

Implications of endothelial shear stress on systemic sclerosis vasculopathy and treatment

T.M. Frech^{1,4,5}, D.R. Machin¹, M.A. Murtaugh¹, G.J. Stoddard¹,
S.I. Bloom³, J.V. Phibbs⁴, A.J. Donato^{1,2,3,5}

¹University of Utah, Department of Internal Medicine, Salt Lake City;

²University of Utah, Department of Exercise and Sport Science, Salt Lake City; ³University of Utah, Department of Nutrition and Integrative Physiology, University of Utah, Salt Lake City;

⁴University of Utah Hospitals and Clinics, Salt Lake City; ⁵VAMC Salt Lake City, GRECC, Salt Lake City, UT, USA.

Tracy M. Frech, MD, MS

Daniel R. Machin, PhD

Maureen A. Murtaugh, PhD

Gregory J. Stoddard, MS

Samuel I. Bloom, BS

Jessica V. Phibbs, BS

Anthony J. Donato, PhD

Please address correspondence to:

Dr Tracy M. Frech,

University of Utah,

Rheumatology, 4b200 SOM,

30N 1900 E,

Salt Lake City, UT 84132, USA.

E-mail: tracy.frech@hsc.utah.edu

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ABSTRACT

There are no Federal Drug Administration approved drugs for the treatment of systemic sclerosis vascular digital ulcers (DU) in the United States, which are thought to be an end-stage result of prolonged ischaemia due to severe, prolonged Raynaud's phenomenon. Most therapeutics for vasodilation used in SSc work different pathways to target the smooth muscle to induce vessel relaxation. Longitudinal studies of vascular function allow insight into the effects of medications used for Raynaud's phenomenon in the SSc patient population. In this review, we discuss vascular tone, the function of the endothelium in SSc, and provide the rationale for longitudinal studies of vascular function and therapeutics that target the endothelial shear stress in addition to vasodilation for treatment and prevention of DU. This review provides the rationale for vasodilatory medication use for treatment of SSc-related DU and justifies access to non-FDA approved medications for this indication.

Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease with heterogeneous multi-organ microvascular manifestations (vasculopathy) and fibrosis. Among the autoimmune diseases, SSc has a high mortality and morbidity and a lack of effective therapeutic options (1). Immunosuppressive agents that are the standard of care for other rheumatic diseases have little efficacy for SSc, and efforts to treat end-stage vasculopathy, such as ace-inhibitors for scleroderma renal crisis and vasodilators for pulmonary arterial hypertension have made the largest impact on survival in this patient population and are well-established treatments (2). Of note, the

aforementioned aspects of end-stage vasculopathy have clear clinical definitions. In contrast, other aspects of SSc-related vasculopathy, such as digital ulcers (DU) have a less clear clinical definition (3-6). Another challenge for SSc-related DU management is there are no Federal Drug Administration (FDA) approved drugs for the treatment, which are thought to be an end-stage result of prolonged ischaemia due to severe, prolonged Raynaud's phenomenon. Our previous work has suggested that a novel and important source of vascular dysfunction is at the endothelial cell level (6, 7), and is accompanied by elevated oxidative stress and attenuated antioxidant capacity (8). We have also found that this endothelial dysfunction (particularly patients with DU) may respond to an endothelial based therapeutic approach (9, 10). In this review, we discuss the rationale for vasodilators in SSc, measurement of therapeutic effectiveness of vascular-based therapeutics with non-invasive vascular imaging (e.g. brachial artery flow mediated dilation [FMD]), and implications of endothelial targeted treatment in SSc.

Vascular tone in systemic sclerosis

The role of enhanced vascular tone in the natural history of SSc is perhaps best demonstrated by the near universal presence of Raynaud's phenomenon in this patient population. This triphasic colour change of the fingers in response to cold or stress, usually precedes the development of puffy hands and skin fibrosis, and is commonly associated with microvascular abnormalities on capillaroscopy, making these findings critical for SSc classification (11). Raynaud's phenomenon is in most cases the first symptom of SSc

(12). Repeat episodes of Raynaud's phenomenon leads to prolonged digital ischaemia that may progress to digital ulceration (DU) or in extreme cases to critical digital ischaemia with gangrene. While SSc is considered a fibrosing disorder, the role of vascular tone in dysregulated endothelium is unclear. Nonetheless, end stage fibrosis via endothelial to mesenchymal transition (Endo-MT) is gaining traction (13). Endo-MT occurs when the endothelial cells delaminate from the cell monolayer, reduce cell-cell contacts, lose endothelial markers such as vascular endothelial-cadherin (VE-cadherin), gain mesenchymal markers like alpha-smooth muscle actin (α -SMA), and acquire mesenchymal cell-like properties. Whether vascular tone promotes this transition is unclear, however, fibrosis occurs at varying rates in different organs. This differential fibrosis highlights that while improvement of vascular function in all SSc patients represents a potential therapeutic goal, the disease duration and degree of organ fibrosis complicates the study and treatment of SSc vasculopathy, particularly in cross-sectional clinical studies. Evaluation of the skin microvasculature in SSc reveals absence of inflammatory cells and presence of features of oxidative stress including, swollen endothelial cells with a duplicated, lamellated appearance of the basement membrane (14-16), and regardless of limited or diffuse cutaneous subsets, disease duration, or internal organ clinical features (16). Endothelial cells are the only mesenchymal cell type that undergo apoptosis in early SSc, whereas vascular smooth-muscle cells and pericytes proliferate vigorously (17, 18). These features suggest that endothelial homeostasis is disrupted, which under conditions of stress, dysregulates the synthesis, degradation and recycling of cellular components. Endothelial cells control vascular tone by vasoactive molecules, of which nitric oxide (NO) produced by endothelial NO synthase is one of the most important for endothelium dependent dilatation (19). NO is the primary vasodilatory molecule released from the vascular endothelium in response

to stimulation by agonists, such as catecholamines; platelet products, including serotonin; autacoids formed in or near the vascular wall (including bradykinin and adiponectin), and physical factors at the vessel surface (haemodynamic shear stress) (20). NO enhances vasodilatation, reduces platelet aggression and adhesion (anti-thrombotic), prevents smooth muscle proliferation, inhibits adhesion of leukocytes and expression of pro-inflammatory cytokines genes (anti-inflammatory), and counters the oxidation of low density lipoprotein (LDL) cholesterol (21). Endothelial dysfunction is induced by a shift in the equilibrium that favours NO deficiency and enhanced reactive oxygen species (ROS) formation. Endothelial dysfunction therefore can be influenced by reduced activity of endothelial NO synthase, which is modulated by the calcium concentration and phosphorylation, or failure of NO diffuse to vascular smooth muscle (22). When NO reaches the vascular smooth muscle, it interacts with soluble guanylyl cyclase to produce cyclic guanosine monophosphate (cGMP). Under homeostasis, NO bioavailability is evidenced by an intact, robust endothelium-dependent dilation (23-26) and is, in part, responsible for mediating the angiogenic capacity, peripheral permeability, and anti-inflammatory properties of a healthy vascular endothelium (26, 27). Endothelial dysfunction, characterised by reduced NO and impaired vasodilator capacity, results in diminished peripheral tissue blood flow (28).

Functions of the endothelium in SSc

Glycocalyx

The endothelial glycocalyx is a gel-like, thin polysaccharide layer that projects from the endothelial cell wall toward the vessel lumen, protecting the vessel and playing a role in mechanotransduction of shear stress (29). The glycocalyx coats the healthy vascular endothelium, and modifies the interaction between the blood and vessel wall and helps to prevent adhesion of leukocytes and platelets. The glycocalyx can dictate the migration pattern of immune cells, which protects against

inflammation, thrombosis, abnormal perfusion and subsequent fibrosis (29). Leukocyte adhesion and infiltration into the vessel wall is an important part of the tissue inflammatory process that leads to oxidative stress and the augmented production of free radicals. As such, penetration into the glycocalyx and the perfused barrier region (PBR) is important in vascular health, particularly where immune cell extravasation is involved. Our previous work using intravital microscopy has demonstrated that mean PBR across all microvessel segments was significantly higher in patients with SSc compared with healthy age-matched controls (2.1 ± 0.0 vs. 1.9 ± 0.0 μ m, respectively; $p=0.012$). We found glycocalyx thickness was significantly lower in patients with SSc compared with controls ($p<0.001$), with PBR was significantly, inversely associated other measures of glycocalyx thickness ($r=-0.41$, $p=0.003$). This implicates that endothelial dysfunction is not only associated with enhanced vascular tone, but also blunted glycocalyx, which could allow for greater immune cell adhesion and infiltration into critical tissues to promote fibrosis.

Leukocyte adhesion/infiltration

While SSc vasculopathy is not classically inflammatory, increased infiltration of immune cells in the perivascular tissue is implicated in the pathogenesis of SSc (30-32). Immune cells are increased in peripheral tissues of patients with SSc (33) and likely result from greater adhesion of immune cells to the vascular endothelium (34). While a healthy endothelium can serve as a barrier to the movement of immune cells from the circulation into tissues, the unhealthy endothelium in SSc can augment the immune dysregulatory process (16), thereby stimulating greater infiltration of immune cells into the peripheral tissues. Oxidative stress also increases vascular endothelial permeability, which is coupled with alterations in endothelial cell signal transduction.

Oxidative stress

Transforming growth factor- β signaling, which is widely considered one of the most important pro-fibrotic factors

in SSc, causes a pro-oxidant shift in redox homeostasis and a concomitant decrease in nitric oxide (NO) signalling (35). Oxidative stress, defined as an excess production of free radicals relative to antioxidant defenses, has been documented in SSc (36). Serum and urinary markers of systemic oxidative stress are greater in SSc compared with healthy age matched controls (37-40). The functional consequences of oxidative stress are widespread, but the vascular endothelium is particularly vulnerable to oxidative damage from ROS (41). NO produced by the endothelium reacts with superoxide to form the ROS peroxynitrite (ONOO⁻), (42) resulting in reduced NO available to signal vasodilatation. ROS production, including superoxide and ONOO⁻ formation, is increased in the circulation and skin of patients with SSc (43, 44). Thus, oxidative stress is implicated as a major contributor to the reduced NO bioavailability and endothelial dysfunction, and leads to the deleterious endothelial phenotype characterised by enhanced permeability, reduced peripheral blood flow, increased immune cell adhesion and infiltration, and increased local vascular inflammation (41, 42). ROS are also considered transducers of fibroblast proliferation, collagen-gene expression, and myofibroblast phenotype conversion in SSc, which leads to pathological fibrosis (45). However, independent of markers of oxidative stress, there is still evidence of universal endothelial dysfunction in SSc (8), which may reflect the importance of disease duration and vascular adaption.

Angiogenesis

Angiogenesis, *i.e.* new vessel growth, is required for the appropriate expansion of the tissue during growth or in times of sustained or frequent tissue hypoxia (46, 47). The angiopoietin(Ang)/Tie2 system is a key regulator of vascular biology and has been reported as an important aspect of SSc vasculopathy (48). A dysregulation of membrane bound (mb) Tie2 and Ang-1, which ensures vessel stability, and Ang-2, which is inducible by vascular endothelial growth factor (VEGF), inflammation, and hypoxia is proposed (48). The

role of shear-stress on this dysregulation is unclear, however, there is clear clinical evidence of a decrease of new blood vessel growth in SSc, despite elevation of potent angiogenic growth factors (49). In healthy tissue, hypoxic stress stimulates the pro-angiogenic transcription factor hypoxia inducible factor 1 α (HIF1 α), leading to increased angiogenic factors, such as VEGF (50) and subsequent angiogenesis. SSc is thought to induce an hypoxic environment in tissues, which increases HIF1 α (51) and VEGF (52-54), but subsequent angiogenesis is blunted because of enhanced angiogenic inhibitors such as endostatin (55). While initially VEGF may be of benefit in reducing damage to small blood vessels, chronic overexpression may be deleterious and result in overproduction of angiostatic molecules including soluble VEGF receptors, endostatin, angiostatin, and soluble endoglin (49). Interestingly, higher levels of endostatin is associated with reduced eNOS activation (56), reduced NO production and apoptosis (57). Furthermore, the angiogenic actions of VEGF signaling are dependent on a functional vascular endothelium and presence of NO (58). Increased serum levels of endothelin-1 (ET-1), asymmetric dimethylarginine (ADMA, which induces oxidative stress), and VEGF are strong predictors of DU (59).

Vascular biomarkers

While vascular biomarkers of progressive vascular injury are reported in SSc (60), the ideal vascular biomarkers can measure pathways fundamental to disease pathogenesis, predicts future development of relevant outcomes, is easily measurable, and changes with effective therapy (49). Autoantibodies are reported at the first diagnosis in more than 95% of SSc patients and have been associated with distinct vascular disease subtypes and with differences in disease severity, and as such, have prognostic value for DU (61). In SSc, where compensatory angiogenesis does not occur normally in spite of an important increase in many angiogenic factors, a clearer understanding of the role of endothelial progenitors (EPCs)

homing ability to a site of ischaemia to contribute to *de novo* vessel formation is critical to understanding pathogenesis (62). While significant advances have been made in understanding the biology of EPCs and molecular mechanisms regulating EPC function, the detailed events that contribute to shear stress-induced protection in EPCs, particularly the mechanisms of signal transduction to repair injured vascular endothelial cells are insufficiently understood (63). In addition, to laboratory vascular biomarkers, clinical vascular biomarkers such as nailfold capillaroscopy (NC) provide evidence that progressive vascular abnormalities (particularly capillary loss) are associated with disease severity (such as DU), however, the role of this important vascular biomarker in the interpretation of progressive vascular shear stress is not clear (64).

In summary, while vascular biomarkers (laboratory and clinical) exist in SSc, it is critical to longitudinally characterise the dysfunctional endothelium in SSc patients. SSc endothelium is characterised by a reduced glycocalyx, impaired vasodilator capacity due to reduced NO bioavailability, and abnormal angiogenesis response, perhaps due to oxidative stress. Targeting SSc vascular pathology may require additive therapeutic approaches, which include reduction of vascular damage and loss of capillaries not compensated by new vessel formation, correction of the imbalance of vasoconstrictive/vasodilatory factors, and reduction of proliferative vasculopathy characterised by prominent intimal proliferation (65). The accurate measurement of endothelial function in response to these therapeutics is critical, particularly with regards to DU treatment.

Measuring endothelial function in systemic sclerosis by flow-mediated dilation

The non-invasive study of the natural history of SSc endothelial function can be assessed non-invasively in humans using duplex ultrasound and techniques related to the brachial artery the flow-mediated dilation (FMD) technique, which has historically been used to

measure subclinical atherosclerosis (7, 66-70). FMD is an indirect measure of endothelial function. This approach involves inflating a cuff on a limb (typically the upper forearm) to a supra-systolic external pressure for several minutes and measuring change in diameter and blood flow in a segment of an artery (typically the brachial artery) proximal to the occlusion following rapid deflation of the cuff. The ischaemia-evoked dilation of resistance vessels distal to the occlusion produces a marked temporary increase in blood flow (reactive hyperaemia, RH) in the proximal conduit arteries that can be quantified and, in turn, causes dilation (FMD) of those proximal conduit arteries. Thus, this procedure not only assesses the ability of peripheral conduit arteries to dilate in response to the physiological stimulus of increases in intravascular shear, but also the vasodilatory ability of the peripheral resistance arteries to a brief bout of ischaemia. Thus, utilising duplex ultrasound and established FMD protocols provides information on endothelial function (brachial artery flow mediated dilation), perfusion (resting forearm blood flow), and vasodilator ability (reactive hyperaemia) and has been demonstrated as a potential early clinical marker of DU risk in RP patients and in SSc patients by our group and others (7, 67). Of note FMD, is different from peripheral endothelial function measured by forearm blood dilatation response to brachial artery occlusion using non-invasive plethysmography in that FMD assesses shear rate. Nonetheless, non-invasive plethysmography provides evidence of complex pathological progression of SSc vasculopathy (71).

FMD is a procedure that requires subject preparation and standardisation (7), and is valuable as a proof-of-concept procedure for identifying aspects of endothelial dysfunction that may be valuable for future research. In our SSc cohort, we have examined all the aforementioned FMD variables at the time of routine care, in order to determine which features are most helpful for understanding DU. Of the 123 SSc patients with baseline FMD, 70 had at least two standardised FMD meas-

urements with clinical characteristics available at the time of the assessment (Table I) as previously described by our group (7). Among these patients, DU was present at baseline in 22 and 10 developed a new DU in up to 56 months of follow-up. The timeline between FMD measures for complete healing for initial DU patients ranged from 3.9 to 24.9 months (mean 6.7 months). Of the 10 patients that developed a new DU at the time of a repeat FMD measure: 2 patients developed one within 6 months; 2 between 6 and 12 months; and 3 over one year after initial measurement. We examined serial FMD values by whether a patient had ever had a DU, and adjusted the analysis for vasodilator use, days between measurements, SSc duration, and age (Table II). We found we found lower baseline flow in those with DU than those without DU ($p=0.01$). When we examined differences between FMD measures over time between those with and without DU, we found significantly lower change in baseline flow ($p=0.002$) and change in shear rate among those with DU than without DU ($p<0.001$). Thus, in this early analysis, the implications of FMD applied to routine clinical care of SSc patients support that vasodilators are acting on vascular smooth muscle to improve blood flow (perfusion) to digits and perhaps reduce DU. The identification of vascular shear rate as an important variable in DU occurrence implies that therapeutics that effect shear at the vessel wall may be an important future target.

Shear stress effect on the endothelium

Vessel wall shear stress induces biologic effects in endothelial cells that can affect the crucial balance between matrix synthesis and breakdown (72). Specifically, high laminar shear stress stimulates ECs to produce NO that might suppress synthetic smooth-muscle proliferation (matrix synthesis) (73). Pertinent to the SSc population, the distribution of laminar shear stress can be significantly affected by disrupted blood flow as well as the velocity of flow in vessels with abnormal shapes (74). Importantly, abnormal

Table I. Clinical features of systemic sclerosis patients with flow mediated dilation.

Patient characteristics (n=70)	Total number or mean
Limited cutaneous SSc	49
Diffuse cutaneous SSc	21
Female	61
Male	9
White	58
Hispanic	8
Other race	4
Systolic blood pressure (mmHg)	114.6 ± 15
Systolic blood pressure (mmHg)	70 ± 9
Mean arterial pressure (mmHg)	85 ± 9
Heart rate (beats/min)	76 ± 1
Duration of SSc at first FMD visit (years)	10 ± 9.8
Smoking	
Never	59
Former	8
Current	3
Age	55.4 ± 11.8
Calcium channel blocker	68
Ace inhibitor	1
Angiotensin receptor blocker	2
Prostacyclin analog	1
Endothelin receptor agonist	0
Phosphodiesterase 5 inhibitor	1
Anti-centromere	58
Anti-topoisomerase	9
Anti-RNA polymerase III	3
Anti-Th/To	1

laminar and oscillatory shear stress can induce pro-inflammatory/matrix-remodelling genes levels, contributing to vascular smooth muscle cells phenotypic switching from a contractile to a synthetic phenotype, and can markedly induce autophagy (75). Impaired endothelial cell autophagy in SSc (18) can further compromise compensatory shear stress-induced NO generation (76). Thus, life-style interventions and therapeutics that can elevate the endogenous endothelial repair response to vascular injury through modulation of vessel shear stress with improved endothelial function have potential value for SSc vasculopathy.

Therapeutics that effect vessel shear stress and improve endothelial cell function

Most therapeutics for vasodilation used in SSc work different pathways to target the smooth muscle to induce relaxation. Dihydropyridine-type calcium antagonist (such as, nifedipine, felodipine, and amlodipine) inhibit influx of calcium across the smooth muscle membrane to prevent vessel contraction.

Table II. Flow mediated dilation in systemic sclerosis patients stratified by presence of digital ulcerations.

Vascular function variables in 70 SSc patients at follow-up FMD	Difference between SSc patients with DU (n=32) and those without DU (n=38)	p-value
Baseline flow	-17.2 ± 6.9	0.01
Absolute FMD	-.03 ± .04	0.50
Normalised absolute FMD	0.00007 ± .00006	0.25
Relative FMD	-0.7 ± 1.3	0.58
Normalised relative FMD	-0.002 ± 0.002	0.33
Shear Rate	-5013 ± 3238	0.12
Peak Hyperaemia	31.0 ± 36	0.39
Vascular resistance	0.57 ± 0.34	0.09
Change in Baseline flow*	-40.8 ± 12.9	0.002
Normalised Absolute FMD change*	-0.00007	.30
Absolute FMD change*	-0.002 ± 0.05	0.96
Relative FMD change*	-0.3 ± 1.53	0.84
Normalised Relative FMD change*	-0.002 ± .002	0.3
FMD Shear Rate change*	-14374 ± 4037	0.0001
FMD Peak Hyperaemia change*	-33.8 ± 37.7	0.36
Normalised FMD change*	-0.00002 ± 0.00004	0.57

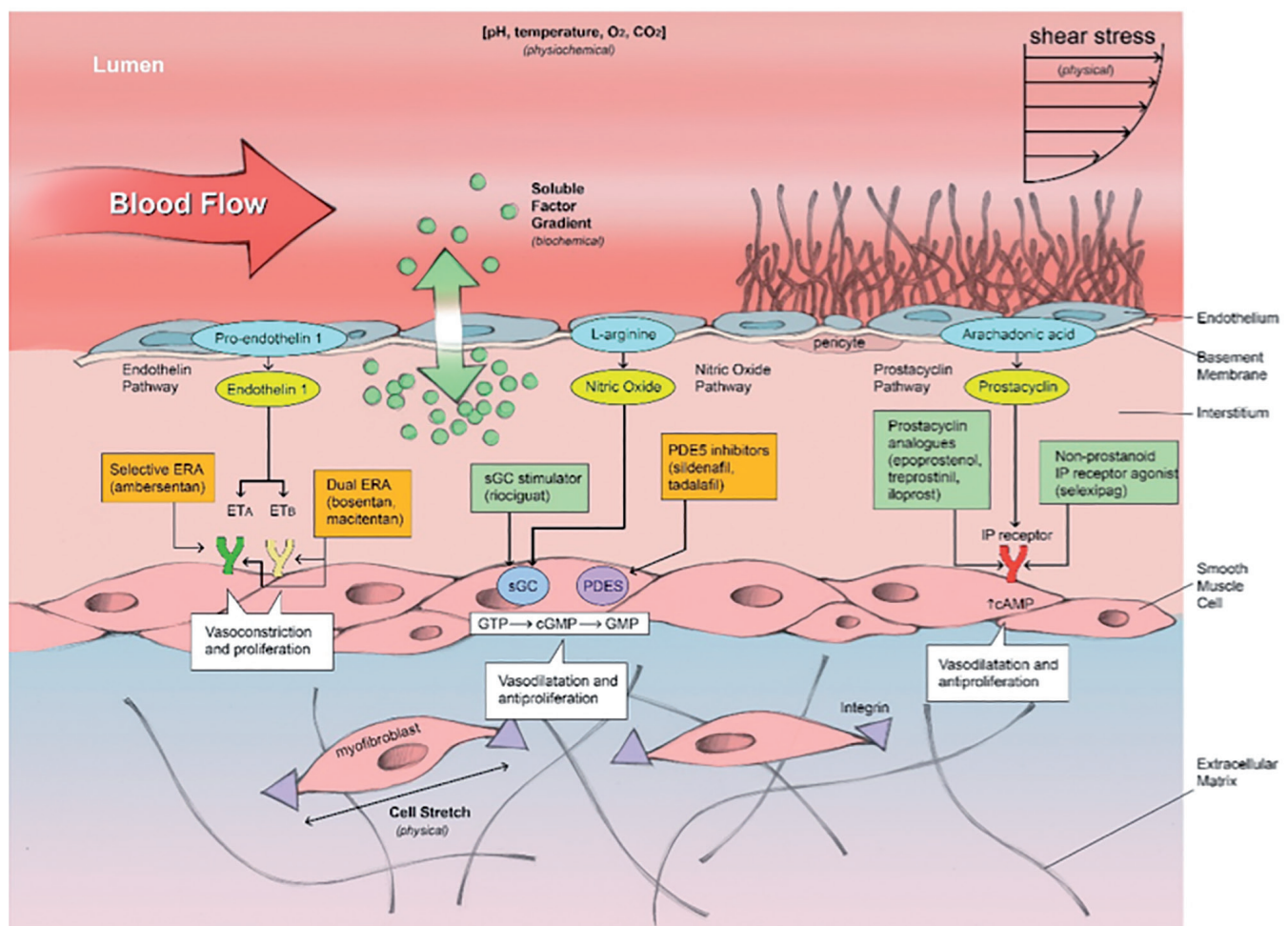
*Change between measures at baseline FMD adjusted for days between measures and duration of SSc.

Endothelin receptor antagonists can be selective (ambrisentan) or dual (bosentan, macitentan) and work through the

endothelin pathway. The prostacyclin pathway, which includes prostacyclin analogs (epoprostenol, treprostenol, il-

oprost) and non-prostanoid IP receptor agonist (selexipag), work through the arachidonic acid pathway. Phosphodiesterase type 5 inhibitors (PDE5I) (including sildenafil and tadalafil), and soluble guanylate cyclase stimulator (riociguat) act through the nitric oxide signalling pathway to induce smooth muscle vasodilation. Soluble guanylate cyclase stimulators might target both vascular remodelling and tissue fibrosis (77). The use of these classes of medications is supported by data, but access to vasodilator therapeutics is often limited by cost (78). Of note, these medications primarily work at the smooth muscle level and do not target endothelial function (Fig. 1).

To target endothelial function, the glycocalyx and activity of nitric oxide synthase may be important targets. While pathological shedding of the glycocalyx in response to mechanical shear

**Fig. 1.** Therapeutics targeting vasodilation in systemic sclerosis.

stress, inflammatory mediators, endotoxins, ischaemia-reperfusion injury, and free radicals is recognised (79), the ability of therapeutics to affect the glycocalyx has been inadequately studied, but warrants attention in SSc. Tetrahydrobiopterin (BH₄) is an essential cofactor for nitric oxide synthase, and inadequate BH₄ leads to uncoupling of nitric oxide synthase and production of highly oxidative radicals. Importantly, the guanosine triphosphate cyclohydrolase/tetrahydrobiopterin (GTPCH)/(BH₄) pathway has been proved to regulate the function of endothelial progenitor cells (EPCs) in response to vessel shear stress (80).

Numerous human studies have reported the beneficial effect of BH₄ supplementation on endothelial dysfunction caused by a variety of vascular diseases, including hypercholesterolaemia (81), diabetes (82), hypertension (83), chronic heart failure (84), and tobacco use (85). Acute oral BH₄ administration improves vascular phenotypes in patients with cardiovascular disease as well as healthy older adults (82, 85-87). In these studies adverse effects were mild, occurred in less than 5% of participants, and included headache, runny nose, nasal congestion, and sore throat. Important for SSc, BH₄ has been studied in animal models of pulmonary hypertension (88) and renal ischaemia/reperfusion injury in an animal model of aortic cross-clamping (89). Mechanistically, BH₄ has shown a positive effect on *in vivo* endothelial repair capacity of early EPC in hypertensive hyperaldosteronism patients (90). Oral BH₄ supplementation for 4 days was sufficient to improve endothelium dependent dilation measured by FMD in patients with hypercholesterolaemia (91). We have recently shown that acute administration of oral BH₄ improves endothelial function in the brachial artery in patients with SSc who had a history of DU and can be used safely with other vasodilators (9). BH₄ has not been studied in other rheumatic diseases.

Conclusions

Understanding SSc-related vasculopathy requires longitudinal studies of endothelial function which capture

vascular shear stress in the context of multi-organ disease severity. There is rationale for vasodilatory medication use for treatment of SSc-related DU and patients should have access to non-FDA approved medications for this indication. The importance of inadequate endothelial cell response to shear stress and development of DU highlights the value of longitudinal functional studies such as FMD in studies of DU. Targets that have been inadequately studied but may influence shear stress include the glycocalyx and BH₄. Importantly, endothelial targeted treatments in SSc can help all patients with Raynaud's phenomenon, and can safely supplement vasodilator therapeutics for DU providing a sound rationale for further study.

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