REVIEW ARTICLE

The clinical effects of L-arginine and asymmetric dimethylarginine: implications for treatment in secondary Raynaud's phenomenon

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Abstract

Secondary Raynaud's phenomenon (RP) is often the sentinel clinical finding in systemic sclerosis and may precede systemic disease by several years. Altered nitric oxide metabolism plays a critical role in both fibrosis and severe secondary RP phenotypes in these patients. Increased flux through inducible nitric oxide synthase (iNOS) drives cutaneous fibrosis. Failure of flux through endothelial nitric oxide synthase (eNOS) contributes to increased vasoconstriction and decreased vasorelaxation. The underproduction of nitric oxide by eNOS is in part due to increased levels of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase. The inhibitory effects of increased ADMA levels may be counteracted increasing serum L-arginine, which is often an effective treatment strategy in these patients. As such, L-arginine-based therapies should be considered in managing secondary RP, particularly given their favourable safety and tolerability profile. While there is no established dosing regimen, studies of oral L-arginine in secondary RP suggest that divided dosing may begin at 1–2 g/day and may be titrated up to 10 g/day. Conversely, primary RP is not associated with increased ADMA production which likely accounts for the failure of L-arginine trials to show benefit in primary RP.

Received: 5 February 2018; Accepted: 6 July 2018

Conflicts of interest

The authors have no conflict of interests to disclose. The authors do not have grants or additional technical support to disclose. This manuscript is not under consideration elsewhere and has not been previously published.

Funding sources

We are grateful for support from the Frances & Benjamin Benenson Foundation and the Lynn & William M. Silverman Foundation.

Introduction

Raynaud's phenomenon (RP) is characterized by an abnormal vascular response to cold temperatures, stress and other factors. Patients typically present with episodic, sharply demarcated bi- or triphasic colour changes of the digits.[1] First, an increased response to sympathetic stimuli induces severe vasoconstriction causing the skin to become white. Subsequently, as tissue oxygenation remains low the digits may become cyanotic giving them a dusky blue appearance. Finally, blood flow is restored causing the tissue to become hyperemic and red.

The pathogenesis of RP is believed to involve local defects in the arteries supplying the digits, though the precise nature of these abnormalities varies depending on the underlying disease state. The majority of RP patients have primary RP and no underlying systemic disease. In these individuals, vasospasm is thought to occur secondary to increased alpha-2 adrenergic responses in the digital and cutaneous vessels and does not result in vascular pathology.[2] Conversely, secondary RP is associated with autoimmune connective tissue disease, most commonly systemic sclerosis (SSc) and is accompanied by structural and biochemical changes in the endothelium and blood vessels.[2] Affected patients are especially prone to severe phenotypes with frequent, painful attacks of RP that can lead to ulceration and tissue necrosis.

The biochemical pathways underlying abnormal endothelial function and structural remodelling in patients with secondary

RP in the setting of SSc are becoming increasingly well understood. Specifically, affected endothelial cells demonstrate increased exocytosis of endothelin 1 and ultralarge von Willibrand factor (ULVWF) which contribute to vasospasm and capillary thrombosis, respectively. Additionally, increased TGF-β, cytokines, endothelin 1 and angiotensin II are believed to drive myofibroblast proliferation, vascular fibrosis and dropout in SSc patients.[2,3]

Also involved is nitric oxide (NO), which plays a central but paradoxical and double edged role in the pathophysiology of secondary RP and systemic sclerosis.[4,5] Decreased endothelial production of nitric oxide leads to impaired vascular relaxation and prolonged vasoconstriction. Conversely, over production of nitric oxide leads to increased generation of reactive oxygen species and plays a pathologic role in fibrosis.[6] Recent advances in the understanding of NO metabolism and its cellular effects in secondary RP may inform and improve clinical management of these patients. This review will focus on the mechanisms underlying NO signalling, its implications in secondary SSc and the therapeutic application of L-arginine in select patients to augment NO signalling.

Role of NO in secondary RP and systemic sclerosis

NO is produced by multiple nitric oxide synthase isoforms including neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS) and endothelium nitric oxide synthase (eNOS).[5,7] Arginine, a semi-essential amino acid, is the substrate for all of these enzymes. Both iNOS and eNOS play critical roles in normal skin physiology as well as SSc and secondary RP pathology. iNOS is induced by increased levels of cytokines and mediators including IL-1, interferon-gamma, TNF-alpha and lipopolysacharide and is expressed in keratinocytes, fibroblasts, macrophages and endothelial cells.[8,9] Healthy individuals do not constitutively express iNOS, and flux through the iNOS pathway is minimal. Nitric oxide generated through the iNOS pathway is physiologically important in wound healing and scar formation, where it is frequently elevated.[10–12] It is also important in combating skin infections, including cutaneous leishmaniasis.[13,14] Flux through the iNOS pathway is pathologically increased in a variety of cutaneous diseases including psoriasis, atopic dermatitis, Stevens–Johnson syndrome and melanoma.[8] Unsurprisingly, many studies have demonstrated elevated NO from the iNOS pathway in SSc, and it is believed to play an important role in mediating fibrosis.[4,6,15–18] This may account for the paradoxical observation of high NO levels in SSc, as iNOS is found in multiple cell lines including fibroblasts and quantitatively produces the majority of NO in its upregulated state.[6,9]

Conversely, eNOS is constitutively active and expressed primarily by endothelial cells.[9] It generates proportionally small quantities of NO and is critical in maintaining vasodilatation via cyclic GMP mediated relaxation of vascular smooth muscle.[9,19,20] Nitric oxide generation through this pathway is impaired in secondary RP and contributes to increased vasoconstriction and failure of vasodilatation. Impairment in local endothelial NO synthesis also contributes to other pathologic processes in SSc including pulmonary artery hypertension (PAH).

Early SSc with mild phenotypes is associated with largely normal eNOS and low iNOS expression.[21] In advanced or severe phenotypes the converse is true, iNOS predominates and eNOS is underexpressed. Functionality of eNOS may be further diminished by increased utilization of necessary cofactor tetrahydrobiopterin (BH4) by high constitutively high iNOS levels.[19] Finally, nitric oxide generated through eNOS is also decreased through the action of asymmetric dimethylarginine (ADMA), an endogenous eNOS inhibitor in the setting of SSc.[22]

Impaired eNOS activity has been shown to contribute to vascular proliferation and pathologic remodelling in several models. Specifically, animal studies on endothelial injury using balloon angioplasty demonstrate decreased eNOS activity following injury, as well as neointimal hyperplasia and shrinking of vascular lumen.[23–25] This is partially recovered by increasing NOS expression through gene therapies.[26] A similar mechanism involving decreased eNOS activity may play a role in medial thickening and vascular dropout in SSc-related secondary RP.[2,3,27] Additionally, nitric oxide is a potent inhibitor of platelet aggregation.[28,29] Impairment in this functionality likely contributes to pathologic platelet aggregation that contributes to capillary thrombosis in SSc.[3]

Perhaps, most critically, impaired endothelial NO generation plays a role in the prolonged digital occlusion and failure of relaxation seen in secondary RP patients.[2] Nitric oxide relaxes vascular smooth muscle by increasing the concentration of cyclic GMP.[20] Clinically, increasing NO or potentiating its effects are useful therapeutic strategies for RP patients. Phosphodiesterase-5 (PDE-5) inhibitors are an established therapy with a recent meta-analysis of randomized control trials (RCTs) supporting their clinical benefit with respect to frequency, duration and severity of attacks.[30] They potentiate the effects of available NO by inhibiting the hydrolysis of cyclic GMP. Another approach is to increase the quantity of NO that is available through various nitrate preparations. Topical nitrates provide exogenous NO after metabolism by mitochondrial aldehyde dehyrogenase.[31] A recent meta-analysis conducted by our research group demonstrated the effectiveness of locally applied topical nitrates in treating RP.[32] Alternatively, local NO production may be increased through L-arginine supplementation.[33] Developments in the biochemical understanding of ADMA and its effect on this pathway are critical in understanding which RP patients may benefit from L-arginine treatments (Fig. 1).

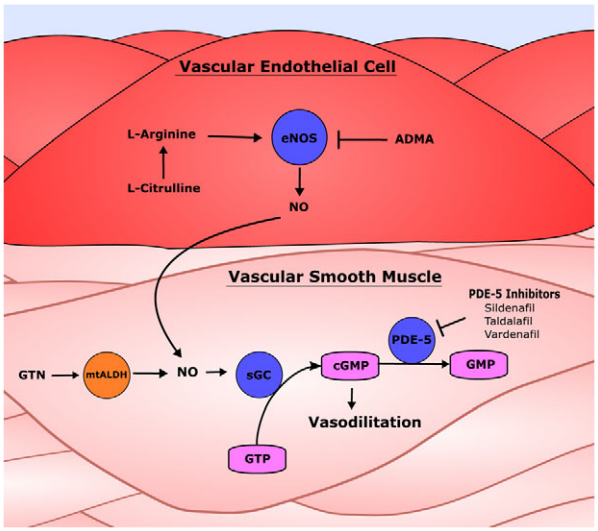

Figure 1 Sources and effects of nitric oxide on vascular smooth muscle. Endothelial cells produce endogenous nitric oxide through endothelial nitric oxide synthase (eNOS) metabolism of L-arginine. Exogenous nitric oxide is produced by topical or systemic nitrates through mitochondrial aldehyde dehydrogenase (mtALDH). Nitric oxide stimulates soluble guanylyl cyclase (sGC) to produce cyclic GMP resulting in vasodilatation. Breakdown of cyclic GMP is inhibited by phosphodiesterase-5 (PDE-5) inhibitors.

Asymmetric dimethylarginine is a critical regulator of NO production in multiple diseases

Asymmetric dimethylarginine is an endogenous competitive inhibitor of nitric oxide synthase and is elevated in a host of vascular pathologies. ADMA is largely absent in healthy individuals, and overexpression may decrease local endothelial production of NO via its inhibitory affects on NOS.[22,34] Furthermore, ADMA levels and the arginine-to-ADMA ratio are early markers of endothelial dysfunction and disease severity in conditions characterized by persistent vasoconstriction or impaired vascular relaxation including essential hypertension, coronary artery disease (CAD), peripheral vascular disease, preeclampsia and pulmonary hypertension.[35–38] Importantly, the inhibitory effects of ADMA may be overcome by increasing serum arginine, the substrate for nitric oxide synthase.[39]

Evidence describing the pathologic role of ADMA is most robust in the cardiovascular literature, where endothelial damage is a sentinel event in atherosclerotic plaque formation.[40,41] Early in the pathogenesis of atherosclerosis, the arginine-to-ADMA ratio becomes deranged and at more advanced disease stages, elevated ADMA levels predict cardiovascular risk and mortality.[42–46] Recent studies demonstrate that in CAD and PVD, administration of L-arginine can improve end-organ blood flow through increased NO generation, overcoming the inhibitory effect of ADMA on NOS.[47–51]

Another disease characterized by endothelial dysfunction and failure of vascular smooth muscle relaxation is preeclampsia. Preeclampsia is caused in large part by failure of endothelial mediated vasodilatation of the placental circulation.[52] ADMA levels and the arginine-to-ADMA ratio are identified as early predictors of the development of preeclampsia.[53,54] Due to its favourable safety profile, L-arginine is an attractive treatment option for pregnant women. Recent meta-analysis of published clinical studies supports the effectiveness of L-arginine in treatments to prevent the development of preeclampsia in healthy and hypertensive women.[55] Of particular interest, pregnant women with SSc are at increased risk of hypertensive complications related to their underlying vascular disease.[56] A small report of four pregnant SSc patients with preeclampsia suggests that L-arginine may decrease pregnancy complications and improve outcomes in this population.[57] Additionally, one patient who developed severe digital ulcerations due to RP saw dramatic improvement after repeated infusions of IV L-arginine.

In SSc-related PAH, ADMA levels are almost universally elevated.[38,58] An increase in ADMA often precedes clinical disease, and ADMA testing is being explored as a screening marker for the development of SSc-related PAH.[58,59] Clinically, ADMA levels in these patients are inversely correlated with the 6-min walk test and exercise capacity and may be used as an indicator of disease severity.[38]

Effects of ADMA in Raynaud's phenomenon and L-arginine treatment

Primary RP is due to an abnormal neuronal response rather than true endovascular pathology and ADMA levels are normal and similar to healthy individuals. Thus, there is extremely limited evidence to support any therapeutic benefit for L-arginine in these patients.[7,60] When ADMA levels are low, eNOS flux is near the V_{max} at physiologic arginine levels.[61] Consequently, clinical studies of L-arginine in healthy individuals generally fail to show beneficial effects on blood flow.[34,62–65] Similarly, clinical trials using L-arginine in primary RP consistently demonstrate no appreciable effect.[66,67] Whether or not there is a long-term protective effect of arginine supplementation in these patients is speculative and beyond the scope of this article.

Studies on ADMA levels in patients with secondary RP and SSc have yielded interesting but inconsistent results likely reflecting diverse pathologic processes, disease stages and clinical phenotypes among these patients. A large study of 187 SSc patients showed similar ADMA levels to controls.[68] A separate RP study including 89 patients with primary RP, dSSc and lSSc demonstrated significantly elevated ADMA levels, in the dSSC group alone.[7] Primary RP, lSSc and healthy controls all exhibited similar ADMA levels.[7] Other recent studies have supported ADMA levels are elevated in dSSc patients.[69] Interestingly, in SSc patients who develop PAH (predominantly lSSc subtype), ADMA is almost universally elevated.[38,58]

Table 1 Clinical studies assessing L-arginine treatments of primary and secondary Raynaud's phenomenon

Study	Year	Methods	*N*	SRP	PRP	Healthy	Outcome
Agostini[67]	1991	Comparison of oral L-arginine (4 g BID) on vasodilatation in PRP and SRP during cold challenge test.	12	7	5	0	L-arginine increased vasodilatation in the cold induced challenge study in the SRP but not PRP study groups in both cooling ($P < 0.05$) and warming ($P < 0.02$) phases of laboratory-based experiment. No effect was seen in PRP study group
Freedman[72]	1999	Laboratory-based repeated-measures study of intra-arterial (brachial) infusions of L-arginine (8.5 g) or sodium nitroprusside (50 μg) on frequency of RP attacks compared with control hand.	15	15 (lSSc 8, dSSc 7)	0	0	Significantly fewer fingers demonstrated attacks of RP as assessed by independent blinded reviewers in the treatment arm ($P = 0.02$) when compared with the control hands. No significant difference was seen between infusions of nitroprusside and L-arginine
Giuggioli[57]	2010	Case series of four women treated with systemic sclerosis who were treated with L-arginine during pregnancy. Two patients were treated with 3.3 g of oral L-arginine daily from weeks 16 forward. Two patients with severe complications received IV L-arginine 40 g/day for 7 days then decreased to 20 g/day.	4	4 (lSSc 3, dSSc 1)	0	0	All patients experienced subjective improvement in RP frequency and severity of attacks. One patient experienced improvement in existing digital necrosis. Two patients with planned pregnancy who were preemptively placed on oral L-arginine experienced no complications. One patient treated with IV L-arginine saw improvement in maternal–fetal haemodynamics. One patient treated with L-arginine following development of intrauterine growth restriction and preeclampsia saw clinical improvement but delivered at 28 weeks and the neonate died from pulmonary complications of prematurity.
Khan[66]	1997	Double-blind crossover trial placebo-controlled trial of L-arginine (8 g/day) on digital blood flow in PRP and healthy control patients. Blood flow was assessed by laser Doppler flowmetry.	10	0	10	10	No significant improvement was seen in blood flow in patients treated with L-arginine compared with placebo in the PRP group or the healthy control group.
Rembold[73]	2003	Case series of four patients with severe RP, two with digital necrosis, treated with L-arginine. All patients were refractory to multiple other therapies including calcium channel blockers, topical nitrates and surgical sympathectomy.	4	4†	3†	0	Two cases experienced dramatic improvement and reversal of digital necrosis on treatment with oral L-arginine (2 g TID and 500 mg QID). Other two reports demonstrated digital cyanosis with some tissue breakdown due to severe RP. Cases were improved significantly on oral L-arginine 500 mg QID. All patients experienced improvement longitudinally over years with return of severe phenotypes on withdrawal of medication.

†Three patients described as having primary RP, but with evidence of digital necrosis suggesting a secondary aetiology.
IV, intravenous; PRP, primary Raynaud's phenomenon; SRP, secondary Raynaud's phenomenon.

It is apparent that factors beyond ADMA contribute to secondary RP given that 95% of SSc patients exhibit RP in the absence of universally elevated ADMA. Nevertheless, a recently published 3-year longitudinal study of biochemical markers in 77 SSc patients demonstrated increasing ADMA levels to be the strongest serologic risk factor for the development of new digital ulcers and a predictor of future ulcers.[70] This suggests that, similar to PAH, ADMA levels may be an indicator of RP severity and an early predictor of digital necrosis in SSc. Additionally, a cohort study of 40 connective tissue (CT) disease patients demonstrated elevated levels of ADMA in secondary RP patients compared with healthy controls, suggesting ADMA levels are linked to RP pathology in multiple CT diseases.[71]

Asymmetric dimethylarginine functions as an inhibitor of eNOS and limits the production of endogenous NO that may be critical for vasodilatation. From a kinetic standpoint, the inhibitory effects of ADMA are overcome by increasing serum-free arginine.[61] However, there are no large RCTs studying L-arginine as a treatment for secondary RP. As discussed, several clinical trials have been performed on primary RP patients who are unlikely to derive benefit given normal levels of ADMA and normal local endothelial NO production. Conflation of the effects in these two distinct diseases may have diminished early optimism for the effectiveness of L-arginine in secondary RP.

Several small observational studies and laboratory-based clinical trials have reported a dramatic benefit in secondary RP patients, including those with severe phenotypes (Table 1). One laboratory-based clinical trial demonstrated intra-arterial infusions of L-arginine or sodium nitroprusside were able to reduce the incidence of RP attacks in response to cooling in a repeated-measures study of 15 patients with SSc compared to no treatment.[72] Strikingly, differences between sodium nitroprusside and L-arginine

treatments were not statistically significant. A second comparative study between primary RP and seven secondary RP SSc patients demonstrated a significant improvement in blood flow to cold challenge after 8 g of daily oral L-arginine in the secondary RP group only.[67]

L-arginine also appears to benefit patients with more advanced RP phenotypes, who are likely to have higher ADMA levels.[70] In a case series of four patients with non-healing necrotic ulcerations due to RP in patients refractory to calcium channel blockers, L-arginine was able to revitalize necrotic appearing tissue and improve symptoms.[73] All patients experienced worsening of symptoms when discontinuing L-arginine which was improved upon resuming therapy. One patient experienced recurrence of necrotic ulcers after discontinuation which was again reversed by L-arginine therapy.

As previously described, a case series of four preeclamptic women with SSc demonstrated significant improvement in RP symptoms with arginine.[57] One patient experienced reversal of digital necrosis following weekly IV infusion of L-arginine. In this study, high ADMA levels from preeclampsia in addition to SSc may have contributed to RP phenotypes and response to L-arginine.

Factors influencing L-arginine therapeutics

Despite the absence of large RCTs, the aforementioned reports demonstrate that some secondary RP patients are able to derive significant clinical benefit from treatments aimed at increasing local endothelial NO levels. In the case of L-arginine, supplementation is meant to overcome ADMA mediated NOS inhibition. However, it is unlikely that secondary RP patients with normal ADMA levels will reap significant benefit, as similar to patients with primary RP, eNOS is saturated at physiologic arginine levels. In the absence of testing serum ADMA levels, some patients may be stratified based on their clinical features. SSc patients with PAH (even mild or preclinical), dSSc and severe RP phenotypes (digital tuft digits or digital necrosis) are more likely to have elevated ADMA levels and therefore may stand to benefit most from treatment with L-arginine. It is possible that patients receiving exogenous NO from other sources (e.g. topical nitrates) may receive a more marginal benefit. One study demonstrated that while L-arginine improved secondary RP when given alone, there was no additional benefit of L-arginine with IV infusion of sodium nitroprusside, suggesting that the NO-mediated vasodilatation may be saturated by sources of exogenous NO.[74] Conversely, PDE-5 inhibitors potentiate the effects of endogenous NO, and patients may experience theoretical synergistic effects of using these medications in concert.

It is important to consider that the pathology of secondary RP is multifactorial and not limited to an acute lack of NO.[2] While luminal shrinking due to myofibroblast proliferation may in part be related to failure of NO generation, pathologic remodelling is unlikely to be reversed by increasing eNOS flux through L-arginine supplementation.[75,76] As SSc progresses, external vascular compression by fibrosis of the tunica adventitia makes meaningful dilatation impossible. These patients generally require surgical stripping of the adventitia to reestablish digital perfusion.[77] Thus, the presence of a severe phenotype does not universally indicate a probable response to L-arginine.

Treatment recommendations

There is no established dosing regimen or route of administration for L-arginine for secondary Raynaud's phenomenon. The medication is generally safe and well tolerated, although patients who take oral L-arginine at 9 g or more daily may experience mild diarrhoea.[78] However individuals may tolerate up to 20 g daily.[79] There is significant intersubject variability in the bioavailability of L-arginine which may alter the effectiveness of oral L-arginine in individuals.[80] The mechanism behind this variability is not completely understood. On average, L-arginine is 20% bioavailable, and ingestion of 10 g of oral L-arginine will triple the plasma concentration.[80] Several reports showed clinical benefits at lower doses of 2–3 g daily; however, quantitative flow studies have only been performed at larger doses of 8 g daily.[57,81] Case reports of patients with necrotic digital ulcers who improved on IV L-arginine often received large 20–40 g infusions daily for several days.[57,73] Ultimately, the dose–response relationship and 'optimal' dose is likely dependent on an individual patient's ADMA level and absorption of L-arginine. Another approach may be to supplement with oral L-citrulline which is an L-arginine precursor and increases serum L-arginine.[82,83]

Additionally, L-arginine may be particularly useful in patients who are unable to tolerate systemic RP treatments that lower blood pressure such as PDE-5 inhibitors or calcium channel blockers. A practical advantage of oral L-arginine is that it is available without a prescription and is likely the cheapest available pharmacologic therapy for RP.

Conclusions

Nitric oxide metabolism and signalling plays an important and diverse role in the pathophysiology of secondary Raynaud's phenomenon. Increased production of NO via the iNOS pathway likely plays a role in progressive fibrosis and oxidative damage while decreased production of NO through the eNOS pathway is important in vascular pathologies including secondary Raynaud's phenomenon. This is in part due to elevated levels of ADMA, an endogenous inhibitor of nitric oxide synthase, which is elevated in many secondary RP patients. The inhibitory effect of ADMA can be counteracted by increasing serum levels of free L-arginine through oral supplementation or IV infusion. Due to its benign side-effect profile and affordability, L-arginine supplementation should be considered in secondary RP patients, particularly those with concomitant pulmonary hypertension, dSSc or digital ulcerations all of which are linked to elevated ADMA. A dose–response relationship is expected, but optimal dosing

ranges and effects are likely highly variable between patients. Further well-conducted randomized clinical trials are needed to clarify the benefits of L-arginine in secondary RP patients with elevated ADMA levels.

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