FUTURE PERSPECTIVES

The studies reported in this thesis confirm the importance of ADMA and its precursor arginine in various pathophysiological processes. Our increased insight into the metabolism of ADMA is accompanied by the realization that reducing the plasma ADMA concentration is potentially an important therapeutic option. Although several publications have shown that some drugs reduce ADMA levels, consistent results have not yet been published (37). There are currently no drugs available that specifically reduce ADMA levels, and thus more research is needed to identify potential ADMA-lowering therapies. Theoretically, four mechanisms can lead to accumulation of ADMA: (1) increased proteolysis, (2) increased methylation of arginine by protein arginine methyltransferases (PRMT), (3) decreased renal excretion, and (4) impaired metabolism by DDAH. Consequently, we should focus on therapies that decrease catabolism, increase the renal excretion of ADMA, increase the activity or expression of DDAH enzymes, or decrease the activity or expression of the ADMA-producing enzyme PRMT.

Several studies have shown that hemodialysis lowers ADMA levels in patients with renal failure (38;39). Recently, Rifai and colleagues (40) demonstrated that plasma levels of ADMA could be effectively lowered by 25% by using an artificial liver support system (Prometheus). Thus there are a number of potential ADMA-lowering therapies in development. However, more research is needed to confirm these results and to investigate the clinical consequences of decreased

ADMA levels. Most studies measured the concentration of plasma only, under the assumption that plasma levels reliably reflected intracellular levels. However, little is known about the intracellular ADMA concentration. More needs to be learned about the intracellular concentration and metabolism of ADMA, and the relationship between intracellular and plasma ADMA concentrations. Lastly, it is open to question whether a reduction in ADMA levels is always favorable. For example, it is known that NO affects tumor progression and the development of tumor metastasis (41). Several research groups have demonstrated a positive correlation between NOS expression and tumor growth. Intracellular factors such as ADMA and DDAH, which regulate NO synthesis, could therefore be important targets for controlling tumor growth (42;43). Future research should focus on the role of ADMA and DDAH in tumor angiogenesis.

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