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Pharmacotherapies and their influence on asymmetric dimethylarginine (ADMA)

Renke Maas

Abstract: Elevated plasma concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) are found in various clinical settings, including renal failure, coronary heart disease, hypertension, diabetes and pre-eclampsia. In healthy people acute infusion of ADMA promotes vascular dysfunction, and in mice chronic infusion of ADMA promotes progression of atherosclerosis. Thus, ADMA may not only be a marker but also an active player in cardiovascular disease, which makes it a potential target for therapeutic interventions. This review provides a summary and critical discussion of the presently available data concerning the effects on plasma ADMA levels of cardiovascular drugs, hypoglycemic agents, hormone replacement therapy, antioxidants, and vitamin supplementation. We assess the evidence that the beneficial effects of drug therapies on vascular function can be attributed to modification of ADMA levels. To develop more specific ADMA-lowering therapies, mechanisms leading to elevation of plasma ADMA concentrations in cardiovascular disease need to be better understood. ADMA is formed endogenously by degradation of proteins containing arginine residues that have been methylated by *S*-adenosylmethionine-dependent methyltransferases (PRMTs). There are two major routes of elimination: renal excretion and enzymatic degradation by the dimethylarginine dimethylaminohydrolases (DDAH-1 and -2). Oxidative stress causing upregulation of PRMT expression and/or attenuation of DDAH activity has been suggested as a mechanism and possible drug target in clinical conditions associated with elevation of ADMA. As impairment of DDAH activity or capacity is associated with substantial increases in plasma ADMA concentrations, DDAH is likely to emerge as a prime target for specific therapeutic interventions.

Key words: asymmetric dimethylarginine (ADMA); cardiovascular disease; DDAH; drug therapy; nitric oxide synthase inhibitor

Introduction

The L-arginine–nitric oxide pathway is involved in the regulation of diverse physiological functions including endothelium-dependent vasodilation, platelet aggregation, neurotransmission, immune defense and apoptosis.¹ Accumulating evidence links asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), to human disease.² Elevated plasma ADMA concentrations are found in various clinical settings ranging from renal failure and liver failure to atherosclerosis, hypertension, diabetes and pre-eclampsia.^{3–11} Moreover, in patients

with cardiovascular disease, end-stage renal disease, or critical illness, elevated plasma ADMA concentrations independently predict progression of atherosclerosis, future vascular events and/or overall mortality.^{12–15}

Several lines of evidence suggest that ADMA, being a NOS inhibitor, may not only be a marker of disease but a player. In human volunteers, infusion of ADMA has been shown impair to endothelium-dependent vasodilation, increase systemic and renal vascular resistance, and decrease cardiac output.^{3,16–18} These data suggest that chronic elevation of ADMA may directly promote vascular disease, which makes ADMA a promising new drug target. Studying the effects of commonly used drugs on plasma ADMA levels may be a first step towards gaining some insights into the mechanisms leading to elevated plasma ADMA concentrations. As ADMA may be both a marker and a player in cardiovascular disease it may therefore be difficult to discern indirect drug effects on ADMA levels related to improvement of

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the underlying condition from direct drug effects (such as drug-induced augmentation of dimethylarginine dimethylaminohydrolase (DDAH) activity).

It is the purpose of this review to evaluate possible mechanisms leading to the elevation of ADMA plasma concentration and critically to review first in vitro and in vivo data concerning potential ADMA-lowering therapies.

Formation and elimination of ADMA: mechanisms and targets for therapeutic interventions

Asymmetric dimethylation of L-arginine residues in proteins

The initial step in the endogenous formation of ADMA (Figure 1) is the asymmetric dimethylation of L-arginine residues of various cellular proteins

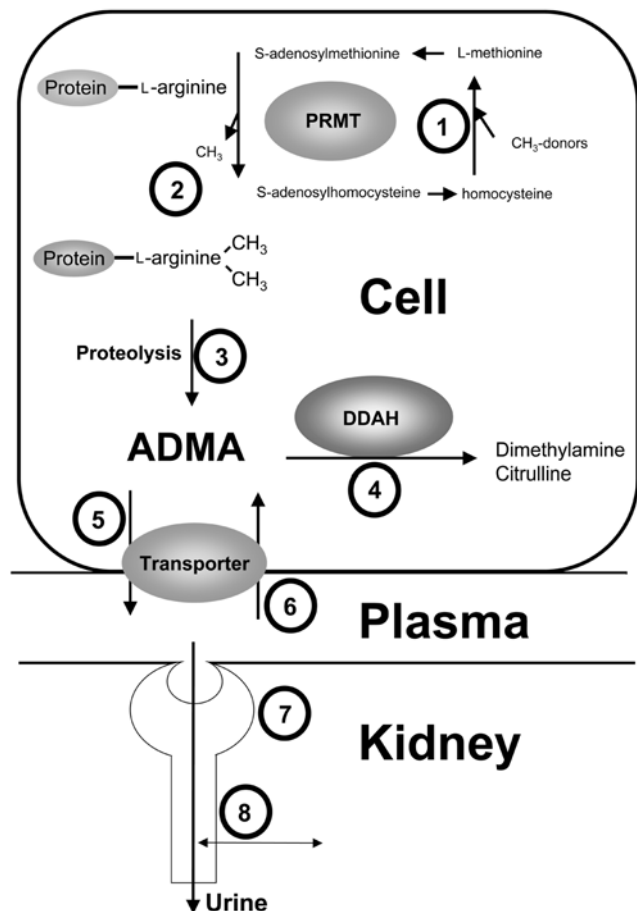


Figure 1 Formation and elimination of ADMA: (1) *S*-adenosylmethionine is required as a source of methyl groups; (2) asymmetric methylation of L-arginine residues in proteins by type I protein-arginine methyltransferases (PRMTs); (3) proteolysis leads to liberation of ADMA; (4) degradation of ADMA by dimethylarginine dimethylaminohydrolase (DDAH); (5) carrier-mediated excretion of ADMA; (6) carrier-mediated uptake of ADMA; (7) renal filtration of ADMA; (8) carrier-mediated secretion and re-uptake of ADMA.

by specific protein L-arginine methyltransferases (PRMTs).^{19,20} Type 1 PRMTs are considered the predominant enzymes responsible for asymmetrical dimethylation,²⁰ and *S*-adenosylmethionine (SAM) derived from L-methionine appears to be the principal methyl group donor for this reaction.²¹ Moreover, elevation of SAM promotes PRMT activity and subsequent formation of ADMA. The product of this methylation reaction, *S*-adenosylhomocysteine (which inhibits PRMT activity) is hydrolysed to L-homocysteine, which, in turn, can be remethylated by methionine synthase to L-methionine.²² This reaction, which closes the methylation cycle, depends on vitamin B₁₂ and 5-methyltetrahydrofolic acid as methyl group donors. In principle, each step of this methylation–remethylation cycle constitutes a potential target for therapeutic approaches to lower SAM levels and thus reduce substrate availability for asymmetric dimethylation of L-arginine residues.

Practical and clinical considerations, however, make formation of SAM and PRMTs unlikely targets. First, post-translational methylation of L-arginine residues is considered a universal biological process affecting hundreds of proteins.¹⁹ In most cases the effects of this methylation are still unknown. Thus, interference with this mechanism may have unforeseen consequences. Secondly, based upon their role in the homocysteine–methionine cycle, one would expect folate and vitamin B₁₂ supplementation to increase synthesis of SAM and subsequent ADMA formation. However, it was shown in patients with increased plasma homocysteine levels that folate lowered elevated plasma ADMA concentrations.²³ In this study, which unfortunately lacked a placebo group, the beneficial effect of folate on ADMA levels was attributed to the reduction of homocysteine by folate. In contrast, in another study, supplementation with folate and B vitamins had no impact on ADMA levels.²⁴

Proteolysis and formation of ADMA

When proteins are degraded, asymmetrically dimethylated L-arginine residues are released as ADMA. Considering the wide variety of asymmetrically dimethylated proteins it is very unlikely that sufficiently specific inhibitors of the proteasome involved in degrading these proteins can be found. Moreover, specific or nonspecific inhibition of protein degradation could lead to accumulation of these proteins or peptide fragments, which potentially could cause a form of storage disease. Hence, proteolysis is unlikely to present a suitable drug target for reducing ADMA levels.

Specific metabolism of ADMA by DDAH

ADMA is selectively degraded by DDAH to citrulline and dimethylamine.²⁵ In humans there are two major isoforms of DDAH (DDAH-1 and DDAH-2) with

Table 1 Major sites of ADMA elimination.

	Kidney	Liver	Endothelium
Predominant DDAH isoform	DDAH-1>2	DDAH-1>>2	DDAH-2>>>1
Organ weight (kg)	0.3 (0.15 × 2)	1.5	~1.5
Increase of ADMA in organ failure	Yes 1.5–10×	Yes 6–12×	Likely but causality not established
Net organ extraction (%)	16 (arterial-venous)	~30 ^a	?

^aEstimate based on rat data.

partly overlapping tissue distributions²⁶ in kidney, liver and small intestine (Table 1). Presently, it is not known what percentage of the endogenously formed ADMA is directly metabolized in the cells where it is formed and how much is released from the cells into other tissues to be metabolized or excreted. It is also not known which DDAH isoform or which organ dominates ADMA degradation in humans in vivo. Rising plasma concentrations in renal³ or liver failure⁴ strongly suggest, however, that at least some tissues release a substantial fraction of the endogenously formed ADMA to be metabolized or excreted elsewhere. With an estimated 'organ weight' similar to that of the liver, the endothelium could be another major site of ADMA metabolism (Table 1).²⁷ Current estimates of the relative expression of the two isoforms are mainly based on mRNA data. DDAH-1 expression tends to dominate in tissues with a high expression of the neuronal isoform of NOS (nNOS), whereas DDAH-2 expression seems to follow the expression pattern of endothelial NOS isoform (eNOS). Analysis of the DDAH-2 gene and its promoter regions revealed potential binding sites for regulators of transcription (STAT, PPAR/RXR, NF-κB and IRF-1) as well as features consistent with the function of a housekeeping gene (absence of canonical TATA boxes and a CpG island around the transcription start sites). Specific activators of DDAH expression are not yet known, but activation of DDAH expression is of course an interesting drug target.

In cell culture all-*trans*-retinoic acid increased DDAH-2 expression.²⁸ Regulation of DDAH-1 expression is even less well understood. In smooth muscle cells interleukin-1 beta upregulates DDAH-1 expression at the protein level.²⁹ DDAH contains four highly redox sensitive cysteine groups, which appear to be crucial for its catalytic function.^{25,30} DDAH activity may also be regulated by *S*-nitrosylation of a reactive cysteine residue (Cys-249),³⁰ providing some form of an indirect negative feedback, especially for inducible NOS (iNOS)-derived nitric oxide (NO).

Therapeutic augmentation of DDAH expression or activity: a word of caution

Although increased NO synthesis due to upregulation of DDAH expression or activity is usually desired in cardiovascular disease, it may have catastrophic

effects when occurring in tumors (by promoting NO-mediated angiogenesis). Indeed, recent studies suggest that DDAH activity is increased in human tumors.³¹ Moreover, overexpression of DDAH-1 or -2 enhances tumor growth and tumor angiogenesis.³²

Transport and exchange of ADMA

When assessing drug effects on ADMA levels, a major drawback of all available clinical data has to be considered. We currently measure the plasma concentration, which may represent only some form of 'cellular spillover'. Little is known regarding the correlation of plasma ADMA and intracellular ADMA concentrations in vivo. Thus, even a normal or normalized plasma ADMA concentration may not completely exclude severe derangement of intracellular ADMA concentrations by disease or drugs.

Given its structural similarities to L-arginine, the cationic amino acid ADMA most likely shares carrier systems with L-arginine to leave or enter cells.³³ Indeed, early in vitro data suggest that transport of ADMA may at least in part be mediated by γ carriers.³⁴ Clinical data regarding the precise transport mechanisms for ADMA and their regulation in vivo are not currently available.

Renal filtration and excretion of ADMA

Elevated plasma levels of ADMA were first reported in patients with chronic renal failure by Vallance and co-workers.³ We now know that the combination of renal filtration, tubular secretion, tubular reabsorption and degradation by renal DDAH result in a net renal extraction of about 16% of circulating ADMA.³⁵ Presently it is difficult, however, to estimate the relative contribution of each mechanism. It is interesting that symmetric dimethylarginine, which is chemically very similar to ADMA but not metabolized by DDAH, has a renal extraction rate of only 10%. This difference may be interpreted as evidence for a substantial contribution of renal DDAH activity to the renal extraction of ADMA.

Exogenous sources of ADMA

Protein-L-arginine methylation is a universal biological principle found in bacteria as well as in plants and animals.³⁶ It is therefore very likely that free and protein-incorporated ADMA³⁷ is also taken in with the

diet, modulated by dietary components, or formed by resident colonic bacteria. This offers several further targets for ADMA-lowering therapies that have not yet been investigated.

Major disease mechanisms leading to elevation of ADMA in human disease

Shear stress

In vitro, shear stress may have divergent effects on ADMA.³⁸ Moderate shear stress was found to upregulate PRMT1 expression while DDAH activity was unchanged, resulting in enhanced cellular ADMA release. In contrast, when cells were exposed to a higher shear stress, PRMT1 and DDAH activity both increased, resulting in normalization of ADMA levels.

High blood glucose and impaired glucose tolerance

Elevation of plasma ADMA levels have been observed in a rat model of diabetes, and DDAH activity was found to be significantly impaired in human cells exposed to high concentrations of glucose.³⁹ Since it was shown that impairment of DDAH activity could be alleviated by polyethylene glycol-conjugated superoxide dismutase, impairment of DDAH activity by glucose-induced oxidative stress was suggested as the major underlying mechanism. In humans the situation appears to be more complex. In volunteers without diabetes, plasma ADMA levels were found to be correlated with individual insulin sensitivity but not with blood glucose level.¹⁰ Clinical data concerning plasma ADMA levels in patients with diabetes are somewhat conflicting: some studies have shown an elevation of ADMA⁴⁰ while others demonstrated a reduction.⁴¹

Oxidative stress

Several independent lines of evidence indicate that impairment of DDAH activity by oxidative stress is the final mechanism leading to elevation of ADMA in diverse clinical conditions.⁴² DDAH contains four highly redox sensitive cysteine groups, which appear to be crucial for its catalytic function.^{25,30}

Oxidized low-density lipoprotein (oxLDL) but not native LDL was found to decrease DDAH activity⁴³ and increase cellular ADMA liberation. In cell culture, oxLDL and high concentrations of native LDL lead to upregulation of PRMTs and cellular ADMA synthesis that could be prevented by antioxidants.⁴⁴

Unfortunately, impairment of DDAH activity by oxidative stress or upregulation of PRMTs may not be easy to overcome in humans. A Finnish study found no association between plasma ADMA levels and dietary vitamin E or C intake.⁴⁵ In an open label study, 800 IU vitamin E over 6 weeks had no significant effect on plasma ADMA.⁴⁶ Moreover, in a very recent

clinical trial *N*-acetyl-cysteine had no effect on plasma ADMA levels.⁴⁷

Hyperhomocysteinemia

In animals⁴⁸ and humans^{49,50} experimental hyperhomocysteinemia is associated with elevation of plasma ADMA. Two (not necessarily exclusive) mechanisms have been proposed to explain this finding:

1. Homocysteine may be remethylated to form L-methionine, which, in turn, serves as a methyl group donor for the methylation of L-arginine.²² Increased ADMA release by endothelial cells under high concentrations of methionine or homocysteine was reduced by the methylation inhibitor *S*-adenosylmethionine.⁴⁴ In monkeys, dietary supplementation with B vitamins normalized homocysteine but did not alter ADMA levels or improve endothelial function.⁴⁸
2. Homocysteine may also directly interact with the sulfhydryl groups of DDAH. In an in vitro binding assay, biotinylated homocysteine was found to bind directly to DDAH-1.⁵¹ This binding could be prevented by an antioxidant.

Drug therapy and ADMA: early clinical data

Statin therapy

Statins are among the most effective drugs for lowering cardiovascular risk associated with elevation of LDL cholesterol. Several lines of evidence suggest that the beneficial effects of statins are at least in part mediated by LDL-independent (pleiotropic) mechanisms such as improvement of endothelial function by increasing the bioavailability of NO. In animal experiments as well as in patients a high LDL cholesterol level was found to be associated with elevated plasma ADMA concentrations.^{48,52} Even though this finding does not seem to be found consistently in all patient populations,⁵³ several investigators have tested the hypothesis that statin treatment may improve vascular function by reducing plasma ADMA (Table 2). In the majority of studies, statin treatment had no impact on plasma ADMA levels.^{54–56} This would clearly argue against a direct connection between ADMA and LDL cholesterol in humans. Only rosuvastatin was shown to lower ADMA⁵⁷ but, given the negative results for the other statins, this finding remains to be replicated in other populations.

Irrespective of the potential effects of statins on ADMA, recent studies suggest that ADMA may modulate the effect of statins on vascular function.⁵⁸

Angiotensin converting enzyme inhibitors and angiotensin receptor blockers

In various conditions such as hypertension, diabetes and coronary artery disease, angiotensin converting

Table 2 Effect of statin therapy on plasma ADMA levels.

Drug (ref.)	Duration (weeks)	n	% change	p
Pravastatin 40 mg/d (Eid et al 2003 ⁵⁴)	8	32	-9	ns
Pravastatin 40 mg/d (Janatuinen et al 2001 ⁵⁵)	26	21	No change	ns
Simvastatin 80 mg/d (Päivä et al 2003 ⁵³)	8	24	No change	ns
Atorvastatin 40 mg/d (Päivä et al 2003 ⁵³)	8	24	No change	ns
Simvastatin 20 mg/d (Pereira et al 2003 ⁵⁶)	8	25	No change	ns
Rosuvastatin 10 mg/d (Lu et al 2004 ⁵⁷)	6	23	-18	<0.001

ns, not significant.

enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) were found to improve endothelial function and NO availability.^{59–61} Several studies have assessed the effect of ACE inhibitors or ARBs on plasma ADMA levels in these patient groups (Table 3).^{62–66} In patients with hypertension, diabetes or syndrome X, the reduction in plasma ADMA achieved by ACE inhibition was in the range of 12–20%, while plasma L-arginine levels did not seem to be affected.⁶³ ARBs were found to reduce plasma ADMA by 10–16%.^{62,66} All of these studies were small and had rather short observation periods of 1–12 weeks. Thus, confirmation of these promising results by larger trials will be required.

The results of a recent study in eNOS knockout mice suggests that ADMA may also exert NO-independent effects via upregulation of ACE and augmentation of oxidative stress through AT₁-dependent pathways.⁶⁷ Thus, ACE inhibitors and ARBs may help to prevent ADMA-induced pathology independently of their effects on plasma ADMA levels.

Hypoglycemic agents

In patients with type 2 diabetes, 3 months of treatment with 0.5–1.0 g/d metformin alone (*n* = 16) or in combination with sulfonylurea (*n* = 15) reduced plasma ADMA concentrations by 28.5% and 32.0% respectively.⁶⁸ Some caution is warranted, however, as no placebo data are available from that study. Furthermore, in contrast to what one would expect

from the *in vitro* data, no relationship was observed between the decrease in ADMA concentration and glycemic control. Thus, it is possible that the reduction of ADMA levels by metformin is mediated via glucose-independent mechanisms. In this respect it may be of interest to note that metformin has structural similarities to ADMA.⁶⁹

In an uncontrolled study the PPAR- γ agonist rosiglitazone (12 weeks of treatment with a final dose of up to 8 mg/d) improved insulin sensitivity and reduced plasma ADMA concentrations by 30% in seven insulin-resistant patients.¹⁰ These data certainly await further confirmation by larger controlled trials because, *in vitro*, neither PPAR- γ agonists (ciglitazone) nor PPAR- γ antagonists (T007) significantly altered cellular ADMA liberation.⁷⁰ Apart from the effects on blood glucose there is, however, at least support from theoretical considerations because a PPAR/PXR binding site has been identified in the promotor region of DDAH-2.²⁸

Hormone replacement therapy

Hormone replacement therapy (HRT) has frequently been shown to improve endothelial function and NO synthesis.^{71,72} Moreover, ADMA levels were found to decrease in normal pregnancy.¹¹ The precise mechanism by which estrogen reduces ADMA has remained obscure so far. The effect of various forms of HRT on ADMA plasma concentration was explored in some small randomized controlled trials (Table 4).^{73–75} In all

Table 3 Effect of ACE inhibitors and angiotensin receptor blockers on ADMA plasma concentration.

Drug (ref.)	Duration (weeks)	n	(% change)	p
Enalapril 20 mg/d (Delles et al 2002 ⁶²)	1	20	-17%	<0.05
Enalapril 10 g/d (Chen et al 2002 ⁶³)	8	10	-20%	<0.01
Enalapril 20 mg/d (Napoli et al 2004 ⁶⁴)	12	38	-16 %	<0.05
Zofenopril 15–30 mg/d (Napoli et al 2004 ⁶⁴)	12	38	-24%	<0.01
Perindopril 4 mg/d (Ito et al 2001 ⁶⁶)	4	7	-12% ^a	<0.05
Perindopril 4 mg/d (Ito et al 2002 ⁶⁵)	1	11	-16 % ^a	0.02
Eprosartan 600 mg/d (Delles et al 2002 ⁶²)	1	20	-16	<0.05
Eprosartan 300 mg/d + enalapril 20 mg/d (Delles et al 2002 ⁶²)	1	20	-18	<0.05
Losartan 50 mg/d (Ito et al 2001 ⁶⁶)	4	7	-10	<0.05

^aData extracted from graphs in publication.

Table 4 Effect of hormone therapy on ADMA plasma concentration.

Drug and dose (ref.)	Duration (weeks)	ADMA (% change; $\mu\text{mol/l}$)	<i>p</i>	L-arginine (% change; $\mu\text{mol/l}$)	<i>p</i>	L-arginine/ADMA ratio (% change)	<i>p</i>	Placebo
17-beta-estradiol 2 mg/d (Post et al 2003 ⁷⁴)	12	-5.2	0.03	-7.3	0.02	-1.6	0.75	Yes
17-beta-estradiol 2 mg/d + dydrogesterone 10 mg/d (Post et al 2003 ⁷⁴)	12	-8.0	0.02	-7.9	0.14	0.3	0.83	Yes
17-beta-estradiol 2 mg/d + trimegestone 10 mg/d (Post et al 2003 ⁷⁴)	12	-21	<0.01	-36	<0.001	-19	<0.01	Yes
Conjugated equine estrogen 0.625 mg/d (Teerlink et al 2003 ⁷⁵)	104	-9.7	<0.01	No change; data not reported	-	Not reported	-	Yes
17-beta-estradiol 100 mg implant (Holden et al 2003 ⁷³)	2	-18	<0.05	Not reported	-	Not reported	-	No

-, data not cited.

of these trials ADMA levels were modestly (5–21%) reduced by 17-beta-estradiol alone or when sequentially combined with cyclogesterone or trimegestone. Unfortunately, L-arginine levels also declined, resulting in no change or even a decrease in the L-arginine/ADMA ratio. Thus, unless it can be proven that the decrease in L-arginine was the result of increased consumption in NO synthesis (and not upregulation of arginase activity), it remains questionable how much benefit can be conferred by HRT on patients with an elevated ADMA.

Conclusion and outlook

Specific ADMA-lowering therapies are not yet available. Promising results from first pilot studies with widely used drugs such as ACE-inhibitors, ARBs, hypoglycemic agents and HRT await confirmation in large placebo-controlled trials.

To develop more specific therapies, further insights into the mechanisms leading to an elevation of ADMA in vivo are needed. Tools such as high-throughput methods for the quantification of ADMA and isoform-specific DDAH inhibitors (and activators) will play a key role. Important insights may also come from the DDAH-transgenic mice⁷⁶ and DDAH1/2 knock out models currently under development. So far, most conditions associated with an elevation of ADMA are chronic, so ADMA-lowering therapies will have to be long term. Future studies will therefore have to address carefully the long-term effects of ADMA-lowering therapies on angiogenesis and tumor growth.

Until specific ADMA-lowering therapies become available, treatment of modifiable risk factors leading to elevation of ADMA or depletion of L-arginine, and

supplementation with L-arginine, may be the best available options for patients with high plasma ADMA concentrations because NO synthesis is correlated with the L-arginine/ADMA ratio.^{77,78}

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