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Cardiovascular Research 73 (2007) 359–367

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Platelet nitric oxide synthesis in uremia and malnutrition: A role for L-arginine supplementation in vascular protection?

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Received 11 May 2006; received in revised form 20 September 2006; accepted 26 September 2006
Available online 30 September 2006
Time for primary review 25 days

Abstract

L-arginine is the physiological precursor for nitric oxide (NO) synthesis, and availability and transport of L-arginine modulate the rates of NO biosynthesis in circulating blood cells and the vasculature. NO is involved in many vascular functions such as vasodilation and inhibition of platelet aggregation and adhesion. We have established that reduced plasma L-arginine and NO production and increased tumour necrosis factor-alpha (TNF-α), fibrinogen, and C-reactive protein levels in malnourished uremic patients are associated with increased aggregability of platelets. Our findings may explain the increased cardiovascular mortality in patients with deficient nutritional status, leading to inflammation, oxidative stress, impaired L-arginine–NO signalling, and platelet activation. The aim of this review is to evaluate whether disturbances in the L-arginine–NO signalling pathway in chronic renal failure and atherosclerosis are affected by malnutrition and inflammation. We have included a brief overview of membrane transporters mediating influx of L-arginine and other cationic amino acids, as these transporters are involved in the potential benefits of L-arginine supplementation and platelet function in malnourished uremic patients.

Keywords: Platelets; Uremia; Chronic renal failure; Malnutrition; L-arginine–nitric oxide signalling pathway; L-arginine; Nitric oxide; TNF-α; C-reactive protein; Fibrinogen; L-arginine supplementation

1. Introduction

Uremia is defined as an irreversible and long-standing loss of renal function causing ill-health, and patients with end-stage chronic renal failure are treated principally by renal dialysis and transplant [1]. However, in underdeveloped countries, renal transplantation is not a viable option for the majority of patients on hemodialysis [1,2]. The most common form of dialysis is hemodialysis, which allows a 10-year mean survival rate of uremic patients in developed countries.

In chronic renal failure (CRF) there is endothelial dysfunction, accelerated atherosclerosis and a high incidence of thromboembolic complications [3]. In this context, cardiovascular disease is the main cause of mortality in CRF patients, who have an annual mortality rate 10–20 times higher than the general population [1–3]. CRF is associated with endothelial cell injury and impaired endothelium-dependent relaxation in the early stages of the syndrome [4,5]. Reduced bioavailability of NO and abundant formation of reactive oxygen species (ROS) within the vascular wall detected in uremic patients seem to be the key determinants of endothelial dysfunction [6–9].

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doi:10.1016/j.cardiores.2006.09.019

Table 1
Effects of malnutrition on plasma L-arginine, C-reactive protein, TNF-α and fibrinogen concentrations in chronic renal failure patients

	L-arginine (μM)	CRP (mg ml^{-1})	TNF-α (pg ml^{-1})	Fibrinogen (g/l)
Controls	125±5 (n=10)	1.14±0.4 (n=10)	1.2±0.13 (n=6)	2.49±0.8 (n=42)
CRF well-nourished patients	86±9* (n=10)	8.14±1.9* (n=10)	1.8±0.13 (n=6)	4.46±0.27* (n=42)
CRF malnourished patients	54±14*,§ (n=10)	14.4±3.7*,§ (n=6)	3.1±0.7* (n=6)	3.47±0.32*,§ (n=36)

CRF, chronic renal failure; CRP, C-reactive protein; TNF-α, tumour necrosis factor-α. Values denote means±S.E. of measurements in control subjects, well-nourished and malnourished uremic patients, *p<0.05 vs control, §p<0.05 vs well-nourished patients, ANOVA plus post hoc Tukey test. Data taken from Ref. [18] and unpublished data for CRP.

Malnutrition associated with an inflammatory state exacerbates cardiovascular mortality in uremic patients, and is further aggravated by impaired nutrition in impoverished communities [2,3,10–12]. The assessment of nutritional status in CRF patients can be performed using an anthropometric parameter, body mass index (BMI), i.e. the ratio of post-dialysis body weight (kg) divided by height square (meters), considering malnourished values as less than 18.5 kg/m^2 [13–15]. Biochemical parameters are also useful to determine the nutritional status in uremia, such as serum concentrations of albumin, pre-albumin and transferrin [13,14].

Low concentrations of plasma amino acids, including L-arginine (Table 1), the precursor for NO synthesis in vascular cells, are present in CRF, especially in malnourished patients [6,16–18]. Long-term hemodialysis in patients with chronic renal failure is associated with muscle wasting and catabolism of muscle protein caused by underlying mechanisms not yet clarified [10,19,20]. Low serum albumin and increased C-reactive protein levels (Table 1) are also associated with increased mortality in patients with chronic renal failure and may reflect widespread inflammation and increased oxidative stress [12,21–23]. Moreover, malnutrition in uremic patients is associated with increased levels of circulating cytokines such as TNF-α (Table 1) and IL-β, further exacerbating the oxidative and inflammatory milieu in uremia [20,24].

Uremia is also associated with profound disturbances in the regulation of NO synthesis. Conflicting data are available on the systemic production of NO in chronic renal failure. Several studies in experimental animals and humans have demonstrated an accumulation of NO metabolites in uremia [6,25,26]. This enhancement in NO production may be related to the type of dialysis membrane used and seems to be more pronounced in patients who present with hypotension during dialysis [27,28]. Interestingly, uremic plasma can increase the production of NO in endothelial cells [29]. In animal models of uremia, the activity and expression of eNOS and iNOS are also upregulated in the systemic vasculature [17].

An important finding in uremia is the altered metabolism of protein and amino acids. Although some studies have reported normal or increased levels of L-arginine in uremic patients [30,31], we and others have demonstrated that in patients with CRF, including those on conservative treatment and those on dialysis, continuous ambulatory peritoneal dialysis (CAPD) or hemodialysis, plasma levels of L-arginine are significantly reduced compared with controls [6,7,32–34]. Since the total amino acid pool seems depleted in uremia, L-arginine may become an essential amino acid in this pathological state. Indeed, L-arginine metabolic pathways are directly involved in the pathophysiology of the uremic syndrome, and by-products of L-arginine metabolism, such as polyamines and urea, are potential uremic toxins [35,36].

2. L-arginine metabolism and modulation of platelet function

L-arginine is a semi-essential amino acid for most mammals and is required during periods of growth, severe stress and injury [35,37]. The sources of L-arginine are endogenous and exogenous. Although macrophages and endothelial cells can generate L-arginine, most synthesis takes place in the liver and kidney by the transfer of an amino group from L-aspartic acid or L-glutamic acid to L-citrulline in a reaction mediated by the enzyme argininosuccinic acid synthetase [35,37,38]. Since the liver utilizes most of the L-arginine produced in the urea cycle, the kidney, together with dietary intake (1–2 g/day), is responsible for maintaining normal plasma levels of L-arginine (80–100 μM) [35]. Experimental studies have demonstrated that the proximal convoluted tubule is the major site of L-arginine synthesis in the kidney [38,39]. In pathological conditions such as chronic renal and heart failure, we have shown that plasma levels of L-arginine are reduced [6,7,40]. In malnourished uremic patients, plasma levels of L-arginine are even lower (Table 1) [18].

NO regulates platelet activation by inhibiting adhesion and aggregation [41–45]. Moreover, L-arginine has been reported to inhibit aggregation of platelets both *in vitro* and *in vivo*, while N^{ω}-monomethyl-L-arginine (L-NMMA), an endogenous arginine analogue and inhibitor of nitric oxide synthase (NOS), increases platelet activation and adhesion [41,42,46–48].

Chronic and acute renal failure is partially reversed by L-arginine supplementation in different animal models (Table 2), implicating reduced L-arginine availability in the genesis and/or progression of uremia, and highlighting a possible beneficial effect of L-arginine supplementation in maintaining renal function and slowing the progression of the disease [25,49–59]. It is still unclear whether chronic L-arginine administration can delay the progression of chronic renal failure in humans [60–62]. Additional larger clinical studies are needed to support a therapeutic role for L-arginine supplementation in renal progression.

Table 2
Effects of L-arginine supplementation on renal and vascular function in chronic renal failure patients and animal models of renal failure

	Dosage and duration of L-arginine supplementation	Summary of findings	Ref.
CRF patients	100 nmol/min L-arginine i.v.	Venodilation in response to acetylcholine prior to dialysis improved	[4]
	2.5 g/m^2 or 5 g/m^2×3/day for 4 weeks	Brachial artery function at rest or during increased flow did not improve in children with CRF	[5]
	0.2 g/kg body wt/day for 6 months	Proteinuria, BP or glomerular filtration rate did not change and was associated with a delayed increment in urinary and plasma NO_3 levels in moderate CRF patients	[25]
	9 g/d for 9 days+18 g/d for 9 more days (18 days) or two months	Favourable effects on systolic and diastolic BP in hypertensive kidney transplant and hemodialysis patients	[61]
	Placebo (6 weeks)+2 periods (6 weeks) with L-arginine (0.1 g/kg/day) or L-arginine (6 weeks)+2 periods (6 weeks) with placebo	GFR, renal plasma flow, proteinuria albuminuria, mean systolic and diastolic BP did not alter significantly, but urinary excretion of urea and NO_3 increased in cyclosporine-treated renal allograft recipients with chronic transplant dysfunction	[62]
Animal models of renal failure	0.1% or 1% L-arginine in drinking water for 5 weeks	Low dose of L-arginine, but not high dose, attenuated the development of hypertension and the progression of renal insufficiency and reduced endothelin-1 in rats with reduced renal mass	[49]
	1.25 g/l L-arginine for 12 weeks	Creatinine clearance and fractional excretion of sodium were completely normalised while proteinuria was reduced in nephrectomized rats treated with L-arginine	[50]
	2% L-arginine for 8 months, rats aged 12–13 months	Glomerular filtration rate was increased by 50% and the number of sclerotic glomeruli was reduced compared with untreated controls	[51]
	1% L-arginine for 25 days	Renal damage (including fibrosis, apoptosis and macrophage infiltration) significantly improved and urinary NO increased in rats with unilateral ureteral obstruction	[52]
	1.25 g/l L-arginine for 3 weeks	BP and NO(x) concentrations were not modified in two kidney-two clip hypertension rats	[60]
	1% L-arginine for 7 days	Renal graft function was restored to levels found in normal donors, and vascular occlusion and inflammation were reduced	[53]
	2 g/kg/day L-arginine	NO biosynthesis was enhanced and animals were protected from cyclosporine induced kidney damage	[54]
	2.25 g/l L-arginine	Significant amelioration in the BUN and creatinine levels in gentamicin-treated rats	[55]
	50 mg/kg/bw i.v. acutely or 0.25% in drinking water for 7 days	Acute L-arginine infusion had beneficial effects on *in vivo* renal ischemia, but was harmful in isolated H/R tubules. Chronic L-arginine was deleterious to *in vivo* and *in vitro* renal ischemia	[56]
	1% L-arginine for 14 days	$O_2^{\cdot-}$ generation was reduced and the expression of NO signalling proteins as well as the recovery phase of ischemic ARF were significantly improved	[57]
	10 g/l L-arginine for 34 weeks	Urinary NO(x) significantly increased and focal and segmental glomerulosclerosis and proteinuria were reduced without affecting renal function in an experimental model of renal transplantation	[58]
	1% L-arginine for 5 to 6 or 15 to 16 weeks	The progression of glomerular sclerosis in rats with subtotal nephrectomy was prevented by ameliorating glomerular capillary hypertension	[59]

Abbreviations: NO, nitric oxide; ARF, acute renal failure; GFR, glomerular filtration rate; BP, blood pressure; NO(x), nitrite/nitrate.

Slow deterioration of kidney function and parallel activation of the L-arginine–NO pathway may account for the upregulation of L-arginine transport in blood cells and increased bleeding time mediated via NO-dependent mechanisms [6,7,18,32,34,63–65]. Incubation of human platelets with L-arginine, but not D-arginine, reduces platelet aggregability and increases cGMP levels [46]. A number of studies have shown that endogenous L-arginine analogues such as L-NMMA and asymmetric dimethylarginine (ADMA), which can competitively inhibit L-arginine transport and NO synthesis, are present in increased concentrations in plasma from uremic patients [66–68]. The first observation that ADMA was increased in uremic patients on hemodialysis has now been confirmed in different subsets of uremic patients not yet on dialysis and on CAPD and in other cardiovascular diseases [66]. Inhibition of L-arginine influx by L-arginine analogues such as L-NMMA (and most likely ADMA) inhibits NO production in human platelets [42]. Oral administration of L-arginine inhibits human platelet aggregation through increased intraplatelet NO synthesis in healthy young adults and in hypercholesterolemic patients, and L-arginine stimulated NO production is inhibited following the administration of L-NMMA [48,69].

In healthy young men, oral supplementation with L-arginine increases platelet cGMP production, impairs platelet aggregation without affecting endothelium-dependent vasodilation, and results in platelet-specific increases in NO synthesis [69]. The pathological importance of platelet-derived NO is highlighted by reports that the bleeding time in rats depends on endogenous platelet-derived NO production, and that platelets obtained from patients with acute cardiac events generate significantly less NO [70,71]. Under these conditions platelets would be more prone to aggregation.

Most studies in platelets from patients with uremia have reported an impaired response to agonists, such as thrombin

and collagen, a decreased response to exogenous NO and a decreased adhesiveness to endothelium both *in vivo* and *in vitro* [72–74]. In addition to abnormal platelet function, endothelium-dependent relaxation is impaired in renal failure patients, and some of the metabolic and functional defects of uremic platelets may be a consequence of impaired endothelial function [75,76]. In this context, plasma from uremic patients stimulates NO release from endothelial cells, and we have found increased platelet-derived NO production in uremic patients [29,77]. We have recently further demonstrated that aggregation of platelets taken from well-nourished uremic patients is impaired compared to controls and malnourished renal failure patients (see Fig. 1D) [18]. To date, there are no reports on the effects of L-arginine supplementation on platelet function in malnourished uremic patients.

3. Adaptive increases in L-arginine transport mediate increased NO synthesis in human platelets in uremia

Transport of cationic amino acids (L-arginine, L-lysine and L-ornithine) in mammalian cells is mediated largely by systems y^+, y^+L, $B^{o,+}$ and $b^{o,+}$ [78,79]. Transport system y^+ is selective for cationic amino acids and is Na^+-independent, whereas transport systems y^+L, $B^{o,+}$ and $b^{o,+}$ can transport cationic and neutral amino acids but differ in their interactions with inorganic monovalent ions [80,81]. System $b^{o,+}$ is Na^+-independent and system $B^{o,+}$ is Na^+-dependent. System y^+L transports cationic amino acids independent of sodium, and neutral amino acids only in the presence of Na^+ [78–82]. System y^+L exhibits a much higher affinity for cationic amino acids (K_m for lysine ~10 μM) than any other cationic amino acid transport system [78,79].

Our studies in human platelets obtained from normal subjects (Fig. 1A) and uremic patients (Fig. 1B) have established that L-arginine is transported via system y^+L, whose activity we reported is elevated 2-fold in platelets taken from well-nourished patients as compared to malnourished uremic patients (BMI: 16±1 vs 22±2 kg/m^2; albumin: 3.4±0.6 vs 3.7±0.2 g/dl) [18]. As shown in Fig. 1A, L-arginine influx in platelets was inhibited significantly by L-leucine and *p*-chloromercuribenzoate (PCMBS), both previously shown to inhibit system y^+L transport activity in erythrocytes (data not shown). Our findings establish for the first time that transport rates of L-arginine are similar in control and malnourished platelets, and that changes in initial rates of transport are correlated with alterations in NO production (Fig. 1C, D) [18].

In recent experiments, we further demonstrated that basal and ADP-stimulated rates of NO production are elevated in uremic platelets and that L-leucine (0.1–5 mM, inhibitor of L-arginine transport via system y^+L) significantly reduced basal rate of NO production in platelets from normal and uremic patients [77]. Moreover, we established that the sulfhydryl reagent *N*-ethylmaleimide (NEM, used to inhibit system y^+ in erythrocytes) stimulates L-arginine transport via system y^+L and NO production in human platelets [63]. As NEM also increases the Na^+ content of platelets by inhibiting Na^+/K^+-ATPase, this would provide an increased driving force for the obligatory exchange of extracellular L-arginine

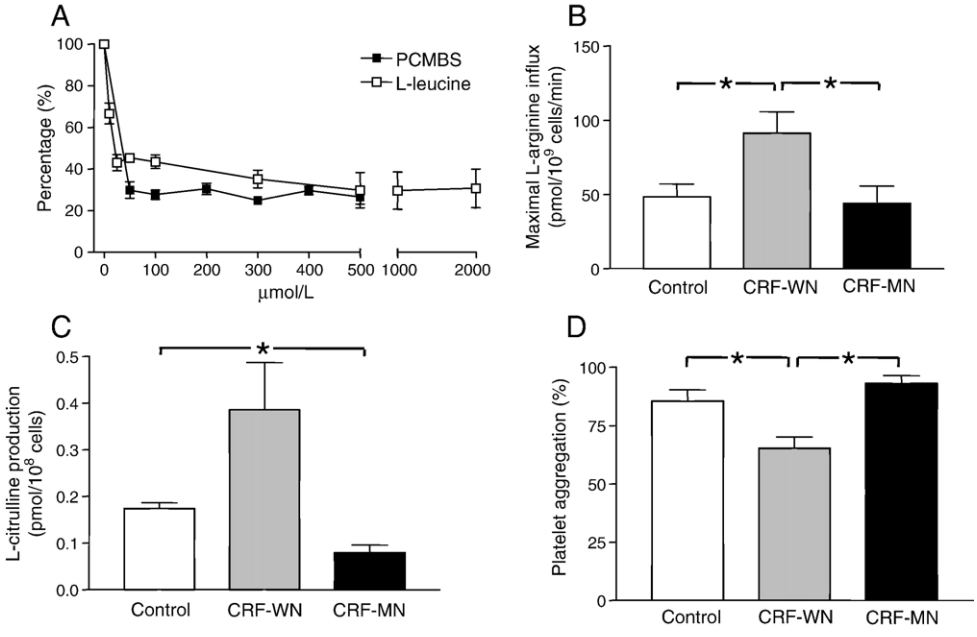

Fig. 1. Characteristics of the L-arginine–nitric oxide pathway in human platelets from control and well-nourished and malnourished chronic renal failure patients. A, inhibition of L-arginine influx by L-leucine (competitive inhibition) and *p*-chloromercuribenzenesulfonate (PCMBS, non-competitive inhibition by mercuryl agent) in washed platelets from control subjects. B, L-arginine transport in washed platelets from controls and well-nourished (CRF-WN) and malnourished (CRF-MN) uremic patients. C, Nitric oxide production assayed by measuring conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline in washed platelets. D, Comparison of collagen (2 μg ml^{-1}) induced platelet aggregation in platelet-rich-plasma from controls and uremic patients. Data denote the means±S.E. of measurements in 6–14 different individuals, *$p<0.05$. Data replotted from Ref. [18] and unpublished data.

(and other cationic amino acids) for intracellular Na^+/neutral amino acids [79,83]. Our observation of an increased production of NO by platelets exposed to NEM is consistent with the report that NEM inhibits thrombin-induced platelet aggregation by increasing intracellular cGMP and cAMP levels in resting and stimulated cells [84]. Interestingly, when we treated human platelets with PCMBS (another sulfhydryl reagent), system y^+L activity was inhibited at a concentration of 50 μM (Fig. 1A).

Since protein kinase C (PKC) is activated by α-adrenergic receptors in human platelets and NEM has been reported to inhibit PKC, it is likely those specific inhibitors (calphostin C, staurosporine) and activators (phorbol ester PMA vs inactive analogue) of PKC may affect L-arginine transport and NO synthesis [85–87]. Although many inhibitors and activators of PKC are not specific and there are multiple PKC isoforms, such experiments would readily establish whether PKC is involved as a modulator of L-arginine transport in uremia [88]. Convincing studies have shown that phosphoinositide 3-kinase (PI-3K) and Akt (protein kinase B) play key roles in platelet activation, leading to activation of NOS, soluble guanylyl cyclase and PKG [89].

4. Uremia-induced changes in NOS activity and in eNOS and iNOS protein levels

NO is one of the regulators of renal hemodynamics, and experimental evidence indicates that renal NO deficiency may occur in CRF, correlating with signs of renal injury [17]. Local conversion of L-arginine to NO in the kidney seems to be a pre-requisite for normal kidney function. All three isoforms of NOS have been identified in the kidney [42], and NO modulates renal blood flow, glomerular surface area available for filtration and glomerulotubular feedback response [17]. Chronic inhibition of NO synthesis will thus affect the function of all of these mechanisms [90]. To our knowledge, data are not available on the altered expression of NOS isoforms in platelets from uremic patients, even though uremic platelets apparently generate more NO and have higher levels of cGMP (NO second messenger) than control platelets [29]. We have measured eNOS and iNOS protein expression in humans platelets from control and well-nourished and malnourished CRF patients and found that both eNOS and iNOS levels were not significantly different in uremic and control platelets (data not shown).

5. Oxidative stress and nitration of platelet proteins in uremia

The reaction of NO with superoxide anions (also generated in platelets during activation) forms the powerful oxidant peroxynitrite. As a consequence, some of the NO formed is destroyed reducing its effectiveness as an antagonist of platelet aggregation [91,92]. Additionally, eNOS can promote superoxide production (eNOS uncoupling) under conditions of diminished availability of the substrate L-arginine or of the essential cofactor tetrahydrobiopterin (BH4) [93]. This transformation of eNOS from a protective enzyme to a contributor to oxidative stress has been demonstrated in animal models of acute and chronic renal failure in the presence of L-arginine and/or BH4 deficiency [18,94–96].

An imbalance between oxidant production and antioxidant defence mechanisms has been documented in patients and animal models of uremia [97–99]. Elevated levels of superoxide and peroxynitrite are present in chronic renal failure [97]. Likewise, reduced expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase have been reported in different stages of CRF [100]. The excessive vascular production or diminished metabolism of ROS by de-regulation of antioxidant enzymes is likely to increase quenching of NO in this pathology [101].

Peroxynitrite has been shown to inhibit or activate platelets depending on the concentration added and can also lead to the nitration of tyrosine residues in proteins [102]. Indeed, during collagen-induced activation of platelets, spontaneous nitration of platelet proteins may occur, specifically that of vasodilator sensitive phosphoprotein (VASP), which is normally an important target protein for NO-induced inhibition of platelet aggregation [91,102]. It is unclear what role this may play in normal platelet function, but it has been suggested that nitration affects signal transduction mechanisms, possibly by influencing tyrosine phosphorylation or dephosphorylation [102]. In a number of inflammatory disorders, extensive nitration of proteins is a common event. Using a competitive ELISA, it has been shown that nitration of proteins increases dramatically in the plasma of children with chronic renal disease, and recent evidence further suggests that tyrosine nitration of alpha-actinin could affect platelet adhesion [5,103,104].

6. Platelet and endothelial function in uremia: a role for L-arginine supplementation

Atherogenesis in advanced chronic renal failure may be the consequence of a synergism of malnutrition, inflammation, oxidative stress, and impaired platelet and endothelial signalling [105,106]. As endothelial dysfunction is a common pathological feature of patients with mild-to-severe renal failure, this abnormality together with platelet activation can lead to atherothrombosis associated with a dysfunction of the L-arginine–NO pathway [4,5,105].

Our underlying hypothesis is that activation of system y^+L activity in human platelets in CRF provides a mechanism for enhanced platelet NO production to reduce aggregation and attenuate both thrombosis and atherosclerosis in uremia [63]. In conditions of malnutrition, the additional increase in oxidative stress and deficiency in plasma L-arginine may further exacerbate the occurrence of atherosclerotic events. Comparative studies of platelet function in malnourished and well-nourished uremic patients complemented by parallel studies of L-arginine supplementation may provide insights

into the mechanisms underlying the cardiovascular alterations observed in CRF.

Increased concentrations of ADMA in the plasma of patients with CRF may provide an alternative explanation for why, in a state of increased transport of L-arginine and NO production such as uremia, NO bioavailability is reduced and endothelium-dependent relaxation is impaired. In support of this hypothesis, increased plasma concentrations of ADMA are associated with important markers of atherosclerosis, such as carotid artery intima-media thickness and cardiovascular mortality in patients with end-stage renal disease [2].

An attractive approach to increase bioavailable NO from the endothelium in uremia would be to supplement L-arginine, however, a therapeutic role for L-arginine supplementation in CRF remains controversial. A single study using an acute infusion of L-arginine (100 nmol/min) reported an improvement of venodilation in response to acetylcholine in CRF patients (Table 2). Chronic administration of L-arginine (9 g/d for 9 days + 18 g/d for 9 more days or two months) also seems to normalise systolic and diastolic blood pressure in uremia [4]. In contrast, in children with CRF chronic administration of L-arginine (2.5–5 g 3×/day for 4 weeks) failed to restore brachial artery function at rest or during increased blood flow [5]. Indeed, two further studies have not shown an improvement of blood pressure following chronic L-arginine supplementation in patients with moderate CRF and chronic transplant dysfunction [25,62]. We believe that larger controlled studies should be undertaken in different stages of CRF to determine the efficacy of L-arginine supplementation in attenuating endothelial dysfunction in these patients and preventing cardiovascular events.

7. The arginine paradox in uremia and atherosclerosis

Accumulating evidence suggests that a supply of L-arginine for NO synthesis may be derived from compartments distinct from the bulk intracellular amino acid pool, e.g. near plasma membrane caveolae or 'lipid rafts' (reviewed in Ref. [78]). Since extracellular L-arginine modulates NO synthesis and therefore platelet activation, it is essential to maintain an adequate supply of L-arginine to avoid platelet aggregation in the pro-thrombotic milieu of uremia. The intracellular concentration of L-arginine (~0.1–4 mM in endothelial cells, macrophages and smooth muscle cells) is usually well above the K_m for NO production [107–109]. Notably, others have shown that variations in intracellular L-arginine concentration over 100-fold cannot change NO production [110]. This observation, that extracellular L-arginine seems to drive NO synthesis despite an excess of intracellular L-arginine, is known as the "arginine paradox" [111]. Although recent studies *in vivo* highlight that bioavailability of exogenous L-arginine modulates vascular NO production [112,113], evidence for a direct association between L-arginine transporter(s) and NOS in plasmalemmal caveolae remains to be reported. In inflammation there is a more compelling case for the dependency of iNOS on extracellular L-arginine supply/transport for NO generation. Exposure of circulating blood cells (and smooth muscle cells) to cytokines leads to an induction of iNOS a lag period of 2 h, reaching a maximum between 6 and 12 h. Induction of iNOS is inhibited by glucocorticoids and depends upon a supply of extracellular L-arginine [109,114,115].

8. Conclusions

Many factors are implicated in the upregulation of system y^+L and NO synthesis in platelets in uremia. Among these the low concentration of plasma L-arginine seems to be a key factor involved in the activation of L-arginine uptake in platelets in well-nourished CRF patients (Fig. 1B). Increased NO production in platelets from well-nourished uremic patients (Fig. 1C) is consistent with the prolonged bleeding time observed in CRF, notably reversed in uremic rats following L-NMMA infusions [116,117]. Platelets from well-nourished uremic patients on hemodialysis synthesize more NO than controls [29,77]. Interestingly, platelets from uremic patients seem to have a diminished response to NO, supporting the hypothesis that NO synthesis in these cells may already be upregulated [73,77]. It is possible that activation of the high-affinity y^+L transport system in uremia provides a unique mechanism for maintaining L-arginine supply for platelet NO synthesis in a milieu of reduced arginine availability and pro-thrombotic factors such as increased fibrinogen concentration (Table 1).

We have established that reduced plasma L-arginine and NO production and elevated cytokine levels (Table 1) are associated with increased aggregability of platelets taken from malnourished uremic patients (Fig. 1D). Our findings may explain the increased cardiovascular mortality in patients with deficient nutritional status, leading to inflammation, oxidative stress, impaired L-arginine–NO signalling and subsequent activation of platelets. Nevertheless, it is still uncertain whether longer-term L-arginine administration can delay the progression of chronic renal failure in humans, and thus further clinical trials will need to evaluate the therapeutic potential of L-arginine supplementation on vascular reactivity in patients with chronic renal failure.

Acknowledgements

We gratefully acknowledge the financial support of a Wellcome Trust Collaborative Research Initiative Grant (A.C.M.R., J.C.E. and G.E.M.) and CNPq-PQ (Brazil). We acknowledge our co-authors in previous publications and thank Dr Monique Moss, Clarissa D. da Silva and Mariana Siqueira for their valuable collaboration in the platelet experiments.

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