Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes

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Abstract

Background. The guanidines asymmetric dimethylarginine (ADMA), a marker of endothelial dysfunction, and its counterpart symmetric dimethylarginine (SDMA), considered inert, are accumulated in chronic kidney disease (CKD). The present study evaluates their effect on monocyte function, since previous data demonstrated leukocyte activation by other guanidino compounds.

Methods. The effect of ADMA and SDMA on reactive oxygen species (ROS) production in human whole blood at baseline and after N-formyl–methionine–leucine–phenylalanine (fMLP) stimulation was evaluated. By using the fluorescent probe Fluo3-AM, the role of changes in monocytic cytoplasmic calcium ([Ca2+]i) was studied. Thapsigargin, and removal followed by addition of extracellular Ca2+ (Ca2+ex), was used to investigate the contribution of store-operated Ca2+-channels (SOCs). SKF96365 was used as a selective inhibitor of the SOCs. A pharmacologic intervention with captopril, known to affect Ca2+ influx, was tested.

Results. SDMA enhanced ROS production in fMLP-stimulated monocytes using heparinized blood, and this effect was abolished in EDTA-anticoagulated blood. In the presence of SDMA, an increased Ca2+ entry from the extracellular milieu resulted in an elevated amplitude of the peak [Ca2+]i change triggered by fMLP. None of these effects were seen with ADMA. Depletion of the intracellular stores with thapsigargin in the absence of Ca2+ex, followed by re-addition of Ca2+ex triggered a significantly larger Ca2+ entry after SDMA treatment versus saline. This effect was prevented with SKF96365, as was the SDMA-enhanced oxidative burst after fMLP. Pre-incubation with captopril also reduced the increased ROS production seen with SDMA.

Conclusions. SDMA, a uraemic retention solute considered inert, stimulates ROS production of monocytes by acting on Ca2+ entry via SOCs. This pro-inflammatory effect may trigger vascular pathology and may be involved in altering the prevalence of cardiovascular disease in CKD.

Keywords: calcium; chronic kidney disease; guanidines; leukocytes; reactive oxygen species

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the general population. Atherosclerosis, the process leading to CVD, is causally related to conventional risk factors such as age, gender, tobacco use, diabetes, dyslipidaemia and hypertension. Impairment of renal function, from a reduction of 50% on or earlier, influences cardiovascular outcome significantly [1,2]. Among patients affected by chronic kidney disease (CKD), CVD is the main cause of morbidity and mortality, at a higher prevalence compared to the general population [3]. Conventional risk factors insufficiently explain this association; it has repeatedly been suggested that factors proper to CKD per se are at play [4–6].

As renal function deteriorates, several compounds, normally excreted by the healthy kidneys, accumulate in the body. Although some of these uraemic retention solutes were shown to have the potential to induce vascular damage [7], our knowledge remains largely incomplete [8]. Recent studies predict a better outcome for haemodialysis patients when a more efficient removal of uraemic solutes is attained [9,10]. Further identification of the culprits and their pathophysiological mechanisms could contribute to the development of specific therapies and interventions, even before renal replacement therapy is needed.

A group of solutes progressively retained in CKD are the guanidino compounds. These small and water-soluble metabolites of L-arginine are mainly known for their neurotoxic effects [11]. More recent data demonstrate an influence of guanidino compounds on mechanisms relevant to vascular damage, such as activation of leukocyte function [12] and decrease of protein binding of homocysteine [13].
The guanidino compound asymmetric dimethylarginine (ADMA) is acknowledged as a marker of endothelial dysfunction because of its inhibitory effect on nitric oxide synthase (NOS) [14]. It has been linked to vascular outcome in CKD [15], but is also considered a strong independent predictor of cardiovascular and overall mortality in other patient groups [16–18]. Symmetric dimethylarginine (SDMA), the structural counterpart of ADMA, has been considered inert. A recent study showed that SDMA inhibited NO synthesis in cultured endothelial cells by competing with the transport of L-arginine, the substrate of NOS; this effect was associated with an increase in reactive oxygen species (ROS) [19]. In a meta-analysis by Kielstein et al., SDMA was put forward as a possible marker of renal function [20].

The present study was undertaken to evaluate whether SDMA affects monocyte free radical production, elucidate the mechanisms involved, compare the effect of SDMA and ADMA and evaluate pharmacologic agents to neutralize observed effects.

**Methods**

**Materials**

Na$_4$EGTA, captopril, thapsigargin and SKF96365 were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Fluo3-AM was delivered by Molecular Probes (Eugene, OR, USA) and Dulbecco’s PBS with and without calcium (PBS+Ca$^2+$), captopril, thapsigargin and SKF96365 were obtained from Sigma-Aldrich Co. (St Louis, MO, USA).

**Sample collection**

Heparinized or EDTA-anticoagulated whole blood from healthy volunteers was collected in Vacutainer$^{	ext{TM}}$ tubes (Becton Dickinson) after informed consent. Exclusion criteria were smoking, infection, medication intake or pregnancy. The study protocol was approved by the local ethics committee.

**Monocytic oxidative burst activity in whole blood**

**Experimental set-up.** The dimethylarginines ADMA and SDMA (Merck) were dissolved separately in saline (0.9% NaCl, Baxter) as recommended by Cohen et al. [21]. Stock solutions (10×) were stored at −20°C; they were diluted 1:10 in whole blood to obtain a concentration corresponding to the uraemic condition of 36.1 μM for ADMA and 6.1 μM for SDMA [22]. Concentrations tested for dose-response effects were 1.5, 3.0, 6.0, 12.0 and 36.0 μM.

**Oxidative burst.** After a 10 minutes incubation at 37°C with saline (control) or a dimethylarginine, the bursttest (Phagoburst$^{	ext{®}}$) (Orpegen Pharma) was applied following manufacturer’s guidelines. The burst activity in monocytes was evaluated at baseline and after stimulation with N-formyl–methionine–leucine–phenylalanine (fMLP, 0.83 μM). Generation of the ROS superoxide anion, hydrogen peroxide and hypochlorous acid was measured by the conversion of the fluorogenic substrate dihydrorhodamine into rhodamine. Erythrocytes were lysed while leukocytes were simultaneously fixed and propidium iodide was added to exclude aggregation artefacts. Within 30 min, samples were analysed with a FACScan$^{	ext{®}}$ flow cytometer (BD).

**FACScan analysis.** Using the CellQuest Pro$^{	ext{TM}}$ software, 10,000 events were counted in every sample. Monocytes were gated in the light scatter dot plot according to size (forward scatter) and granularity (side scatter). Within this gate, the percentage of rhodamine-positive cells and their mean fluorescence intensity (MFI) were evaluated.

**Cytoplasmic Ca$^{2+}$ changes in monocytes using whole blood**

To study the effect of the dimethylarginines on the fMLP-induced changes in the monocyte cytoplasmic calcium concentration ([Ca$^{2+}$]i), an assay based on a method to study Ca$^{2+}$ mobilization in whole blood platelets [23] was established. This enabled evaluation of monocyte [Ca$^{2+}$]i, fluxes in a physiologic milieu (whole blood).

Heparinized whole blood was diluted in a PBS+Ca$^{2+}$ buffer with 1 mM sodium pyruvate and incubated with 5 μM Fluo3-AM, a fluorescent probe for Ca$^{2+}$, for 30 min at room temperature. Addition of the human monoclonal PE-labelled antibody against the leukocyte common antigen CD45 enabled the distinction between leukocytes and erythrocytes. After washing, cells were resuspended in a PBS buffer. Two different buffers were used to explore the source of observed [Ca$^{2+}$]i changes: PBS+Ca$^{2+}$ allowing both release of Ca$^{2+}$ from the intracellular stores and entry from the extracellular milieu and PBS–Ca$^{2+}$+5 mM EGTA that prevents Ca$^{2+}$ entry (remaining <0.2 nM free Ca$^{2+}$, as calculated with WebMaxC application). Following preparation, samples were incubated with saline or dimethylarginine for 10 min at 37°C and analysed by flow cytometry. During 30 s, the baseline Fluo3-Ca$^{2+}$ fluorescence was established. Next, 0.83 μM fMLP was added and changes in [Ca$^{2+}$]i were recorded up to 180 s.

**FACScan analysis.** Cytoplasmic Ca$^{2+}$ changes were analysed using the CellQuest Pro$^{	ext{TM}}$ software. Only CD45-positive leukocytes were counted. Monocytes were gated according to light scatter and fluorescence properties, and the MFI of the Fluo3-Ca$^{2+}$ complex per interval of 30 s was evaluated for a total time of 180 s.

**Study of store-operated Ca$^{2+}$ entry in monocytes**

Thapsigargin, an inhibitor of the Ca$^{2+}$-ATPase on intracellular membranes, causes depletion of the stores resulting in a rise in [Ca$^{2+}$]i, and an activation of the store-operated Ca$^{2+}$ channels (SOCs) [24] in the plasma membrane. To study the effect of the dimethylarginines on the SOCs, whole blood was prepared as described above. After washing, cells were resuspended in PBS–Ca$^{2+}$+5 mM EGTA with or without dimethylarginines and baseline [Ca$^{2+}$]i was measured.

FACScan analysis. Using the CellQuest Pro$^{	ext{TM}}$ software, 10,000 events were counted in every sample. Monocytes were gated in the light scatter dot plot according to size (forward scatter) and granularity (side scatter). Within this gate, the percentage of rhodamine-positive cells and their mean fluorescence intensity (MFI) were evaluated.
Table 1. Effect of the dimethylarginines on monocytic oxidative burst activity in heparinized whole blood

<table>
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<tr>
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<th>Baseline</th>
<th>fMLP</th>
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<tr>
<td>Saline</td>
<td>4.3 ± 1.0</td>
<td>10.5 ± 4.2**</td>
</tr>
<tr>
<td>SDMA</td>
<td>6.6 ± 3.2</td>
<td>16.0 ± 6.7**††</td>
</tr>
<tr>
<td>ADMA</td>
<td>7.0 ± 3.7*</td>
<td>13.3 ± 4.8**††</td>
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Data are shown as the percentage of ROS producing monocytes and are represented as mean ± SD, n = 8. *P < 0.05, **P < 0.01 versus saline; ††P < 0.01 versus baseline.

Next, 2 µM thapsigargin was added to empty the intracellular stores and after 180 s thapsigargin was removed and cells were resuspended in PBS+Ca²⁺ in the absence or presence of dimethylarginines. [Ca²⁺], changes as a consequence of Ca²⁺ entry via the SOCs were measured for another 180 s. To determine the activation of SOCs in response to dimethylarginines, SKF96365 (30 µM), a specific SOCs inhibitor [25], was applied.

**In vitro pharmacological strategy**

Captopril was evaluated for its potential to neutralize the effects observed with dimethylarginines, in the burst test and the Ca²⁺ assay. It was added 10 min before the compounds at a concentration of 10 µM. Captopril was also used in the thapsigargin experiments described above.

**Statistical analysis**

Data are expressed as means ± standard deviation (SD). Statistics were performed using a nonparametric paired Wilcoxon test. P-values below 0.05 were considered significant.

**Results**

**Effect of SDMA and ADMA on monocytic oxidative burst activity in heparinized whole blood.** As shown in Table 1, <5% of the basal monocytes produced ROS. ADMA increased the percentage of rhodamine-positive monocytes slightly.

Stimulation with fMLP resulted in an increased percentage of monocytes producing ROS versus baseline, under control conditions and in the presence of the dimethylarginines. SDMA enhanced the fMLP-stimulated burst activity significantly compared to control, and this effect was dose dependent in a range from 1.5 to 36.0 µM as shown in Figure 1. Since no significant effects were observed for ADMA, the effect seen with SDMA can be considered specific.

**Role of Ca²⁺ entry in fMLP-stimulated oxidative burst: heparinized versus EDTA-anticoagulated blood.** The oxidative burst in response to fMLP occurs via a Ca²⁺-dependent pathway. fMLP activates, via its G-protein-coupled receptor (GPCR), phospholipase C resulting in the generation of inositol 1,4,5-triphosphate, which in turn triggers the release of Ca²⁺ from the intracellular stores. Store depletion subsequently results in the activation of SOCs via which extracellular Ca²⁺ (Ca²⁺ex) enters the cell to help refilling the stores. This fMLP-induced rise in [Ca²⁺], occurs immediately, has a transient time course and is essential for the activation of NADPH oxidase, the enzyme responsible for the production of ROS [26,27].

Since SDMA increased the fMLP-stimulated burst activity (Table 1), the role of changes in [Ca²⁺], on the oxidative burst in the presence of SDMA was further evaluated. The fMLP-stimulated ROS production in heparinized whole blood was compared to the effect obtained in whole blood anticoagulated with EDTA, a Ca²⁺-chelator. Figure 2 shows that for the control sample (saline, open bars), the stimulation of the monocytic oxidative burst activity with fMLP was attenuated in EDTA-anticoagulated whole blood (low Ca²⁺ex) compared to heparin (normal Ca²⁺ex). In addition, the stimulating effect of SDMA (full bars) on the fMLP-stimulated free radical production observed in heparinized blood (see above) disappeared in EDTA-treated blood (16.0 ± 6.7 versus 8.0 ± 6.0, P < 0.05). These results suggest that the SDMA-enhanced ROS production is Ca²⁺-dependent and the involvement of Ca²⁺-related pathways was submitted to further evaluation.

**Changes in monocytic [Ca²⁺], after fMLP stimulation in whole blood.** Registrations of [Ca²⁺], in monocytes after fMLP stimulation are represented in Figure 3 over a time period of 180 s, in the presence and absence of Ca²⁺ex.
Fig. 3. Cytoplasmic Ca\(^{2+}\) changes in monocytes incubated with saline (squares) or SDMA (circles, A) or ADMA (circles, B) after fMLP stimulation, in the presence (open symbols) and absence (full symbols) of Ca\(^{2+}\)ex. MFI of the Fluo3-Ca\(^{2+}\) complex is evaluated per 30 s. Addition of fMLP is indicated by the arrow. The peak amplitudes (PA) are marked by the vertical double-sided arrows on panel A (1 = PA saline with Ca\(^{2+}\)ex, 2 = PA saline without Ca\(^{2+}\)ex, 3 = PA SDMA with Ca\(^{2+}\)ex, 4 = PA SDMA without Ca\(^{2+}\)ex). *P < 0.05, **P < 0.01 versus baseline; ††P < 0.01 versus corresponding control condition; ◦◦P < 0.01 versus PA 4; n = 8.

Under control conditions with Ca\(^{2+}\)ex present (saline, open squares), fMLP triggered a transient rise in [Ca\(^{2+}\)]\(_i\) that completely recovered to baseline levels. Upon removal of Ca\(^{2+}\)ex (full squares), the [Ca\(^{2+}\)]\(_i\) trace was shifted to lower levels but the peak amplitude (PA), an indicator of the capacity of the cells to increase [Ca\(^{2+}\)]\(_i\) upon stimulation, was not significantly altered (PA saline with Ca\(^{2+}\)ex (1): 86.4 ± 42.0 versus PA saline without Ca\(^{2+}\)ex (2): 62.2 ± 22.3, ns). These experiments suggest that the fMLP-triggered [Ca\(^{2+}\)]\(_i\) transient is related to the release of Ca\(^{2+}\) from the intracellular stores, most likely the endoplasmic reticulum. The recovery to baseline [Ca\(^{2+}\)]\(_i\) was faster without Ca\(^{2+}\)ex in comparison to the condition with Ca\(^{2+}\)ex (delta interval(60–90) s: 74.0 ± 30.3 versus delta interval(30–60) s: 49.1 ± 28.3; P < 0.02; n = 8), as expected because additional Ca\(^{2+}\) entry (via SOCs and others) will occur with Ca\(^{2+}\)ex present, thereby slowing down recovery [26].

In the presence of Ca\(^{2+}\)ex, incubation with SDMA (circles, Figure 3A) resulted in the elevation of mononuclear [Ca\(^{2+}\)]\(_i\) changes as compared to saline (166.3 ± 73.8 versus 136.5 ± 55.6, P < 0.01; n = 8) specifically affecting the peak amplitude, while not influencing the recovery kinetics in the 60–90-s interval. In the absence of Ca\(^{2+}\)ex, no significant changes were observed in the fMLP-induced [Ca\(^{2+}\)]\(_i\) kinetics for SDMA versus control (PA SDMA with Ca\(^{2+}\)ex (3): 107.5 ± 58.9 versus PA SDMA without Ca\(^{2+}\)ex (4): 51.9 ± 33.9; P < 0.01).

These data suggest that SDMA enhances Ca\(^{2+}\) entry in monocytes after fMLP stimulation, resulting in an increased oxidative burst. Most notably, ADMA did not influence the [Ca\(^{2+}\)]\(_i\) changes triggered by fMLP (Figure 3B).

**Effect of SDMA on store-operated Ca\(^{2+}\) channels.** Stimulation with fMLP triggers store-Ca\(^{2+}\) release followed by store refilling aided by Ca\(^{2+}\) entry via SOCs [27]. It was investigated whether SDMA influenced this Ca\(^{2+}\) entry pathway. For that purpose, thapsigargin was used in combination with a Ca\(^{2+}\) -removal/Ca\(^{2+}\) -re-addition protocol.
As shown in Figure 4, the addition of thapsigargin in the absence of Ca\textsuperscript{2+}ex triggered a transient [Ca\textsuperscript{2+}], change due to leakage of Ca\textsuperscript{2+} out of the store and subsequent store emptying. Resuspension in a Ca\textsuperscript{2+}-containing buffer triggered an immediate [Ca\textsuperscript{2+}], rise due to entry via SOCs previously activated by store depletion (squares, full line).

In monocytes incubated with SDMA (circles, full line), the switch to medium with Ca\textsuperscript{2+} resulted in a significantly stimulated [Ca\textsuperscript{2+}], rise and thus Ca\textsuperscript{2+} entry via SOCs. The addition of SKF96365 at the time of resuspension, to inhibit SOCs [25], blocked the Ca\textsuperscript{2+} rise and thus the Ca\textsuperscript{2+} entry via the SOCs gradually (squares, dotted line). SKF96365 also completely abrogated the [Ca\textsuperscript{2+}], rise in the presence of SDMA significantly (circles, dotted line), indicating that SDMA specifically stimulates Ca\textsuperscript{2+} entry in monocytes via the SOC pathway.

**Does inhibition of SOCs influence the oxidative burst?**

To assess whether the observed increase in Ca\textsuperscript{2+} entry via SOCs in the presence of SDMA is playing a role in the increased fMLP-stimulated ROS production, the SOC inhibitor SKF96365 was used in the bursttest. It was demonstrated that SKF96365 inhibited the SDMA-enhanced fMLP-stimulated oxidative burst significantly (Figure 5). These results indicate that SDMA evokes an increased fMLP-stimulated ROS production by enhancing the Ca\textsuperscript{2+} entry via the SOCs.

**Pharmacologic intervention with captopril affects the SDMA effect.** Captopril is a known inhibitor of the angiotensin-converting enzyme (ACE) and is therefore clinically used to treat hypertension. It has also been described to inhibit Ca\textsuperscript{2+} influx in vascular smooth muscle cells and to reduce ROS production in mononuclear leukocytes [28,29].

When captopril was used in the bursttest (Figure 5), the drug did not induce any change in the fMLP-stimulated oxidative burst activity per se. The rise in fMLP-stimulated ROS production after incubation with SDMA was completely abrogated in the presence of captopril.

From this result, the question arose whether captopril, like SKF96365, would block the SOCs. For that purpose, thapsigargin experiments with SDMA were repeated in the presence of this drug. The addition of captopril during resuspension of the monocytes treated with thapsigargin caused no significant difference in [Ca\textsuperscript{2+}], versus saline. Furthermore, the combination captopril and SDMA resulted in the same [Ca\textsuperscript{2+}], course as with SDMA alone (data not shown). In sum, captopril inhibits the fMLP-stimulated burst with SDMA but via a mechanism not related to SOC Ca\textsuperscript{2+} entry.

The effect of captopril on fMLP-induced [Ca\textsuperscript{2+}], changes was further tested. Figure 6 illustrates that the [Ca\textsuperscript{2+}], peak after fMLP in the presence of Ca\textsuperscript{2+ex} as well as in the absence of Ca\textsuperscript{2+ex} for captopril together with SDMA is significantly lower than the peak seen with SDMA alone (see also Figure 3A). Because captopril does not act on the SOCs, these data suggest that captopril inhibits SDMA-enhanced ROS production by blocking the release of calcium from the intracellular stores.

**Discussion**

The present study was performed to evaluate the effect of the dimethylarginines ADMA and SDMA on monocytic cell function, a critical cell type in atherosclerosis [30], and to elucidate the molecular mechanisms involved. Both compounds have an elevated concentration in CKD, but...
until now, SDMA was considered biologically inert [14]. One recent study, however, by Bode-Böger et al., showed that SDMA reduced endothelial NO synthesis [19].

The main findings of this study are that SDMA, unlike ADMA, induce monocyte activation by enhancing ROS production after fMLP stimulation (Table 1, Figures 1 and 2). This effect was linked to an enhanced Ca²⁺ entry into the monocytes (Figure 3A). Making use of store depletion with thapsigargin, it became clear that SDMA acts on the SOCs, thereby augmenting Ca²⁺ entry (Figure 4), an observation confirmed by blocking the effect with SKF96365 (Figure 4 and 5). Captopril inhibited the increased ROS production (Figure 5) as well as the increase of [Ca²⁺]i (Figure 6). The latter effect of captopril was not SOCs related.

It is of note that the effect of SDMA occurs in combination with fMLP (Table 1), an agent known to induce Ca²⁺ changes in leukocytes preceding a moderate stimulation of the oxidative burst activity [26]. The mild activated status of immune cells after fMLP stimulation is the representative for the moderate inflammation present in the majority of uraemic patients [6]. Furthermore, the mechanism induced by fMLP is a characteristic for CKD because it generates oxidative stress, which is present from the earlier stages of CKD on [31], and because it is related to cellular Ca²⁺ changes. Masry and Fadda describe CKD as a state of cellular calcium toxicity and attribute it at least partially to the role of parathyroid hormone [32]. A study in haemodialysis patients performed by Koorts et al. showed an elevated basal intracellular Ca²⁺ level in neutrophils [33]. They also demonstrated an increased Ca²⁺ flux after fMLP stimulation in uraemic neutrophils [33], in parallel with our data on SDMA in monocytes (Figure 3A).

Other uraemic retention solutes also seem to play a role in enhanced [Ca²⁺]i concentrations. Phenylacetic acid [34] and p-hydroxyhippuric acid [35] inhibit the plasmaemal Ca²⁺-ATPase of the erythrocytes, and indoxyl sulfate, guanidino succinate (GSA) and spermine increased [Ca²⁺]i in the uraemic brain through an interaction with voltage- or ligand-gated Ca²⁺ channels [36].

The involvement of SOCs in the uraemic pathophysiology has not been investigated. Nevertheless, SOCs are involved in many pathological conditions. For example, stimulated SOCs have a role in the cell proliferation of colon cancer cells [37] and in Alzheimer’s disease where SOC Ca²⁺ entry is decreased [38].

The activation of the monocytic cells by fMLP occurs via the formyl peptide receptor (FPR), a member of the G-protein coupled receptor (GPCR) superfamily. Other chemotactic factors like complement C5a fragment, leukotriene B4, the platelet-activating factor and a superfamily of chemokines all induce cell activation via a GPCR from the same phylogenetic origin as FPR [39]. Furthermore, also receptors for compounds of importance in CKD like angiotensin, endothelin and cholecystokinin belong to this GPCR family [40]. Therefore, it is likely that a state similar to fMLP stimulation occurs in vivo in CKD patients.

SDMA is one of the guanidino compounds derived from protein metabolism. Although its structural counterpart ADMA has repeatedly been linked to cardiovascular outcome [15–18], SDMA is considered relatively inert [14]. A meta-analysis performed by Kielstein et al., however, suggested that SDMA could be a reliable marker of renal function [20] and the same group demonstrated that SDMA reduces endothelial NO synthesis in vitro. In contrast to ADMA, which inhibits NO production by directly interfering with the NOS, SDMA acts as a competitor for the transport of L-arginine, the substrate for NOS; this finally results in an increased endothelial ROS production [19]. Although focusing on a different cell type, our data clearly put forward a role for SDMA in ROS production (Table 1, Figures 1 and 2). These data corroborate with the toxicity of the global group of guanidino compounds, which have been considered neurotoxic [11] and are also linked to an effect on the immune system [12].

In spite of being small and water soluble and therefore believed to be easily removable by modern dialysis strategies, recent data suggest that guanidino compounds have a different kinetic behaviour in comparison to urea, a classical marker of small water-soluble molecule behaviour during dialysis. Their dialytic removal is substantially hampered because of an increased distribution volume [41,42] that suggests to consider alternative removal strategies, essentially based on slow but overall efficient removal as pursued with prolonged dialysis sessions [43].

More than dialysis treatment, however, it is important to pursue interventions that improve cardiovascular outcome in renal patients long before replacement therapy is necessary. Future approaches should focus on non-dialytic interventions that improve outcome in earlier stages of kidney disease. The present study indicates that the negative impact of SDMA can be prevented by pharmacologic intervention with captopril.

The question to be raised is whether ACE inhibition also has a favourable impact on clinical outcome in patients with CKD. Extensive information demonstrates a vasculoprotective impact of inhibition of the renin–angiotensin system [44]; of note, however, such an effect may also have a protective impact on the degradation of kidney function [44] and it might be difficult to discern these two from each other.

In conclusion, the present data suggest that in CKD, SDMA, but not ADMA, can increase the monocytic ROS production by stimulating Ca²⁺ entry via the SOCs. This is the first report on effects of uraemic toxins at the level of SOCs, implying store-operated Ca²⁺ entry in the pathophysiology of vascular disturbances associated with CKD. The administration of an ACE inhibitor can counter ROS production and may possibly counteract the proatherogenic effects of SDMA.
References


