SYMMETRICAL DIMETHYLARGININE (SDMA) AND VENOUS ULCER OF THE LOWER LIMBS

ILINCA NICOLAE*, MIRCEA TAMPA*,**, MARIA GRIGORE*, CRISTINA IULIA MITRAN**,***, MĂDĂLINA IRINA MITRAN**,***, LUCREȚIA DULGHERU*, SIMONA ROXANA GEORGESCU*,**

Summary

Post-translational modifications (methylation, phos-phorylation, glycosylation, acetylation) of proteins play an important role in controlling cellular processes. Methylation of protein-embedded arginine results in the formation of monomethyl arginine (MMA), symmetrical dimethylarginine (SDMA) and asymmetrical dimethy-larginine (ADMA). The accumulation of ADMA and MMA in cells and in the blood stream has been associated with various pathological conditions, while SDMA has been regarded as a physiologically inert compound. Today it is proven that SDMA inhibits the production of NO by blocking the cellular uptake of L-arginine. In the present study, we have shown that the level of SDMA is significantly increased in patients with venous leg ulcer (17 cases), compared to the control group (15 cases). It should be noted that this is the first study examining serum variations of SDMA in dermatological conditions. In conclusion, our data show that SDMA may contribute to the pathophysiology of venous leg ulcer.

Keywords: *venous leg ulcer, posttranslational modifications, arginine methylation, symmetrical dimethy-larginine.*

Received: 2.02.2019 *Accepted: 20.01.2020*

Introduction

Chronic venous insufficiency (IVC) affects about 2% of the general population. One in five patients with IVC develops venous ulcer, with venous ulcers accounting for 80% of the ulcers in the lower limb. Venous ulcers are most commonly located at the level of the calf (lower leg), in the medial area. From a clinical point of view, they are painless in most cases, they can be superficial or deep, with various sizes, usually with irregular edges accompanied by other changes such as oedema, stasis dermatitis. At the base of the ulcer, granulation tissue or fibrin deposits can be seen. The pathogenesis of venous ulcer of the calf is not fully understood, many factors being involved [1-3].

L-arginine is the common substrate of nitric oxide synthases (NOS, E.C.1.14.1.39) and arginases (E.C.3.5.3.1). NOS catalyses L-arginine to generate nitric oxide (NO) and L-citrulline, while arginases catalyse the conversion of L-arginine to L-ornithine and urea. Therefore, the increased activity of arginase can decrease the bioavailability of L-arginine by substrate competition and may decrease NO production, leading to endothelial dysfunction and circulatory complications [4-17]. The existence of three different NOS isoforms was highlighted: neuronal NOS (nNOS), endothelial (eNOS) and inducible NOS isoenzyme (iNOS). Another way of regulating NO production is mediated by methylated arginine: l-NG-monomethyl arginine (MMA), asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA) (Figure 1). MMA and ADMA are potent competitive endogenous inhibitors of NOS, while SDMA inhibits NO production mainly by blocking the uptake of L-arg [4-7]. Increased

* Department of Dermatology, 'Victor Babeș' Clinical Hospital for Infectious Diseases, Bucharest, Romania.
** Department of Dermatology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania.
*** 'Cantacuzino' National Medico-Military Institute for Research and Development, Bucharest, Romania.

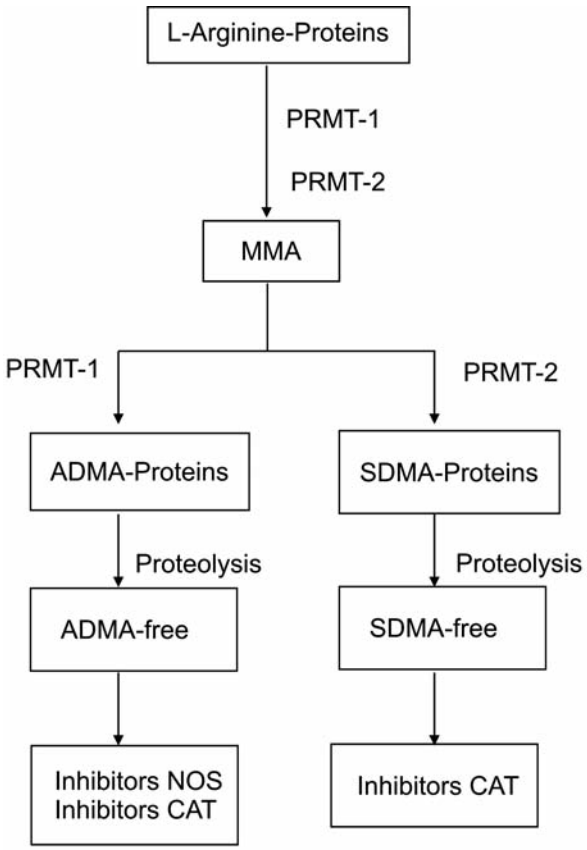

Figure 1. Posttranslational intranuclear synthesis of methylarginines catalysed by protein-arginine methyltransferases (PRMTs), adapted from Chandrasekharan et al. [7]

plasma levels of both ADMA and SDMA are associated with an increased risk of cardiovascular disease in the general population [7].

Asymmetrical dimethylarginine (ADMA) and L-NG-monomethyl arginine (MMA) are potent inhibitors of NOS, while symmetrical dimethylarginine (SDMA) is a competitive inhibitor of cationic transporters (CAT) and inhibits arginine supply to cells.

NO is involved in cellular and molecular events of wound healing, i.e. vasodilation, angiogenesis, inflammation, cell proliferation, tissue fibrosis, immune response and remodelling [17]. The importance of NO was demonstrated by delayed wound healing in animal models with genetically modified NO synthesis. NO exerts antimicrobial activity [18]. Based on the arguments presented, it can be concluded that SDMA could influence the pathogenesis of venous leg ulcer. This possible finding, which will be analysed in the present paper, may contradict the assumption that SDMA is an inert molecule [6].

Experimental part

Patient selection.

17 patients with venous leg ulcer, without metabolic syndrome and without major signs of infection were monitored. Patients with diabetes, atherosclerotic disease, rheumatoid arthritis, systemic vasculitis, renal pathology and obesity were excluded. The diagnosis of venous leg ulcer was established on clinical, paraclinical and histological criteria (when it was necessary to exclude other diagnoses), presented in the Results section.

Laboratory techniques

Blood samples were collected in the morning. Centrifugation of blood samples was done at 3000g, for 10 minutes, one hour after harvesting. Serums were separated and stored at minus 80ṁ C until used for analysis. Homolysed, jaundice, lactating, and contaminated samples were removed.

SDMA determination was performed using the competitive ELISA variant (Elabscience, USA). The method has sensitivity (0.09nmol/ml), reproducibility (95-97%), repeatability (coefficient of variation below 10%), specificity for SDMA, has no cross-reactions or interferences with other structural analogues, has a wide range of detection (0.16-10 nmol / ml), it is cheap (50 Ml quantities of reagents are used), non-irradiant, fast (takes several hours), adapted for a wide range of biological samples (serum, plasma, urine, tissue homogenate, cell lysates). The technique uses a primary antibody, specific for SDMA, not labelled enzymatically, and a secondary antibody, specific for the primary antibody, enzymatically labelled. The intensity of the yellow colour, measurable at 450 nm, is inversely proportional to the SDMA concentration in the sample. A high SDMA concentration in the sample decreases the photometric signal. The SDMA from the samples is calculated based on the standard curve elaborated under identical experimental conditions. The SDMA

values obtained in the patients with venous ulcer (17 cases) were compared with the reference values obtained in a control group (15 cases).

Results

The diagnosis of venous ulcer was based on anamnestic data (IVC history), local clinical examination (lower limb ulceration, painless, with different sizes, with clean edges), venous Doppler examination (IVC signs). Histopathologic ally, venous leg ulcer can be characterized by granulation tissue and fibrin deposits (Figure 2).

Discussions

Current findings [4] show that ADMA and SDMA levels are elevated, and Arg/ADMA and Arg/SDMA ratios are significantly reduced in patients with chronic wounds, indicating reduced availability of NO and arginine, respectively. In our study we have shown that the level of SDMA is significantly increased in patients with venous leg ulcer, compared to the control group. It should be noted that this is the first study addressing the behaviour of SDMA in the pathogenesis of venous leg ulcer. SDMA has been considered an inert metabolite, but because it can be transported into cells, the effect of

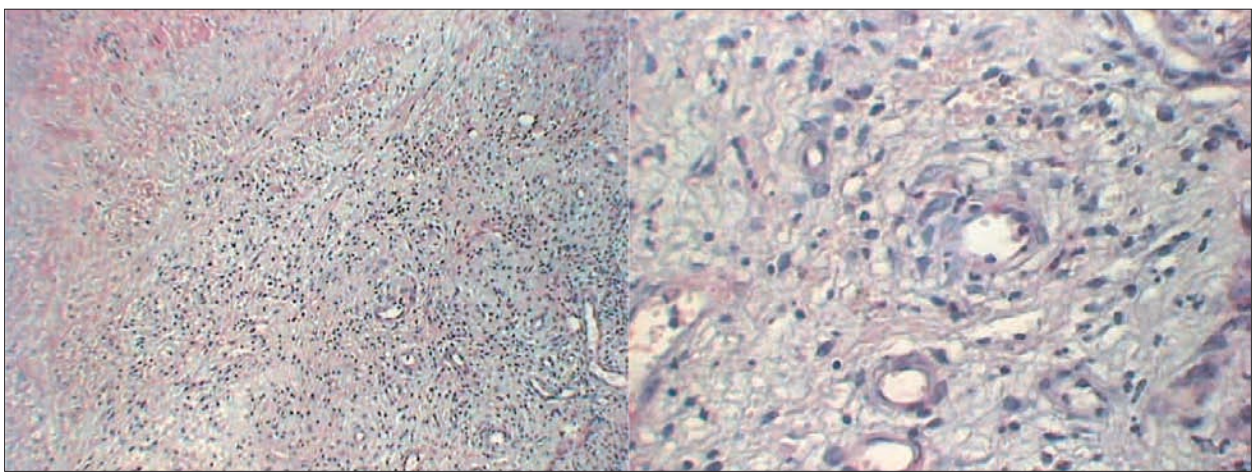

Figure 2. Biopsy fragment of venous leg ulcer in which granulation tissue is observed (haematoxylin and eosin stain)

SDMA determination in patients with venous leg ulcer was performed under conditions identical to those determined in the control group. The two selected groups had similar demographic characteristics. In both groups, serum levels of glucose, urea, creatinine and lipid profile were within normal limits. Serum markers of inflammation, red blood cell sedimentation rate (ESR) and C-reactive protein (CRP) showed statistically significant variations between the two groups ($p < 0.05$) (table 1).

In our study, serum levels of SDMA were significantly increased in patients with leg venous ulcer compared with the control group (Table 2).

SDMA on glomerular endothelial cells has been studied [6]. SDMA suppressed VEGF phosphorylation, eNOS activity, nitric oxide production, but not VEGFR2 activation and signalling leading to eNOS activation. SDMA caused eNOS uncoupling and increased superoxide production in response to VEGF. All of these effects were blocked by preventing cellular uptake of SDMA with a molar excess of arginine. These data show that SDMA interferes with nitric oxide production by eNOS uncoupling, a process associated with the occurrence of oxidative stress in the renal endothelium [6, 13, 16, 18]. Our data show that SDMA is not an inert metabolite and could contribute to the pathophysiology of venous leg ulcer.

Table 1. Characteristics of the study participants

Parameters	Leg ulcer (17 cases)	Control (15 cases)
Women/Men	9/8	8/7
Age (years)	60.9(55-72)	59.2(50-70)
Smokers/Non-smokers	3/14	2/13
BMI (kg/mp)	25.2(23.5-28.1)	23.4(21.4-25.5)
Duration of the disease (months)	over 3 months	-
Blood glucose (mg/dl)	88(72-109)	76(68-93)
Urea (mg/dl)	38 (23-55)	27(18-38)
Creatinine (mg/dl)	87(55-118)	66(53-98)
Triglyceride (mg/dl)	94(66-140)	79(47-90)
Cholesterol (mg/dl)	193(143-237)	132(126-215)
ESR (mm/h)	17(9-40)	4(1-9)*
CRP (mg/dl)	0.85(0.2-2.3)	0.1(0.0-0.2)*

* $p < 0.05$ (leg ulcer versus control), p-level of statistical significance.

SDMA may have an indirect effect on the synthesis of NO [6-10]. SDMA inhibits the transport of cationic amino acids that mediate intracellular uptake of L -arginine and inhibit renal uptake of tubular arginine. These two mechanisms could indirectly inhibit NO synthesis by blocking the uptake of L-arginine. In vitro, SDMA inhibits the production of NO in endothelial cells. In addition, plasma levels of SDMA are negatively associated with the L-arginine/ADMA ratio, an indicator of NO production in vivo [7]. SDMA increases ROS production in monocytes stimulated with N-formyl-Met-Leu-Phe (fMLP) by modulating calcium influx via store-operated calcium channels (SOC). SDMA induces increased endothelial ROS production, an effect associated with reduced Arg uptake and inhibition of NO production [9, 10].

Table 2. SDMA level in patients with leg ulcer and control group

SDMA (nmol/ml)	Venous ulcer	Control	p value
Range of variation	0.59-1.38	0.45 – 0.61	<0.001
Average value	1.04	0.51	

p-level of statistical significance (venous ulcer versus control).

SDMA is a methylated derivative of L-Arginine that is strictly excreted in the urine, thus the plasma level of SDMA is strongly correlated with renal function [11]. In 18 studies with more than 2,136 patients, systemic SDMA concentrations were correlated with inulin clearance and serum creatinine. There is evidence of a positive, significant multivariate relationship between SDMA and cardioembolic stroke [12, 16]. It is not known whether this association between SDMA and cardiovascular diseases is the result of its indirect effects on NO synthesis and/or its relationship with renal function. Moreover, there are indications that increased levels of SDMA correlate with renal impairment, liver failure, and increased cardio-vascular risk [6, 7, 12, 14-16].

Conclusions

The results of this study demonstrate the accumulation of SDMA in the blood flow in patients with venous leg ulcer, compared with the control group. The authors estimate that SDMA induces distinct biological responses in patients with venous leg ulcer. Our data show that SDMA is not an inert metabolite and could contribute to the pathophysiology of venous leg ulcer.

Bibliography

1. Weller C, Evans S. Venous leg ulcer management in general practice: Practice nurses and evidence based guidelines. *Australian family physician*. 2012 ;41(5): 331.
2. Dean S. Leg ulcers: causes and management. Australian family physician. 2006; 35(7): 480.
3. Collins L, Seraj S. Diagnosis and treatment of venous ulcers. *American family physician*. 2010; 81(8): 989.
4. Korpacka MK, Wiœniewski J, Fleszar M et al, Metabolites of the Nitric Oxide (NO) Pathway Are Altered and Indicative of Reduced NO and Arginine Bioavailability in Patients with Cardiometabolic Diseases Complicated with Chronic Wounds of Lower Extremities: Targeted Metabolomics Approach (LC-MS/MS), *Oxidative Medicine and Cellular Longevity*, 2019; 13, 5965721

5. Dorantes LC, Ayala MC. Skin Acute Wound Healing: A Comprehensive Review, *Abs. Int. J. Inflamm.,* 2019, 15.
6. Feliers D, Lee DY, Gorin Y et al. SDMA alters endothelial NO activity glomerular endothelial cells. *Cell Signal*, 2015; 27(1):1-5.
7. Chandrasekharan UM1, Wang Z1, Wu Y et al. Elevated levels of plasma SDMA and increased arginase activity as potential indicators of cardiovascular comorbidity in rheumatoid arthritis. Arthritis Res. Ther. 2018; 20(1):123.
8. Tain YL, Hsu CN, Toxic dimethylarginines: ADMA and SDMA. Toxins (Basel). 2017; 9(3). pii: E92.
9. Tsikas, D. Does the inhibitory action of asymmetric dimethylarginine (ADMA) on the endothelial nitric oxide synthase activity explain its importance in the cardiovascular system? The ADMA paradox. *J. Controversies Biomed. Res.* 2017; 3, 16–22.
10. Tsikas, D., Bollenbach, A., Hanff, E., Kayacelebi, A.A. Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and homoarginine (hArg): The ADMA, SDMA and hArg paradoxes. *Cardiovasc. Diabetol.* 2018; 17, 1.
11. Blanc, R.S.; Richard, S. Arginine Methylation: The Coming of Age. *Mol. Cell* 2017; 65, 8–24.
12. Schlesinger, S.; Sonntag, S.R.; Lieb, W.; Maas, R. Asymmetric and symmetric dimethylarginine as risk markers for total mortality and cardiovascular outcomes: A systematic review and meta-analysis of prospective studies. PLoS ONE 2016; 11
13. Emrich, I.E.; Zawada, A.M.; Martens-Lobenhoffer, J.et al Symmetric dimethylarginine (SDMA) outperforms asymmetric dimethylarginine (ADMA) and other methylarginines as predictor of renal and cardiovascular outcome in non-dialysis chronic kidney disease. *Clin. Res. Cardiol.* 2018; 107, 201–213.
14. Zobel, E.H., von Scholten, B.J., Reinhard, H., Persson, F., Teerlink, T., Hansen, T.W., Parving, H.H., Jacobsen, P.K., Rossing, P. Symmetric and asymmetric dimethylarginine as risk markers of cardiovascular disease, all-cause mortality and deterioration in kidney function in persons with type 2 diabetes and microalbuminuria. *Cardiovasc. Diabetol.* 2017; 16, 88.
15. Damaso EO, Damaso A, Esparragon FR et al, ADMA and SDMA in CKD: A clinical Approach, Molecular Sciences, 2019; 20, 3668.
16. Chandrasekharan JT, Salpeter SR, Boeger SB et al. SDMA as endogenous marker of renal function-a meta-analysis. *Nephrology Dialysis Transplantation,* 2006; 21(9): 2446–2451
17. Luo J, Chen A. NO: a newly discovered function on wound healing. *Acta Pharm. Sinica,* 2005; 26, 259-264.
18. Adler B, Friedman A. NO therapy for dermatologic disease. Future SciOA, 2015 Toxins (Basel). 2017; 9(3): 92.

Conflict of interest
NONE DECLARED

Correspondance address: Mircea Tampa
Department of Dermatology, 'Victor Babeș' Clinical Hospital for Infectious Diseases, Bucharest, Romania
Department of Dermatology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania.
tampa_mircea@yahoo.com