REVIEWS

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Asymmetric Dimethylarginine Synthesis and Degradation Under Physiological and Pathological Conditions

Asymetryczna dimetyloarginina – synteza i rozkład w warunkach fizjologicznych i patologicznych

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Abstract

Asymmetric dimethylarginine (ADMA) is an endogenous methylated amino acid derived from arginine. ADMA can inhibit the activity of all isoforms of nitric oxide synthase (NOS). Protein arginine methyltransferases (PRMTs) catalyze the synthesis of ADMA and dimethylarginine dimethylaminohydrolase (DDAH) is responsible for the metabolism of this compound. ADMA enters cells through cationic amino-acid transporters (CATs), which are known to be y^+ carriers. Many factors can regulate the synthesis, transport, metabolism, or excretion of ADMA. In various pathological states such as hypercholesterolemia, hyperglycemia, hyperhomocysteinemia, hypertension, coronary artery disease, heart failure, and stroke, plasma levels of ADMA may increase two- or even tenfold, contributing to inhibition of NO synthesis and endothelial dysfunction. Impaired liver or renal function could also have an impact on the plasma concentration of ADMA. In some situations such as neurological disorders, decreased levels of ADMA are noted. It is very important to discover which states or drugs can increase or decrease the level of ADMA and what the mechanism of that action is (**Adv Clin Exp Med 2010, 19, 233–243**).

Key words: ADMA, PRMTs, DDAH.

Streszczenie

Asymetryczna dimetyloarginina (ADMA) to endogenny aminokwas powstający z argininy. ADMA hamuje aktywność wszystkich izoform syntetaz tlenku azotu (NOS). Metylotransferazy argininowe (PRMTs) katalizują syntezę ADMA, a dimetyloaminohydrolaza dimetyloargininy (DDAH) odpowiada za rozkład tego związku. Kationowe transportery aminokwasów (CATs) znane, jako y+ transportery pozwalają na gromadzenie się ADMA we wnętrzu komórki. Wiele czynników wpływa na syntezę, transport, metabolizm i wydalanie ADMA z organizmu. Wśród chorób należy wymienić hipercholesterolemię, hiperglikemię, hiperhomocysteinemię, chorobę wieńcową, niewydolność serca i udar. Wszystkim tym stanom towarzyszy 2-, a nawet 4-krotny wzrost stężenia ADMA we krwi, zahamowanie syntezy NO i dysfunkcja śródbłonka. Upośledzona czynność wątroby i nerek, narządów odpowiedzialnych za wydalanie ADMA, również wpływa na wzrost stężenia tego związku w organizmie. W niektórych stanach, np. w chorobach neurologicznych, stężenie ADMA jest zmniejszone. Jest bardzo ważne, aby poznać, które choroby lub substancje są odpowiedzialne za zwiększone lub zmniejszone stężenie ADMA oraz jaki jest mechanizm tego zjawiska (**Adv Clin Exp Med 2010, 19, 233–243**).

Słowa kluczowe: ADMA, PRMTs, DDAH.

Arginine is an amino acid essential for normal growth and development. Endogenous synthesis is adequate in healthy people, but might be insufficient in many pathological states. Asymmetric and symmetric dimethylarginines (N^G,N^G-dimethyl-L-arginine – ADMA, and N^G,$N^{G'}$-dimethyl-L-arginine – SDMA) as well as monomethylarginine (N^G-monomethyl-L-arginine – L-NMMA) are

endogenous methylated amino acids derived from arginine. ADMA and SDMA were isolated from human urine in 1970 by Kakimoto and Akazawa [1]. Both ADMA and L-NMMA can inhibit nitric oxide (NO) synthesis [2]. L-NMMA was initially developed as a pharmacological tool which could elicit an endothelium-dependent increase in the tone of rabbit aortic rings and inhibit the endothelium-dependent relaxation induced by acetylcholine [3, 4]. Later it was also shown to be present in neurons and the brain [5]. The concentration of ADMA in plasma is much higher than that of L-NMMA and it is suggested that ADMA is much more important than this monomethylated derivative of arginine [6]. In contrast to ADMA, SDMA fails to inhibit NO synthesis, but it may compete with arginine for transport across cell membranes [2].

ADMA is an endogenous inhibitor of all isoforms of NO synthase, which was demonstrated in many experiments *in vitro* and *in vivo* [2, 7]. It was also shown that endothelial (eNOS, type III) and neuronal (nNOS, type I) nitric oxide synthase are predominantly inhibited by ADMA [7–9], but the inducible isoform (iNOS, type II) is inhibited to a lesser extent [10, 11]. Plasma levels of ADMA in healthy persons vary between 0.3–0.5 μmol/l [2], but in pathological states it may increase two- or even tenfold, contributing to inhibition of NO synthesis and endothelial dysfunction [12]. It was concluded that a concentration range of 3–10 μM is enough to inhibit vascular NO production [13]. Consequently, under normal conditions eNOS is inhibited by 10%, but in pathological situations by 30–70% [6]. It is worth emphasizing that the concentration of ADMA inside cells is much higher than outside. It was reported that the intracellular level of ADMA in endothelial cells obtained from rabbit carotid artery is ten times higher than in plasma [14].

ADMA, an inhibitor of NOS, inhibits NO synthesis. It was also shown that ADMA can potentiate the expression of genes encoding endothelial adhesion molecules [15], augment mononuclear cell adhesiveness, and participate in the inflammatory reaction [16–18]. Pretreatment of endothelial cells with ADMA increases the expression of monocyte chemotactic protein-1 (MCP-1). This response could be attenuated by anti-MCP as well as L-arginine [17].

Role of ADMA in Clinical Disorders

Elevated plasma ADMA levels have been found to be associated with impaired endothelium-dependent vasodilatation [19]. Elevated plasma levels of ADMA were detected in patients with vascular diseases [20–22] and significantly correlated with carotid intima-media thickness. ADMA is suggested to be a marker of atherosclerosis [23]. Oxidative stress is also responsible for increased synthesis and/or inhibited metabolism of this compound [24] and elevated levels of ADMA are observed in patients with hypercholesterolemia, hyperglycemia, hyperhomocysteinemia, diabetes, hypertension, and heart failure [17, 22, 25, 26]. In hypercholesterolemia, plasma ADMA level better correlates with endothelial dysfunction than LDL [20, 27]. ADMA was shown to be an independent and strong predictor of left ventricular ejection fraction (LVEF) and an inverse relationship between ADMA and LVEF was demonstrated [28]. A high level of ADMA independently predicts future cardiovascular risk in patients with coronary artery disease [29] and adverse cardiovascular events in patients undergoing percutaneous coronary intervention [30]. Furthermore, ADMA is a weak independent marker of acute stroke and a strong marker of transient ischemic attack (TIA) [31].

An association between ADMA level and smoking was also reported [32]. The level of this compound also increases with aging [19]. In critically ill patients with clinical evidence of more than two organ failures, ADMA is a strong and independent risk factor of mortality [33]. Renal insufficiency [34] and liver cirrhosis [35] are further examples of disorders with higher levels of ADMA as both organs are responsible for the elimination of ADMA. Significant correlation was observed between the concentration of ADMA in graft and liver function after transplantation [36].

On the other hand, ADMA is suggested to play a role as an inhibitor of the overproduction of NO, mainly by the regulation of iNOS activity [10, 11]. Furthermore, by modulating nNOS activity, ADMA may prevent neurotoxic injuries [5]. ADMA concentration has been reported to be significantly lower in patients with Alzheimer's disease [37]. Increased NO synthesis and oxidative stress have been implicated in this state and in aging [3]. Treatment with NOS inhibitors could be protective in these diseases, which progress with NO overproduction. L-NMMA was revealed to reverse hypotension in septic shock, prevent inflammatory reactions, and decrease both pain reaction and headache [38]. Elevated levels of ADMA were also found in patients with hematological malignancies, although the significance of this finding remains unclear [39].

Factors Regulating ADMA Level in the Organism

PRMTs

Endogenous methylarginines are derived from the methylation of arginine residues on various proteins. Protein methylation is a posttranslational modification of many intracellular proteins involving the addition of a methyl group to arginine residues in the polypeptide chain by the action of protein arginine methyltransferases (PRMTs). It is a covalent and irreversible reaction. PRMTs are a family of enzymes containing eight isoforms with different substrate specificities. They can transfer two methyl groups provided by S-adenosyl methionine to some proteins in either a symmetric (leading to SDMA) or an asymmetric configuration (leading to ADMA). Additionally, they can also catalyze monomethylation, which leads to the synthesis of L-NMMA [40, 41].

PRMTs can also be divided in two groups: types I and II. Substrates for type I include histones and RNA-binding proteins such as heterogeneous nuclear ribonuclear protein (hnRNP) A1, fibirillarin, and nucleolin and for type II myelin basic protein (MBP) and spliceosomal D1 and D3 proteins. Type I protein methylases produce ADMA and type II produce SDMA. Both types are responsible for the production of L-NMMA [3, 40]. The activity of such proteins as hnRNP, which can regulate the maturation and stability of mRNA and its export to the cytoplasm, is modified by methylation. Approximately 65% of total ADMA is synthesized in hnRNP [42].

The proteolysis of proteins containing endogenous methylarginines is responsible for the release of free methylarginine residues into the cytoplasm [1]. In several studies the production of methylarginines was used as an index of *in vivo* protein degradation [43]. Several disorders such as muscular dystrophy are associated with enhanced protein catabolism and a subsequent higher level of ADMA [44]. Increased protein degradation has also been reported in stress, ischemic heart, diabetes, and during fasting or infections [3, 45–47]. Exposure of cultured hepatoma cells to 43°C also evoked a transient acceleration in protein degradation [48]. It is suggested that in cellular stress, such as ischemia/reperfusion (I/R) conditions or endotoxemia, proteolysis could be upregulated to remove abnormal proteins [3].

The gene expression of PRMTs is increased in cultured endothelial cells after administration of both native low-density lipoprotein (nLDL) and oxidized low-density lipoprotein (oxLDL) [26]. These compounds, similarly to glucose and homocysteine, are also responsible for a higher activity of arginine methylating enzymes, which results in increased ADMA concentration [26, 41, 49, 50].

Argininase

Another important aspect is the activity of argininase. This enzyme catabolizes arginine to ornithine and urea [51]. A decreased level of arginine results in imbalance between ADMA and arginine. Degradation by alanine glyoxalate aminotransferase (AGT-2) could also be involved in ADMA metabolism, but this enzyme has a low binding affinity to ADMA [52].

Transporter Systems

The transport of ADMA is a consecutive factor influenced by ADMA concentration. ADMA, SDMA, and L-NMMA enter cells through cationic amino-acid transporters (CATs), which are known to be y^+ carriers. Arginine and other cationic amino acids such as ornithine and lysine are also transported by CATs [53]. All methylarginines, similarly to arginine, appear to be handled in a similar manner and could compete with each other for this route of transport. It is worth noting that this transporter system co-locates with caveolin-bound eNOS, which suggests that the activity of this transporter may be important in determining the local concentrations of L-arginine and methylarginines [54]. Some factors can impact on ADMA transport. This system has been reported to be upregulated by certain pro-inflammatory cytokines [55]. Additionally, changes in the expression of CAT mRNA may influence dimethylarginine transport. It is suggested that extensive expression of CAT-2A mRNA in the liver may indicate a higher uptake of dimethylarginines by this organ [56, 57].

The y^+ system is not the only route of entry for arginine and methylarginine into cells, but the relative importance of the other amino-acid transporters is uncertain [8]. The y^+ transporter system is responsible for the concentration of methylarginines within the cell and, consequently, the intracellular level of ADMA exceeds its extracellular concentration [55]. The results of one kinetic study demonstrated that intracellular methylarginine levels are 5–10 times higher than outside the cell [6].

Dimethylarginine Dimethylaminohydrolase

The liver plays an important role in the metabolism of ADMA. This organ is able to take up

a large amount of ADMA [58]. In patients with liver injury, an increase in urinary ADMA excretion was reported [59]. In patients undergoing a major hepatic resection, increased levels of ADMA occurred in the postoperative course, especially when liver function was severely impaired [60]. DDAH (Dimethylarginine dimethylaminohydrolase) is an enzyme responsible for the metabolism of ADMA. In contrast to ADMA, SDMA is not degraded by DDAH [8, 52, 61]. The way of ADMA elimination was discovered in 1987, when it was found that a much smaller amount of ADMA than SDMA was excreted in urine for 12 hours after intravenous injection [61, 62]. Significant differences in DDAH activity depending on animal species have been described [63–65]. Similarly to humans, over 90% of ADMA is metabolized by DDAH in rats [63].

DDAH metabolizes L-NMMA and ADMA to citrulline and monomethylamine or dimethylamine, respectively [3, 8, 40, 41]. This reaction is present in both liver endothelial cells [66, 67] and hepatocytes [68].

DDAH recovered from various living organisms display great similarity. The bacterial catalytic center of DDAH is the same as the human one [69]. Human DDAH I has 93% homology with the rat enzyme at the amino-acid level and displays similar enzymatic properties [70]. The mouse and bovine amino-acid sequences of DDAH I and DDAH II show 92% and 95% homology, respectively, with the human ones [71].

Studies on bacterial DDAH isolated from *Pseudomonas aeruginosa* reported that the active site contains cysteine, histidine, and glutamic acid as a catalytic triad [69]. In mouse, sheep, and human DDAH I and II, the catalytic site is composed of cysteine, asparaginic acid, and histidine. These amino acids appeared to be conserved in all mammalian species [72, 73]. DDAH contains a flexible loop that can adopt distinct conformations and may act as a lid to open or close a channel for substrates to the active site [72]. The catalytic reaction occurs between the carbon atom of the guanidine group of ADMA and the sulfur atom of cysteine in the active site of DDAH [69]. It is also reported that DDAH is a zinc (Zn)-containing enzyme. Although this metal is not involved in the catalytic process, it is essential to stabilize the enzyme in its active form [74].

DDAH has two isoforms, I and II, with 62% homology at the amino-acid level and 63% homology at the nucleotide level [3]. They are located mainly in cytoplasm, but DDAH II is also recovered from the membrane fraction [75]. DDAH I is expressed at equivalent levels in fetal and adult tissues, but DDAH II is expressed at relatively high levels in all fetal tissues and then decreases [76]. DDAH I is found in the brain, liver, adrenal glands, testis, kidney, skeletal muscle, pancreas, and in peritoneal neutrophils and macrophages and its distribution correlates generally with that of nNOS. The distribution of DDAH II is more widespread, with the highest expression in the heart, aorta, placenta, and kidney, and it loosely correlates with the distribution of eNOS [8, 70, 76]. DDAH II is also found in such immune tissues as spleen, thymus, peripheral leukocytes, lymph nodes, and bone marrow that express iNOS [76]. It is suggested that DDAH II, concomitantly with iNOS, may play a role in the modulation of host defense or immune tolerance in autoimmune diseases such as rheumatoid arthritis because the gene for DDAH II is located in the region of MHC III. It was also found that the gene for DDAH I is essential during embryogenesis [77].

The average ADMA concentration is approximately 1–2 μM/g of protein and the total production is 300 μM of ADMA in 24 hours. Two hundred fifty μM of ADMA is metabolized by DDAH and only 50 μM is excreted with urine. Complete inhibition of this enzyme can lead to an increase in plasma ADMA level by approximately 5 μM. Therefore, modulation of DDAH activity plays an important role in maintaining ADMA at a safe level [78]. Many factors are able to modulate DDAH activity or change the gene expression for this enzyme. The sulfhydryl group of cysteine in the active site predisposes this enzyme to easy oxidation or nitrosation and it loses its activity [73]. Some antioxidants can preserve DDAH activity after exposure to such factors as a high level of glucose or homocysteine, which indicates that DDAH activity could be modulated by cardiovascular risk factors in a reactive oxygen species (ROS)-sensitive manner [49, 50]. In both *in vitro* and *in vivo* studies, DDAH has been reported to be inhibited by extensive amounts of NO. Six-fold increases in NO produced by iNOS after cytokine treatment of endothelial cells are sufficient to inhibit DDAH activity, probably followed by increased ADMA concentration [73].

Only DDAH I appears to be responsible for ADMA degradation. Loss of DDAH I activity by using specific inhibitors leads to an accumulation of ADMA. Both plasma and tissue levels of ADMA also increased in *Ddah*$^{+/-}$ mice with deletion of the *Ddah1* gene. In *Ddah*$^{+/-}$ mice, some symptoms of endothelial dysfunction, including increased contraction in response to phenylephrine, reduced relaxation in response to acetycholine or calcium ionofore A23187, and increased relaxation in response to sodium nitroprusside, were observed. Hemodynamic effects such as increased mean arte-

rial blood pressure, decreased cardiac output and heart rate, and elevated right ventricular pressure were also revealed. No increase in the expression of DDAH II in these mice was noted [77]. However, the importance of this isoform has also been evaluated. It was shown that only DDAH II expression increased after birth and reached a peak on day 1 in newborn piglets. This isoform decreased in animals with pulmonary hypertension [79].

Depletion of DDAH Activity

The concentration of ADMA, related to disturbances in DDAH, is elevated in many pathological states. The expression of DDAH II gene is decreased by such factors as coupling factor 6 (CF6), which is responsible for increased ROS generation and vasoconstriction. CF6 is also responsible for downregulation of the expression of DDAH II protein [80]. Lipopolysaccharide [81] and high concentrations of glucose that generate ROS [82] could also evoke decreased concentrations of DDAH II protein, but oxLDL or inflammatory cytokines such TNFα are rather responsible for the depletion of DDAH I protein expression [41, 20]. An elevated level of ADMA may be explained by decreased activity of DDAH evoked by the stimulation of TNF-α production in such states as hypercholesterolemia or other inflammatory diseases [41].

Oxidative stress has also been found to affect DDAH activity by modifying a critical cysteine in the enzyme's active site [41, 50, 69]. Although there are papers which do not confirm this effect [83], there is growing evidence suggesting that in hypertension, hypercholestrolemia, hyperglycemia, and hyperhomocysteinemia, oxidative stress is the main factor affecting DDAH activity leading to increased ADMA concentration [20, 25, 41, 50, 69, 84]. On the other hand, it was also demonstrated that inhibition of DDAH activity causes enhanced endothelial superoxide radical formation [85].

Enhancement of DDAH Activity

It has been demonstrated that overexpression of human DDAH I mRNA in transgenic mice results in increased NOS activity and a reduction of ADMA [86]. The expressions of *ddah2* gene and DDAH II protein are increased by many substances, such as all-trans-retinoic acid [66], pioglitazone [87], and estradiol [88]. The protein expression of DDAH I is increased by inflammatory cytokines with pro-oxidative properties, such as IL-1β [10, 89]. It is suggested that IL-1β could increase NO synthesis not only by increasing DDAH activity, but also directly by stimulation of the expression of iNOS [10]. A large production of NO derived from cytokine-induced iNOS can react with superoxide to form peroxinitrite and augment cell injury [90]. Under these conditions, ADMA appears as a regulator of extensive production of NO [11]. Some authors even suggest that in patients with sepsis, DDAH inhibition could potentially be used therapeutically to reduce vascular collapse [77]. Similarly to IL-1β, lipopolysaccharide (LPS) also induces the expression of iNOS [77]. The arterial plasma concentration of ADMA was decreased in rats submitted to LPS-induced endotoxemia [58]. It was also demonstrated that on the first postoperative day in patients undergoing colorectal surgery, the concentration of ADMA was lower than in healthy subjects, which could be explained as the action of endotoxin derived from the gut [60]. It is worth noting that overexpression of DDAH I could enhance tumor growth and angiogenesis [91].

Impaired Liver Function

NO synthesized constitutively by eNOS is responsible for maintaining sinusoidal tone and flow in liver sinusoidal cells [92, 93]. NO derived from endothelial cells evokes relaxation of hepatic stellate cells, partially through the activation of guanylyl cyclase [92–94]. ADMA is one of the regulators of NO synthesis. Other important factors include the phosphorylation of eNOS, eNOS binding to the inhibitory protein claveolin, and protein kinase B binding to G protein-coupled receptor kinase 2 [94].

Impaired liver function may lead to increased plasma levels of ADMA [33, 57]. However, there are some differences among various diseases. It is suggested that the pathogenesis of impaired NO production could differ and depends on the etiology of the liver injury. Elevation of ADMA is observed in such states as decompensated alcoholic cirrhosis [35] and acute alcoholic hepatitis [95]. In rats with portal hypertension evoked by bile duct ligation, increased ADMA levels with diminished production of NO were also observed [96, 97]. In another study on rats with cholestatic cirrhosis induced through bile duct excision, decreased hepatic activity of eNOS and higher concentrations of ADMA were observed [98, 99]. Conversely, no elevation of ADMA levels was observed in drug-evoked cirrhosis. In thiocetamide-induced liver injury, decreased eNOS activity and protein levels were observed in association with a low level of ADMA [96]. Such a result is comparable to another study in which similar decreases in eNOS activ-

ity and protein expression in CCl_4-induced cirrhosis were shown [99]. It is suggested that these decreases may depend on inhibited expression of mRNA for eNOS. The decrease in eNOS activity associated with unaltered eNOS protein expression could be explained as a dysfunction of eNOS cofactors [96, 97].

Ischemia/reperfusion (I/R) is considered to be the main cause of liver cell damage during, for example, liver transplantation, which has become a method of choice in end-stage liver disease. The pathogenesis of liver damage during transplantation is very complex and occurs in two stages: the injury is initially caused by ischemia, while later the damage is aggravated by reperfusion of the organ [100]. Experimental data suggest that NO is one of the components of I/R injury and can modulate ROS metabolism by reaction with free radicals such as superoxide in the formation of peroxinitrite [101–103]. eNOS is responsible for the basal production of NO that maintains the normal vascular tone within the sinusoids [104]. Conversely, a higher level of NO produced by iNOS is involved in the inflammatory process and promotes I/R injury [101, 103, 105]. Vasoconstriction is one of the conditions which affects liver function in I/R. It results from deterioration of the balance between NO and endothelin [36, 106]. NO production is limited because of, for example, the release of NOS inhibitors such as ADMA [36, 40]. Many various factors may influence NO level in the liver. Low NO levels after the ischemic period may be related to a low intracellular level of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydropterin (eNOS cofactors) and limited oxygen support [106], increased argininase activity, which removes arginine required for NO synthesis [107], and increased release of NOS inhibitors from the ischemic organ during the reperfusion period [108]. In I/R injury, increased protein methylation is upregulated to remove altered proteins [3]. Other data suggest that proteolysis in enhanced after I/R and protease activity correlates with graft function [109]. On the other hand, oxidative stress inhibits the activity of the ADMA-metabolizing enzyme DDAH by modifying a critical cysteine in the enzyme's active center [41, 69, 73]. Correlation between methylated arginine derivative concentrations and liver function and survival after liver transplantation was observed [36].

Cardiovascular depression, known as post-reperfusion syndrome (PRS), is another problem in liver transplantation. It is suggested that the pathogenesis of this syndrome is related to the interaction between various vasoactive substances released from ischemic liver during reperfusion [110]. Since vasodilation is the main phenomenon, it is possible that NO is one of them [111]. However, it was shown that hypotension existed with inhibition of iNOS, which suggests that mediators other than NO also play important roles in such a syndrome [36].

Impaired Renal Function

In contrast to SDMA, which is eliminated only with urine [57, 61, 62], the main organ responsible for ADMA elimination is the liver [57]. Additional sites of ADMA release are the kidney and gut [57, 58]. With impaired liver function, urinary ADMA excretion is increased [59], which indicates that renal function could also have an impact on plasma ADMA levels [8]. Inverse correlation between creatinine clearance and the concentration of methyl-arginines in human and rat plasma was observed [58]. In patients with renal failure, the higher level of ADMA results from poor excretion [2, 112].

In patients with renal failure, an elevated level of ADMA could be associated with various pathophysiological changes [2]. Inhibition of NOS and affection of NO production can lead to vasoconstriction and glomerular hypoperfusion [113] and increased mean arterial blood pressure [34]. A higher level of ADMA may also be connected with immune dysfunction in uremia [2]. It has also been shown that elevated ADMA levels are an independent predictor of cardiovascular disease and mortality in patients with end-stage renal disease [2, 112].

Conclusions

ADMA has significant clinical relevance and it seems to be reasonable to investigate all possible factors responsible for its level in the organism. Various drugs can regulate the synthesis, transport, metabolism, or excretion of this compound. Increased ADMA level is observed in many disorders with endothelial dysfunction and it is also an important risk factor for cardiovascular events. It is reasonable to search for drugs increasing the activity of DDAH and/or inhibiting PRMT and decreasing ADMA levels, what may lead to increased NO synthesis and improvement in the function of different organs. On the other hand, overproduction of NO may be a reason for some pathological changes in tissues. In such a situation, drugs acting in an opposite way may be useful.

References

[1] **Kakimoto Y, Akazawa S:** Isolation and identification of NG,NG- and NG,N'G-dimethylarginine, N-epsilon-mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. J Biol Chem 1970, 245, 5751–5758.

[2] **Vallance P, Leone A, Calver A, Collier J, Moncada S:** Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992, 339, 572–575.

[3] **Tran CT, Leiper JM, Vallance P:** The DDAH/ADMA/NOS pathway. Atheroscler Suppl 2003, 4, 33–40.

[4] **Palmer RM, Ashton DS, Moncada S:** Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988, 333, 664–666.

[5] **Cardounel AJ, Zweier JL:** Endogenous methylarginines regulate neuronal nitric oxide synthase and prevent excitotoxic injury. J Biol Chem 2002, 277, 33995–34002.

[6] **Cardounel AJ, Cui H, Samouilov A, Johnson W, Kearns P, Tsai AL, Berka V, Zweier JL:** Evidence for the pathophysiological role of endogenous methylarginines in regulation of endothelial NO production and vascular function. J Biol Chem 2007, 282, 879–887.

[7] **Vallance P, Leone A, Calver A, Collier J, Moncada S:** Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992, 20 Suppl 12, S60–S62.

[8] **Leiper J, Vallance P:** Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. Cardiovasc Res 1999, 43, 542–548.

[9] **MacAllister RJ, Whitley GS, Vallance P:** Effects of guanidino and uremic compounds on nitric oxide pathways. Kidney Int 1994, 45, 737–742.

[10] **Ueda S, Kato S, Matsuoka H, Kimoto M, Okuda S, Morimatsu M, Imaizumi T:** Regulation of cytokine-induced nitric oxide synthesis by asymmetric dimethylarginine: role of dimethylarginine dimethylaminohydrolase. Circ. Res 2003, 92, 226–233.

[11] **Kang ES, Tevlin MT, Wang YB, Chiang TM, Cardenas R, Myers LK, Acchiardo SR:** Hemodialysis hypotension: interaction of inhibitors, iNOS, and the interdialytic period. Am J Med Sci 1999, 317, 9–21.

[12] **Sydow K, Münzel T:** ADMA and oxidative stress. Atheroscler Suppl 2003, 4, 41–51.

[13] **Boger RH, Bode-Boger SM, Thiele W, Junker W, Alexander K, Frolich JC:** Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. Circulation 1997, 95, 2068–2074.

[14] **Masuda H, Goto M, Tamaoki S, Azuma H:** Accelerated intimal hyperplasia and increased endogenous inhibitors of NO synthesis in rabbits with alloxan-induced hyperglycaemia. Br J Pharmacol 1999, 126, 211–218.

[15] **Chen BM, Xia LW, Zhao RQ:** Determination of N(G),N(G)-dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997, 692, 467–471.

[16] **Chan JR, Boger RH, Bode-Boger SM, Tangphao O, Tsao PS, Blaschke TF, Cooke JP:** Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. Arterioscler Thromb Vasc Biol 2000, 20, 1040–1046.

[17] **Boger RH, Bode-Boger SM, Tsao PS, Lin PS, Chan JR, Cooke JP:** An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. J Am Coll Cardiol 2000, 36, 2287–2295.

[18] **Holm T, Aukrust P, Aagaard E, Ueland T, Haugstad TS, Kjekshus J, Simonsen S, Froland SS, Gullestad L, Andreassen AK:** Hypertension in relation to nitric oxide, asymmetric dimethylarginine, and inflammation: different patterns in heart transplant recipients and individuals with essential hypertension. Transplantation 2002, 74, 1395–1400.

[19] **Asagami T, Abbasi F, Stuelinger M, Lamendola C, McLaughlin T, Cooke JP, Reaven GM, Tsao PS:** Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. Metabolism 2002, 51, 843–846.

[20] **Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP:** Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998, 98, 1842–1847.

[21] **Surdacki A, Nowicki M, Sandmann J, Tsikas D, Boeger RH, Bode-Boeger SM, Kruszelnicka-Kwiatkowska O, Kokot F, Dubiel JS, Froelich JC:** Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999, 33, 652–658.

[22] **Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T:** Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. Life Sci 1998, 62, 2425–2430.

[23] **Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T:** Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 1999, 99, 1141–1146.

[24] **Cooke JP:** Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol 2000, 20, 2032–2037.

[25] **Boger RH, Bode-Boger SM, Sydow K, Heistad DD, Lentz SR:** Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia. Arterioscler Thromb Vasc Biol 2000, 20, 1557–1564.

[26] **Boger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Boger SM:** LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. Circ Res 2000, 87, 99–105.

[27] **Yu XJ, Li YJ, Xiong Y:** Increase of an endogenous inhibitor of nitric oxide synthesis in serum of high cholesterol fed rabbits. Life Sci 1994, 54, 753–758.

[28] **Zoccali C, Mallamaci F, Maas R, Benedetto FA, Tripepi G, Malatino LS, Cataliotti A, Bellanuova I, Boger R, CREED Investigators:** Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine in hemodialysis patients. Kidney Int 2002, 62, 339–345.

[29] **Schnabel R, Blankenberg S, Lubos E, Lackner KJ, Rupprecht HJ, Espinola-Klein C, Jachmann N, Post F, Peetz D, Bickel C, Cambien F, Tiret L, Munzel T:** Asymmetric dimethylarginine and the risk of cardiovascular Events and death in patients with coronary artery disease: results from the AtheroGene Study. Circ Res 2005, 97, e53–e59.

[30] **Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC:** Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. Eur Heart J 2003, 24, 1912–1919.

[31] **Wanby P, Teerlink T, Brudin L, Brattström L, Nilsson I, Palmqvist P, Carlsson M:** Asymmetric dimethylarginine (ADMA) as a risk marker for stroke and TIA in a Swedish population. Atherosclerosis 2006, 185, 271–277.

[32] **Wang J, Sim AS, Wang XL, Salonikas C, Naidoo D, Wilcken DE:** Relations between plasma asymmetric dimethylarginine (ADMA) and risk factors for coronary disease. Atherosclerosis 2006, 184, 383–388.

[33] **Nijveldt RJ, Teerlink T, van der Hoven B, Siroen MP, Kuik DJ, Rauwerda JA, van Leeuwen PA:** Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. Clin Nutr 2003, 22, 23–30.

[34] **Moncada S, Palmer RM, Higgs EA:** Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991, 43, 109–142.

[35] **Lluch P, Torondel B, Medina P, Segarra G, Del Olmo JA, Serra MA, Rodrigo JM:** Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. J Hepatol 2004, 41, 55–59.

[36] **Martin-Sanz P, Olmedilla L, Dulin E, Casado M, Callejas NA, Perez-Pena J, Garutti I, Sanz J, Calleja J, Barrigon S, Bosca L:** Presence of methylated arginine derivatives in orthotopic human liver transplantation: relevance for liver function. Liver Transpl 2003, 9, 40–48.

[37] **Abe T, Tohgi H, Murata T, Isobe C, Sato C:** Reduction in asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in the cerebrospinal fluid during aging and in patients with Alzheimer's disease. Neurosci Lett 2001, 312, 177–179.

[38] **Vallance P, Leiper J:** Blocking NO synthesis: how, where and why? Nat Rev Drug Discov 2002, 1, 939–950.

[39] **Szuba A, Chachaj A, Wróbel T, Dzietczenia J, Mazur G, Antonowicz-Juchniewicz J, Kuliczkowski K, Andrzejak R:** Asymmetric dimethylarginine in hematological malignancies: a preliminary study. Leuk Lymphoma 2008, 49, 2316–2320.

[40] **Vallance P, Leiper J:** Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004, 24, 1023–1030.

[41] **Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP:** Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 1999, 99, 3092–3095.

[42] **Leiper J, Vallance P:** The synthesis and metabolism of asymmetric dimethylarginine (ADMA). Eur J Clin Pharmacol 2006, 62 (Suppl 13), 33–38.

[43] **Young VR, Alexis SD, Baliga BS, Munro HN, Muecke W:** Metabolism of administered 3-methylhistidine. Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivative. J Biol Chem 1972, 247, 3592–3600.

[44] **Inoue R, Miyake M, Kanazawa A, Sato M, Kakimoteto Y:** Decrease of 3-methylhistidine and increase of NG,NG-dimethylarginine in the urine of patients with muscular dystrophy. Metabolism 1979, 28, 801–804.

[45] **Millward DJ:** Protein turnover in skeletal muscle. II. The effect of starvation and a protein-free diet on the synthesis and catabolism of skeletal muscle proteins in comparison to liver. Clin Sci 1970, 39, 591–603.

[46] **Goldberg AL, St John AC:** Intracellular protein degradation in mammalian and bacterial cells: Part 2. Annu Rev Biochem 1976, 45, 747–803.

[47] **Dice JF, Walker CD:** Protein degradation in metabolic and nutritional disorders. Ciba Found Symp 1979, 75, 331–350.

[48] **Parag HA, Raboy B, Kulka RG:** Effect of heat shock on protein degradation in mammalian cells: involvement of the ubiquitin system. EMBO J 1987, 6, 55–61.

[49] **Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP:** Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 2002, 106, 987–992.

[50] **Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP:** Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 2001, 104, 2569–2575.

[51] **Wu G, Morris SM Jr:** Arginine metabolism: nitric oxide and beyond. Biochem J 1998, 336, 1–17.

[52] **Ogawa T, Kimoto M, Sasaoka K:** Dimethylarginine:pyruvate aminotransferase in rats. Purification, properties, and identity with alanine:glyoxylate aminotransferase 2. J Biol Chem 1990, 265, 20938–20945.

[53] **Bogle RG, MacAllister RJ, Whitley GS, Vallance P:** Induction of N^G-monomethyl-L-arginine uptake: a mechanism for differential inhibition of NO synthases? Am J Physiol 1995, 269, C750–C756.

[54] **McDonald KK, Zharikov S, Block ER, Kilberg MS:** A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric oxide synthase may explain the "arginine paradox". J Biol Chem 1997, 272, 31213–31216.

[55] **Malandro MS, Kilberg MS:** Molecular biology of mammalian amino acid transporters. Annu Rev Biochem 1996, 65, 305–336.

[56] **Hattori Y, Kasai K, Gross SS:** Cationic amino acid transporter gene expression in cultured vascular smooth muscle cells and in rats. Am J Physiol 1999, 276, H2020–H2028.

[57] **Nijveldt RJ, Teerlink T, Siroen MP, van Lambalgen AA, Rauwerda JA, van Leeuwen PA:** The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). Clin Nutr 2003, 22, 17–22.
[58] **Nijveldt RJ, Siroen MP, Teerlink T, van Lambalgen AA, Rauwerda JA, van Leeuwen PA:** Gut and liver handling of asymmetric and symmetric dimethylarginine in the rat under basal conditions and during endotoxemia. Liver Int 2004, 24, 510–518.
[59] **Carnegie PR, Fellows FC, Symington GR:** Urinary excretion of methylarginine in human disease. Metabolism 1977, 26, 531–537.
[60] **Nijveldt RJ, Teerlink T, Siroen MP, van der Hoven B, Prins HA, Wiezer MJ, Meijer C, van der Sijp JR, Cuesta MA, Meijer S, van Leeuwen PA:** Elevation of asymmetric dimethylarginine (ADMA) in patients developing hepatic failure after major hepatectomy. JPEN J Parenter Enteral Nutr 2004, 28, 382–387.
[61] **Ogawa T, Kimoto M, Watanabe H, Sasaoka K:** Metabolism of NG,NG-and NG,N'G-dimethylarginine in rats. Arch Biochem Biophys 1987, 252, 526–537.
[62] **Kielstein JT, Boger RH, Bode-Boger SM, Schaffer J, Barbey M, Koch KM, Frolich JC:** Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. J Am Soc Nephrol 1999, 10, 594–600.
[63] **Ogawa T, Kimoto M, Sasaoka K:** Occurrence of a new enzyme catalyzing the direct conversion of NG, NG-dimethyl-L-arginine to L-citrulline in rats. Biochem Biophys Res Commun 1987, 148, 671–677.
[64] **McDermott JR:** Studies on the catabolism of NG-methylarginine, NG, NG-dimethylarginine and NG, NG-dimethylarginine in the rabbit. Biochem J 1976, 154, 179–184.
[65] **Schwartz S, Clare R, Devereux K, Sheung CF:** Pharmacokinetics, disposition and metabolism of 546C88 (L-N G3-methylarginine hydrochloride) in rat and dog. Xenobiotica 1997, 27, 1259–1271.
[66] **Achan V, Tran CT, Arrigoni F, Whitley GS, Leiper JM, Vallance P:** All trans retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylarginine dimethylaminohydrolase. Circ Res 2002, 90, 764–769.
[67] **Scalera F, Borlak J, Beckmann B, Martens-Lobenhoffer J, Thum T, Tager M, Bode-Boger SM:** Endogenous nitric oxide synthesis inhibitor asymmetric dimethyl L-arginine accelerates endothelial cell senescence. Arterioscler Thromb Vasc Biol 2004, 24, 1816–1822.
[68] **Mishima T, Hamada T, Ui-Tei K, Takahashi F, Miyata Y, Imaki J, Suzuki H, Yamashita K:** Expression of DDAH1 in chick and rat embryos. Brain Res Dev Brain Res 2004, 148, 223–232.
[69] **Murray-Rust J, Leiper J, McAlister M, Phelan J, Tilley S, Santa Maria J, Vallance P, McDonald N:** Structural insights into the hydrolysis of cellular nitric oxide synthase inhibitors by dimethylarginine dimethylaminohydrolase. Nat Struct Biol 2001, 8, 679–683.
[70] **Leiper J, Santa Maria J, Chubb A, MacAllister RJ, Charles IG, Whitley GS, Vallance P:** Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem J 1999, 343, 209–214.
[71] **Palm F, Onozato ML, Luo Z, Wilcox CS:** Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems. Am J Physiol Heart Circ Physiol 2007, 293, H3227–H3245.
[72] **Frey D, Braun O, Briand C, Vasak M, Grutter MG:** Structure of the mammalian NOS regulator dimethylarginine dimethylaminohydrolase: A basis for the design of specific inhibitors. Structure 2006, 14, 901–911.
[73] **Leiper J, Murray-Rust J, McDonald N, Vallance P:** S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. Proc Natl Acad Sci USA 2002, 99, 13527–13532.
[74] **Knipp M, Charnock JM, Garner CD, Vasak M:** Structural and functional characterization of the Zn(II) site in dimethylargininase-1 (DDAH-1) from bovine brain. Zn(II) release activates DDAH-1. J Biol Chem 2001, 276, 40449–40456.
[75] **Birdsey GM, Leiper JM, Vallance P:** Intracellular localization of dimethylarginine dimethylaminohydrolase overexpressed in an endothelial cell line. Acta Physiol Scand 2000, 168, 73–79.
[76] **Tran CT, Fox MF, Vallance P, Leiper JM:** Chromosomal localization, gene structure, and expression pattern of DDAH1: comparison with DDAH2 and implications for evolutionary origins. Genomics 2000, 68, 101–105.
[77] **Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S, Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, Stidwill R, McDonald NQ, Vallance P:** Disruption of methylarginine metabolism impairs vascular homeostasis. Nat Med 2007, 13, 198–203.
[78] **Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, Vallance P:** Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003, 23, 1455–1459.
[79] **Arrigoni FI, Vallance P, Haworth SH, Leiper JM:** Metabolism of asymmetric dimethylarginines is regulated in the lung developmentally and with pulmonary hypertension induced by hypobaric hypoxia. Circulation 2003, 107, 1195–1201.
[80] **Tanaka M, Osanai T, Murakami R, Sasaki S, Tomita H, Maeda N, Satoh K, Magota K, Okumura K:** Effect of vasoconstrictor coupling factor 6 on gene expression profile in human vascular endothelial cells: enhanced release of asymmetric dimethylarginine. J Hypertens 2006, 24, 489–497.
[81] **Xin HY, Jiang DJ, Jia SJ, Song K, Wang GP, Li YJ, Chen FP:** Regulation by DDAH/ADMA pathway of lipopolysaccharide-induced tissue factor expression in endothelial cells. Thromb Haemost 2007, 97, 830–838.

[82] **Sorrenti V, Mazza F, Campisi A, Vanella L, Li Volti G, Di Giacomo C:** High glucose-mediated imbalance of nitric oxide synthase and dimethylarginine dimethylaminohydrolase expression in endothelial cells. Curr Neurovasc Res 2006, 3, 49–54.

[83] **Chobanyan K, Thum T, Suchy MT, Zhu B, Mitschke A, Gutzki FM, Beckmann B, Stichtenoth DO, Tsikas D:** GC-MS assay for hepatic DDAH activity in diabetic and non-diabetic rats by measuring dimethylamine (DMA) formed from asymmetric dimethylarginine (ADMA): evaluation of the importance of S-nitrosothiols as inhibitors of DDAH activity *in vitro* and *in vivo* in humans. J Chromatogr B Analyt Technol Biomed Life Sci 2007, 858, 32–41.

[84] **Xiong Yan, Fu YF, Fu SH, Zhou HH:** Elevated levels of the serum endogenous inhibitor of nitric oxide synthase and metabolic control in rats with streptozotocin-induced diabetes. J Cardiovasc Pharmacol 2003, 42, 191–196.

[85] **Lin PS, Boger RH, Bode-Boger SM, Tsao PS, Cooke JP:** Asymmetric dimethylarginine enhances superoxide production in human endothelial cells. Circulation 1997, 96 (suppl.), I-45 (abs.).

[86] **Jacobi J, Sydow K, von Degenfeld G, Zhang Y, Dayoub H, Wang B, Patterson AJ, Kimoto M, Blau HM, Cooke JP:** Overexpression of dimethylarginine dimethylaminohydrolase reduces tissue asymmetric dimethylarginine levels and enhances angiogenesis. Circulation 2005, 111, 1431–1438.

[87] **Wakino S, Hayashi K, Tatematsu S, Hasegawa K, Takamatsu I, Kanda T, Homma K, Yoshioka K, Sugano N, Saruta T:** Pioglitazone lowers systemic asymmetric dimethylarginine by inducing dimethylarginine dimethylaminohydrolase in rats. Hypertens Res 2005, 28, 255–262.

[88] **Monsalve E, Oviedo PJ, Garcia-Perez MA, Tarin JJ, Cano A, Hermenegildo C:** Estradiol counteracts oxidized LDL-induced asymmetric dimethylarginine production by cultured human endothelial cells. Cardiovasc Res 2007, 73, 66–72.

[89] **Boota A, Zar H, Kim YM, Johnson B, Pitt B, Davies P:** IL-1 beta stimulates superoxide and delayed peroxynitrite production by pulmonary vascular smooth muscle cells. Am J Physiol 1996, 271, L932–L938.

[90] **Buttery LD, Springall DR, Chester AH, Evans TJ, Stanfield EN, Parums DV, Yacoub MH, Polak JM:** Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. Lab Invest 1996, 75, 77–85.

[91] **Kostourou V, Robinson SP, Cartwright JE, Whitley GS:** Dimethylarginine dimethylaminohydrolase I enhances tumour growth and angiogenesis. Br J Cancer 2002, 87, 673–680.

[92] **Shah V:** Cellular and molecular basis of portal hypertension. Clin Liver Dis 2001, 5, 629–644.

[93] **Rockey DC, Chung JJ:** Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: endothelial dysfunction in portal hypertension. Gastroenterology 1998, 114, 344–351.

[94] **Langer DA, Shah VA:** A gas, an amino acid, and an imposter: the story of nitric oxide, L-arginine, and ADMA in portal hypertension. Hepatology 2005, 42, 1255–1257.

[95] **Mookerjee RP, Davies NA, Sen S, Hodges SJ, Turner C, Dalton RN, Williams R et al:** Decreased hepatic extraction of asymmetric and symmetric dimethylarginine (ADMA/SDMA-NOSinhibitors) in acute alcoholic hepatitis may contribute to impaired end-organ function. Hepatology 2005, 42, 419A. Abstract 566.

[96] **Laleman W, Omasta A, Van de Casteele M, Zeegers M, Vander Elst I, Van Landeghem L, Severi T, van Pelt J, Roskams T, Fevery J, Nevens F:** A role for asymmetric dimethylarginine in the pathophysiology of portal hypertension in rats with biliary cirrhosis. Hepatology 2005, 42, 1382–1390.

[97] **Shah V, Toruner M, Haddad F, Cadelina G, Papapetropoulos A, Choo K, Sessa WC, Groszmann RJ:** Impaired endothelial nitric oxide synthase activity associated with enhanced caveolin binding in experimental cirrhosis in the rat. Gastroenterology 1999, 117, 1222–1228.

[98] **Perez MJ, Macias RI, Duran C, Monte MJ, Gonzalez-Buitrago JM, Marin JJ:** Oxidative stress and apoptosis in fetal rat liver induced by maternal cholestasis. Protective effect of ursodeoxycholic acid. J Hepatol 2005, 43, 324–332.

[99] **Van de Casteele M, Omasta A, Janssens S, Roskams T, Desmet V, Nevens F, Fevery J et al.:** *In vivo* gene transfer of endothelial nitric oxide synthase decreases portal pressure in anaesthetised carbon tetrachloride cirrhotic rats. Gut 2002, 51, 440–445.

[100] **Cobreros A, Sainz L, Lasheras B, Cenarruzabeitia E:** Hepatotoxicity of ethanol: protective effect of calcium channel blockers in isolated hepatocytes. Liver 1997, 17, 76–82.

[101] **Fan C, Zwacka RM, Engelhardt JF:** Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med 1999, 77, 577–592.

[102] **Liu P, Yin K, Nagele R, Wong PY:** Inhibition of nitric oxide synthase attenuates peroxynitrite generation, but augments neutrophil accumulation in hepatic ischemia-reperfusion in rats. J Pharmacol Exp Ther 1998, 284, 1139–1146.

[103] **Peralta C, Rull R, Rimola A, Deulofeu R, Roselló-Catafau J, Gelpí E, Rodés J:** Endogenous nitric oxide and exogenous nitric oxide supplementation in hepatic ischemia-reperfusion injury in the rat. Transplantation 2001, 71, 529–536.

[104] **Shah V, Haddad FG, Garcia-Cardena G, Frangos JA, Mennone A, Groszmann RJ, Sessa WC:** Liver sinusoidal endothelial cells are responsible for nitric oxide modulation of resistance in the hepatic sinusoids. J Clin Invest 1997, 100, 2923–2930.

[105] **Simonsen U, Rodriguez-Rodriguez R, Dalsgaard T, Buus NH, Stankevicius E:** Novel approaches to improving endothelium-dependent nitric oxide-mediated vasodilatation. Pharmacol Rep 2009, 61, 105–115.

[106] **Barker JE, Knight KR, Romeo R, Hurley JV, Morrison WA, Stewart AG:** Targeted disruption of the nitric oxide synthase 2 gene protects against ischaemia/reperfusion injury to skeletal muscle. J Pathol 2001, 194, 109–115.

[107] **Shiraishi M, Hiroyasu S, Nagahama M, Miyaguni T, Higa T, Tomori H, Okuhama Y, Kusano T, Muto Y:** Role of exogenous L-arginine in hepatic ischemia-reperfusion injury. J Surg Res 1997, 69, 429–434.
[108] **Martin-Sanz P, Bosca L, Olmedilla L, Perez-Pena J, Garutti I, Sanz J, Calleja J, Avellandal M, Ortega A, Aleixandre A, Barrigon S:** Presence of a nitric oxide synthase inhibitor in the graft efflux during reperfusion in human liver transplantation. Clin Transplant 1999, 13, 221–230.
[109] **Schemmer P, Bunzendahl H, Herfarth C, Klar E, Thurman RG:** Arginine-specific proteolytic activity predicts graft survival. Transplant Proc 2001, 33, 833–834.
[110] **Aggarwal S, Kang Y, Freeman JA, Fortunato FL Jr, Pinsky MR:** Postreperfusion syndrome: Hypotension after reperfusion of the transplanted liver. J Crit Care 1993, 8, 154–160.
[111] **Bennett-Guerrero E, Feierman DE, Barclay GR, Parides MK, Sheiner PA, Mythen MG, Levine DM, Parker TS, Carroll SF, White ML, Winfree WJ:** Preoperative and intraoperative predictors of postoperative morbidity, poor graft function, and early rejection in 190 patients undergoing liver transplantation. Arch Surg 2001, 136, 1177–1183.
[112] **Zoccali C, Bode-Boger SM, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger RH:** Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001, 358, 2113–2117.
[113] **Zatz R, De Nucci G:** Effects of acute nitric oxide inhibition on rat glomerular microcirculation. Am J Physiol 1991, 261, F360–F36.

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