Q J Med 2013; **106**:839–848
doi:10.1093/qjmed/hct111 Advance Access Publication 21 May 2013

Targeting the endothelin axis in scleroderma renal crisis: rationale and feasibility

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Received 31 December 2012 and in revised form 24 March 2013

Summary

Background: We have studied endothelin-1 (ET-1) levels and ET-1 ligand and receptor tissue expression in scleroderma renal crisis (SRC) and undertaken a pilot open label safety study of bosentan, a non-selective ET-1 receptor antagonist, in SRC [Bosentan in Renal Disease-1 (BIRD-1)].

Methods: Serum levels of ET-1 were measured in healthy controls ($n = 20$) or systemic sclerosis (SSc) ($n = 80$) with or without SRC, including cases of pulmonary arterial hypertension (PAH). Renal biopsies ($n = 27$) from patients with SRC were stained for endothelin ligand and receptors. Six cases of SRC received 6 months bosentan. Outcome measures were compared with SRC cases managed at our centre from 2000 to 2004 ($n = 49$).

Results: Serum ET-1 was elevated in SRC but less than in PAH. ET-1 and both endothelin A and endothelin B receptor expression was increased in SRC biopsies in glomeruli, interstitium and hallmark vascular lesions of SRC. In the BIRD-1 cohort, serum ET-1 was elevated in all cases at SRC (median healthy controls 0.50 pg/ml; SRC 1.48 pg/ml; $P < 0.0005$), and increased further with bosentan therapy (1.46 vs. 3.05 pg/ml; t-test $P < 0.05$). Bosentan was well tolerated with no significant drug-related serious adverse events and long-term outcomes were favourable compared with historic cases. Three patients developed rebound hypertension on withdrawal of bosentan and one appeared to further benefit from maintenance therapy.

Conclusions: Upregulation of ET-1 ligand axis suggests that ET-1 receptor blockade is logical and treatment with bosentan appears to be safe in SRC. Future studies to assess therapeutic benefit and compare selective or non-selective receptor antagonists are justified.

Scleroderma renal crisis (SRC) is a life-threatening complication of systemic sclerosis (SSc), which leads to accelerated phase hypertension and acute renal failure. SRC had a very high mortality until the advent of treatment with angiotensin converting enzyme (ACE) inhibitors, following which the mortality fell appreciably (from 85% at 1 year to 24% at 1 year in a single centre[1]). SRC is more common in patients with diffuse cutaneous SSc (dcSSc) and is also associated with anti-RNA polymerase III antibodies.[2] It occurs in ~5% of all SSc cases[3] though has been reported in up to 20% of dcSSc patients.[4] Walker *et al.*[5] report SRC in only 2% Scl70+ patients in their cohort from the EUSTAR database.

There are multiple reports of elevated circulating endothelin-1 (ET-1) in SSc.[6–10] Vancheeswaran *et al.*

suggested that plasma ET-1 levels were greatest in patients with lcSSc and pulmonary vascular disease or in extensive dcSSc. Others have reported contradictory data with respect to clinical phenotypes. Although this elevation might be caused by endothelial damage, the clear efficacy of both selective and non-selective endothelin receptor antagonists as treatment for pulmonary arterial hypertension (PAH) (including SSc-associated PAH) provides very persuasive evidence for a pathogenic role for ET-1 in causing or maintaining SSc-PAH.[11–15] Further evidence for the value of blocking ET-1 in SSc-related vasculopathy comes from studies in digital ulcers, where bosentan treatment reduces the rate of new ulcer formation.[15] The vascular lesions of pulmonary, digital and renal scleroderma have marked microscopic similarities. The clinical success of blocking ET-1 activity in two of these lesions support the concept that blockade in the third should be explored.

This study explores expression of ET-1 and the specific endothelin receptor ETRA and ETRB in cases of SRC and assesses the feasibility and safety of adding 6 months of bosentan therapy within 6 weeks of the onset of SRC to ACE inhibitor. To provide context and explore potential therapeutic benefit outcomes are compared with a historic cohort of SRC cases recently managed at our centre.

Methods

Study cohort

SSc cases fulfilled the American College of Rheumatology preliminary classification criteria,[16] and SRC was defined by new onset of blood pressure >150/85 obtained at least twice over a consecutive 24 h period as well as documented decrease in the renal function as defined by a decrement of at least 30% in the calculated glomerular filtration rate (GFR). When possible, a repeat serum creatinine and recalculation of the GFR was obtained to corroborate the initial results. The following were considered as supporting evidence for the diagnosis of SRC: microangiopathic haemolytic anaemia (MAHA) on blood smear; retinopathy typical of acute hypertensive crisis; new onset of haematuria (excluding other causes); flash pulmonary oedema; oliguria or anuria; renal biopsy showing characteristic changes.

The cases included in this study included confirmed SRC that presented to our unit between 2000 and 2004 ($n = 49$), for whom serum samples and renal biopsies were available for analysis ($n = 27$) and a further six cases that were enrolled into Bosentan in Renal Disease-1 (BIRD-1), an open label clinical trial of bosentan added to standard therapy in SRC. For some subjects, serum samples were available from within 6 months before the SRC.

Measurement of serum levels of endothelin-1 and NT-pro-BNP

ET-1 was assayed in plasma using a chemoluminescent immunoassay (R&D systems Endothelin-1 Quantiglo; QET00B). Briefly, 100 µl of plasma was added to each well and incubated for 1.5 h at room temperature on a horizontal orbital microplate shaker. All wells were aspirated and washed four times. A total of 200 µl of ET-1 conjugate was added to each well and incubated for 3 h at room temperature on the shaker. Wells were washed four times, and 100 µl of Working Glo Reagent was added to each well. The plate was incubated for 10 min at room temperature in the dark. The relative luminescence unit of each well was determined using a luminometer set with the following parameters: 1.0 min lag time, 0.5 s/well read time, summation mode and auto gain on. Values were obtained from a standard curve using values from 0.34 to 250 pg/ml. N-terminal pro-B-type natiuretic peptide was measured with Roche Modular Analytics E-170 immunoassay.

Histological analysis and immunostaining of renal biopsies

Routine histology using haematoxylin and eosin and collagen staining using Sirius red were performed on all biopsy specimens. Immunostaining of formalin fixed 3 µm paraffin sections from patients with SRC ($n = 27$) and healthy controls ($n = 6$) was performed using mouse anti-ET-1 primary (Abcam), rabbit polyclonal anti-ETRA (Abcam), anti-ETRB primary (Abcam) antibodies and corresponding IgG controls. Human pancreas or appendix was positive controls. Biopsies were also stained using primary antibodies specific for transforming growth factor β1 (TGFβ1), α smooth muscle actin (αSMA), von Willebrand Factor (vWF) and CD34 (Abcam) using standard protocols.

BIRD-1 clinical trial protocol

This pilot study addresses the hypothesis that adding 6 months of bosentan therapy at standard doses started within 6 weeks of the onset of SRC to ACE inhibitor therapy is safe and well tolerated, and investigates whether there may be a beneficial effect on a small number of SRC cases. The outcome measures for this group were mortality, renal function at 6 and 12 months (including need for dialysis) and number of maintenance anti-hypertensive

therapy at 6 and 12 months. The protocol was approved by the local ethics committee of the Royal Free Hospital.

To be eligible for recruitment for BIRD-1, patients had to be age 18 or older and have SRC as defined above presenting in the preceding 6 weeks. They were not eligible if they have previously used an endothelin receptor antagonist; had significant abnormalities in liver function testing (biochemical markers more than three times upper limit of normal); had risk of pregnancy (women must be post-menopausal, surgically or naturally sterile, or use a reliable form of contraception during the study treatment, and for 3 months afterwards); had moderate or severe hepatic impairment (i.e. Childs-Pugh class B or C); had a body weight <40 kg; had conditions that prevent compliance with the protocol or failure to adhere to therapy; had any other life-threatening condition; had known hypersensitivity to bosentan; were receiving glibenclamide, cyclosporin A or tacrolimus within 1 week of screening, or expecting to receive any of these agents during the study; or have received an investigational agent in the month prior to screening.

Patients were treated with bosentan tablets 62.5 mg twice daily for 1 month, and then up-titrated to 125 mg twice daily for a further 5 months. All trial assessments were performed by the same investigator. Blood pressure was assessed after 5 min of rest while sitting. Maintenance anti-hypertensive therapy was assessed as the number of anti-hypertensive agents taken by the patient at the end of the clinic visit.

Statistical analysis

Data were analysed using Minitab 16. All significance tests were two-sided. Proportions were compared with Fisher's exact test. Continuous variables were expressed as mean values [interquartile range (IQR)]. Non-parametric data were tested with Mann–Whitney *U*-test.

Results

Measurement of circulating serum ET-1 in SRC and controls

All patients for serum measurement of ET-1 with SRC fulfilled criteria for SRC as previously published.[1] The demographic and clinical features of the cohort from which serum samples were available are summarized in Table 1. Serum samples from patients with SRC were divided into those pre-SRC ($n = 23$), during SRC [taken from onset up to 2 months after diagnosis ($n = 27$)] and those taken post-SRC ($n = 14$). Wherever possible, the same cases were included in the pre-, SRC and post-SRC cohorts. In some cases, samples were not available for the pre- and post-samples. Samples taken pre-SRC were all taken after the diagnosis of SSc had been made. The time period pre-SRC samples were taken before SRC is as follows—median was −10 months; IQR −34 to −3. For the samples post-SRC, the median time was 10 months, IQR 5.5–14. BIRD-1 patients were a separate cohort and were not included in any of the other groups.

All PAH patients had the diagnosis of PAH confirmed on a right heart catheter study. Blood samples for the analysis were obtained at the time of diagnostic right heart catheter. The patients were treated with PAH specific therapy according to standard protocols, including endothelin receptor antagonists in some cases. The median pulmonary arterial pressure (PAP) was 44 mmHg, IQR 34–52 and range 23–63. Nine patients had significant pulmonary fibrosis (PF) thought to be contributing to the PAH; with forced vital capacity measured at <70%; or significant changes on a high-resolution computed tomography scan. These cases were compared with SSc cases without PAH or SRC ($n = 27$) including eight with significant PF.

The levels of ET-1 in the serum samples included in this study are shown in Table 1 and Figure 1. ET-1 levels were elevated at the time of SRC and in PAH. Levels correlate with mPAP in the PAH cohort as have been previously reported (data not shown).

Immunohistochemical analysis of SRC

The immunohistochemical staining of SRC biopsy specimens and control tissue provides unique insight into potentially important pathogenic mechanisms of SRC and particularly the rationale for ET-1 and the endothelial axis being relevant. A total of 27 renal biopsy samples were available for analysis with details summarized in Table 1 for which serum levels of ET-1 had been assayed and the clinical and demographic features of the biopsy subgroup reflected the overall study cohort. Biopsies obtained during routine clinical management were examined together with appropriate control tissue. This study explored expression of markers of vascular and endothelial structures and remodelling as well as expression of ET-1 ligand and the two high-affinity endothelin specific receptors ETRA and ETRB.

The hallmark vascular lesions of SRC show major proliferative changes with almost complete obliteration of the lumen in many specimens. In the vascular lesions from cases with SRC, the luminal single layer of endothelial cells shows expression of CD34

Table 1 Demographic and clinical features of the study cohort and serum ET-1 levels in SSc

Cohort demographics: serum and renal biopsy analysis						
N	Control 20	SSc 27	PAH 26	Pre-SRC 21	SRC 27	Post-SRC 14
Age (mean) (SD)	56.2 (14.0)	52.8 (16.9)	61.2 (9.5)	45.7 (12.0)	52.3 (9.8)	52.9 (12.0)
Median time since SSc diagnosis in months (range)	N/A	89.7 (8–320)	127.5 (35–558)	18.7 (2–60)	15.6 (0–72)	22.5(17–81)
Subset by patients		10d; 17 l	3d; 23 l	19d; 2l	19d; 8l	8d; 6l
ANA	N/A					
ARA		8	2	17	13	7
ATA		9	3	2	8	3
ACA		9	14	0	1	0
Other		1	7	2	5	4
PF	0/20	8/27	8/26	9/21	11/27	4/14
ET-1	0.499	0.544	1.506	0.358	1.478	1.323
Median (range, pg/ml)	(0.184–0.827)	(0.126–1.963)	(0.475–7.11)	(0.093–2.520)	(0.059–3.60)	(0.126–3.02)

ANA, anti-nuclear antibody; RNA, RNA polymerase III antibody; ATA, anti-topoisomerase antibody; ACA, anticentromere antibody.

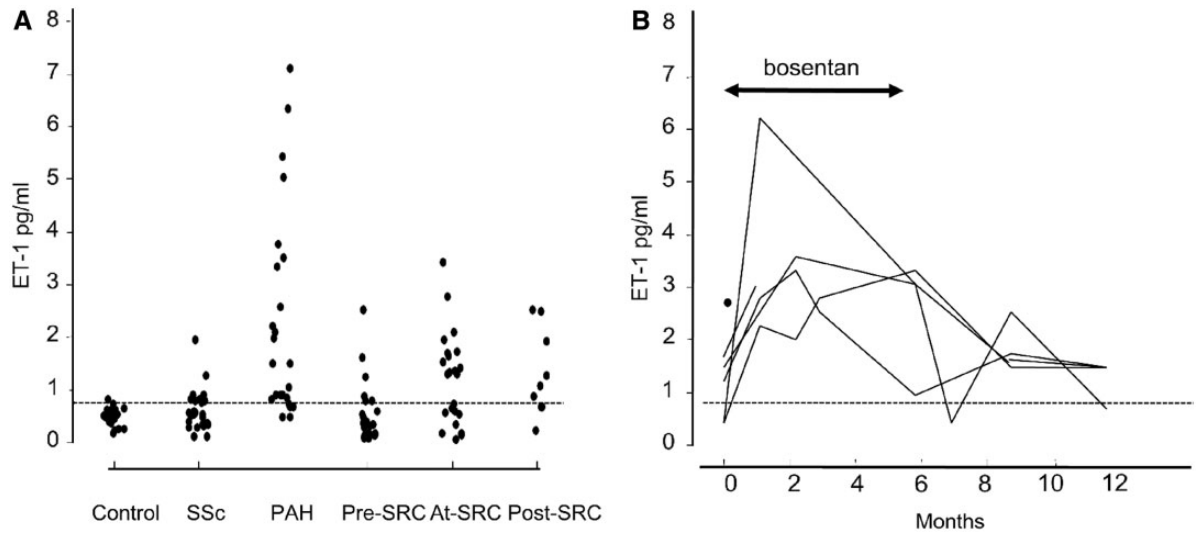

Figure 1. ET-1 levels in SSc with vascular complications and during BIRD-1. (**A**) Endothelin levels in SSc with major vascular complications. Healthy volunteer data are also included. For SRC where possible samples taken in the 6 months prior to SRC as well as during and after SRC are included. (**B**) The plasma level of ET-1 was measured at study entry and regularly during follow up. Baseline levels were variable but all cases increased after starting bosentan. Mean level fell in those cases followed for 12 months and were similar at final assessment to those at baseline.

and CD31. The neointima does not express CD34. In addition, there are CD34 positive areas in the adventitia of the vessel. At higher magnification, these are small tubular structures which in many lesions are present in greater numbers than seen in control tissue. In some samples, these are also present at the centre of the lesion. No significant differences were seen in the staining outside the vascular lesions.

vWF is a surface marker of endothelial cells but is also expressed by platelets and so location in tissues provides further evidence for an endothelial aetiology in SRC. In control tissue, vWF is found on the endothelial layer, and in platelets. In SRC lesions, the expression of vWF is different, however, with expression in the neointima in most (4/6) lesions. Similar small tubular structures in the centre and adventitia are seen as with CD34. The expression away from the vascular lesions is not different between the control and SRC tissue.

αSMA is a marker of vascular smooth muscle cells and also likely to be expressed in myofibroblasts that

are central to the fibroproliferative pathway of SSc. The latter are especially likely to be reflected in the interstitial tissue away from vascular lesions. However, co-localization of vWF with αSMA provides an elegant and clear confirmation that αSMA is also expressed in the vascular structures inducing the neointima. αSMA expression in SRC vascular lesions is expanded compared with control vessels, with expression in the neointima from the media in most lesions (13/17) demonstrates lesions with and without αSMA expression in the neointima in the same biopsy sample. Similar small tubular structures in the centre and adventitia are seen as with CD34. 3/17 lesions demonstrate extension of αSMA into the adventitia. There is also more expression of αSMA in the interstitium of tissue away from the vascular lesions in SRC. vWF and αSMA dual staining show co-localization in the centre of lesions.

Sirius red staining demonstrates more fibrosis in SRC cases than in control tissue, although the degree of fibrosis varies significantly between samples. The neointima in vascular lesions similarly vary, with 9/12 showing + or ++ staining, and three showing little or no staining. Interestingly, there was a more generalized intimal staining in some biopsy samples that might reflect background interstitial fibrosis distant from the SRC pathology.

Consistent with the fibrotic changes noted above, TGFβ1 ligand was expressed in glomeruli, adventitia, and in the interstitium in control kidney. The expression of TGFβ1 in glomeruli includes the cortex and the cortico-medullary junction. In SRC, TGFβ1 is significantly upregulated in glomeruli, tubules and the interstitium. There is no significant expression of TGFβ1in SRC vascular lesions however. Immunostaining data are summarized in Table 2 and Figures 2 and 3.

ET-1 expression is clearly identified in the SRC biopsies with extensive and intense staining in some cases. Representative sections are indentified in Figure 3. Expression in control renal tissue was minimal. Similarly, there was clearly demarcated expression of both ETRA and ETRB in SRC. This was notable within renal tubules as well as vessels staining for ETRB. In control renal tissue, some low-level expression within the normal perivascular connective tissue was apparent.

BIRD-1 study cohort and subject disposition

In addition to the cases outlined earlier, nine patients with SRC were screened for possible inclusion in this open label clinical trial. Three cases were ineligible; one case was referred 8 weeks after SRC; one case had concurrent metastatic malignant melanoma and one case had accelerated hypertension without sufficient renal decrement for entry. Thus, six patients entered the study. Patients were followed at 1, 3, 6 and 12 months after entry, with the study drug discontinued at 6 months, and the trial ending at the last patient's last visit in April 2008. A treatment period of 6 months was chosen on the basis of a previous safety study[15] and also we were confident that a treatment effect would be seen within this period of time.

BIRD-1 cohort demographics are summarized in Table 3. All six patients recruited were caucasian. One was taking an immunomodulatory drug prior to SRC (case 5—mycophenolate mofetil). Two were treated with steroids at SRC—case 4 with 20 mg prednisolone daily followed by 2 g of intravenous methylprednisolone for presumed glomerulonephritis, and case 5 with low dose prednisolone

Table 2 Semi-quantitative analysis of endothelin ligand and receptor expression in SRC

	Control arterioles $n=42$ from 6 samples			SRC vascular lesions $n=64$ from 27 biopsies				
Marker	Intima	Media	Adventitia	Intima	Neo-intima	Media	Adventitia	Vasa vasorum
CD34	++	−	−	++	−	−	−	++
CD31	+	−	−	+/−	−	−	−	+/−
vWF	++	−	−	++	+/++	+/−	−	+
ET-1	−	+/−	−	+/−	+/−	+/−	+/−	+/−
ETRA	−	−	−	+/−	+/−	+/−	+/−	+/−
ETRB	++	−	+/−	+/−	+/−	+	+	+
TGFβ1	−	+	++	−	+/−	+/−	++	−
SMA	−	++	+\−	−	+/−	++	+	+
Sirius red	−	+	+	−	+/−	+	+/++	N/A

−, negative; +, staining; ++, intense staining.

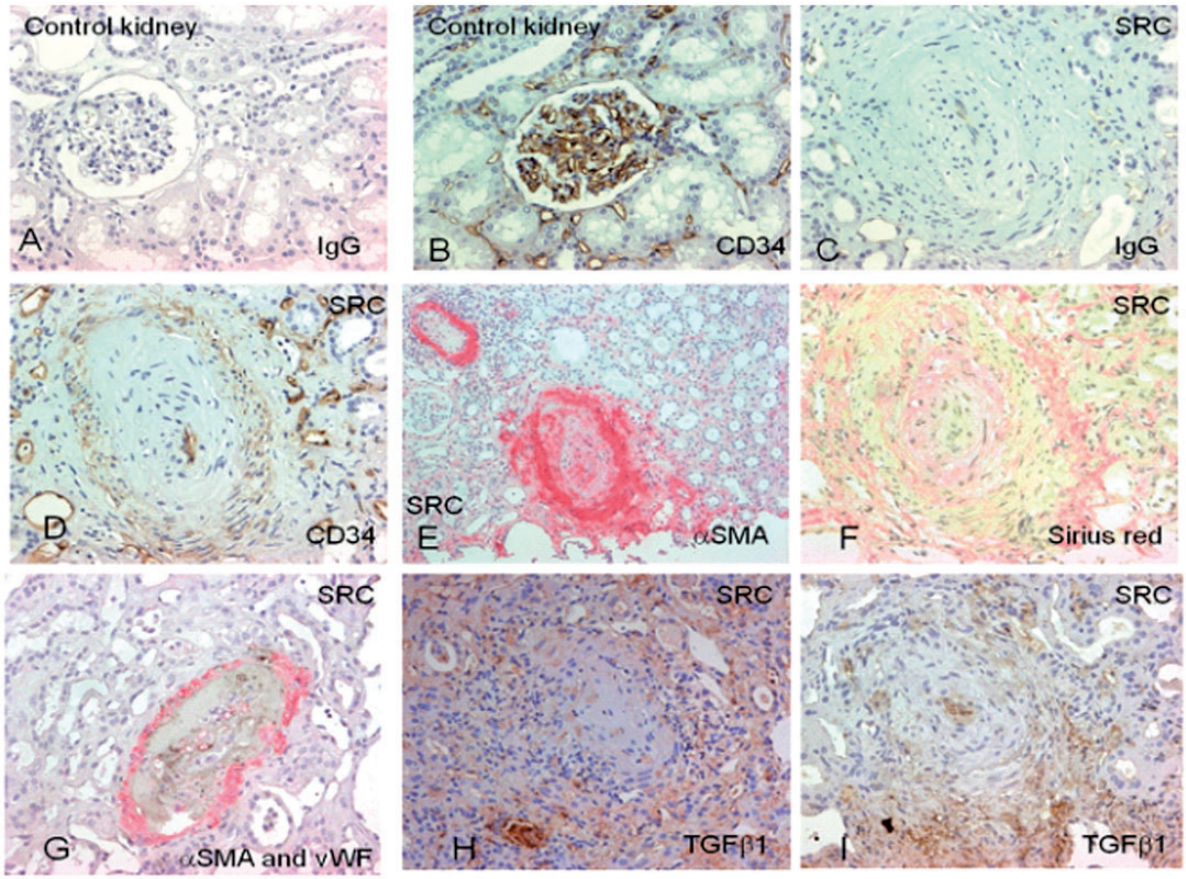

Figure 2. Vascular markers and fibrosis in SRC. The histological features of the SRC biopsies can be compared with those of the control normal renal tissue taken from a nephrectomy specimen stained with IgG to confirm absence of non-specific staining (**A**). The normal pattern of endothelial staining is shown in panel (**B**) that is stained for CD34. Panel (**C**) confirms there is no staining in SRC after incubation with IgG. CD34 staining demonstrates new vessel formation in the adventitia and the remnant of the lumen that has almost completely been occluded (**D**). Staining with aSMA shows vascular smooth muscle as well as myofibroblasts within the endoproliferative lesion that is the hallmark of SRC (**E**). The increase in collagen deposition is the outer vessel wall is confirmed using Sirius red (**F**). Co-localization of aSMA and vWF highlights new vessel formation (**G**). There is evidence of TGFβ1 ligand expression in and around the proliferative lesion of SCR (**H, I**).

(5 mg daily). None were treated with ACE inhibitors or angiotensin II blockers (ATIIB) prior to the diagnosis of SRC. All had been started on ACE inhibitors at diagnosis and were commenced on bosentan between 1 and 6 weeks after diagnosis of SRC.

Comparator cohort of SRC cases

To enable the outcome of cases treated in the BIRD-1 protocol to be put into context with other cases of SRC managed at our centre, we undertook a comparative analysis of SRC cases presenting between 2000 and 2004 and managed at our centre. The comparator cohort was selected to permit the most recently treated SRC cases in our centre to be used, with near complete 12-month follow-up data and to avoid the potential confounder from changes in management of SRC that might have been relevant if a more historic group was analysed. These cases were chosen to be representative as they occurred just prior to the cut-off for inclusion into the BIRD-1 study. This comparator cohort is summarized in Table 4. Notably, 25 cases had steroid therapy in the month prior to SRC; seven records were incomplete. For those with clinical data, 4 patients received treatment with ACE inhibitors or ATII blockers prior to SRC, 12 had received immunomodulatory therapy prior to or at SRC. All cases were treated with ACE inhibitors at SRC.

Clinical outcomes in bosentan-treated patients

No serious drug-related adverse events were seen during the period of bosentan treatment. All patients had leg oedema, and anaemia was also universal

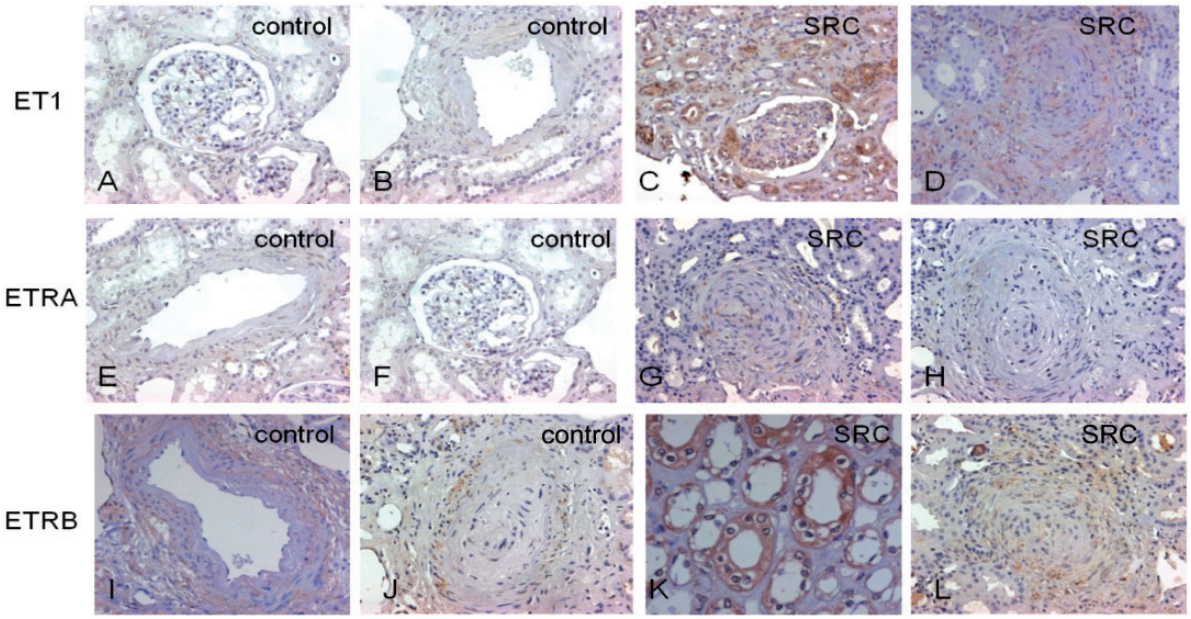

Figure 3. Endothelin ligand and receptor expression in SRC. Immunostainings for endothelin are shown in panels (**A–D**), with very low levels of expression in control renal samples but intense staining in SRC in the parenchyma, glomerulus and tubular structures. Panels (**E–H**) demonstrate ETRA expression that is within the proliferative lesion of SRC but essentially absent in control tissue. Some SRC sections have very high level ETRB expression (**K, L**) compared with control sections for healthy kidney (**I, J**).

Table 3 Clinical and demographic features of the BIRD-1 study cohort

Patient no	1	2	3	4	5	6
Age	59	62	39	64	43	41
Sex	F	F	M	F	F	F
Subset	d	d	l	d	d	d
Autoantibody	ATA; Ro	ATA	U3RNP	ATA	ARA	ARA
Months since SSc onset	4	5	43	2	72	6
Skin score at SRC	26	23	17	23	23	25
MAHA	y	y	n	y	y	y
Low platelets	n	y	y	y	y	n
Haematuria	y	y	y	Anuric	y	n
Pulmonary oedema	y	y	n	y	n	y
Retinopathy	n	y	y	nk	y	y
BP at Presentation	160/110	253/139	210/140	150/90	190/110	206/124
Creatinine at presentation (μmol/l)	185	251	94	226	163	
BP at trial entry	100/50	150/70	140/95	126/56	135/86	110/67

d, diffuse; l, limited; nk, not known; y, yes; n, no.

but was present prior to commencing bosentan. No patients developed dose-limiting changes in liver function tests. One patient (patient 1) withdrew from the study. She commenced bosentan, and 10 days later required dialysis for progressive uraemia and hyperkalaemia. She was admitted 10 days after commencing dialysis, with an encephalopathic state, normal brain imaging and a normal brain magnetic resonance imaging scan. The bosentan was withdrawn without affecting her clinical condition. The encephalopathy resolved over a 2-week period without sequelae and was considered to be related to her scleroderma. At 1 year after SRC she remained on dialysis, although her renal physician was planning a trial without dialysis in the near future. Patient 4 developed pneumonia, atrial fibrillation, *Clostridium difficile* diarrhoea, severe thrombocytopenia, hypotension and severe oedema. She elected

Table 4 Clinical outcome for BIRD-1 study and comparator SRC cohort

	BIRD-1 cohort ($n=6$)	Comparator cohort ($n=49$)
Median (range) BP at presentation	194/118 (150–253/90–140)	195/114 (130–250/80–180)
Median (range) serum creatinine at presentation (μmol/l)	185 (94–251)	191 (82–1123)
Dialysis		
Ever	3/6 (50%)	34/49 (69%)
At 12 months	2/5 (40%)	25/49 (51%)
eGFR median (range) ml/min for cases not on dialysis		
At 3 months	66 (43–85)	31 (21–83)
At 6 months	68 (59–107)	36.5 (19–66)
At 12 months	72 (62–107)	41 (23–70)
Mortality at 1 year	1/6 (16%)	6/49 (12%)

to discontinue bosentan, 5 weeks into the trial. This did not ameliorate her hypotension. She then chose to discontinue dialysis, and died of multi-organ failure a week later. Other adverse events included atrial fibrillation and pericarditis (DC cardioverted and self-limiting, respectively, in patient 3); and pleural effusion (patient 2).

Three of the five patients showed signs of clinical deterioration on discontinuing bosentan. Patient 2, 3 and 5 developed further hypertension requiring the addition of one or two further anti-hypertensive agents. Patients 2 and 5 complained of an increase in symptoms of Raynaud's phenomenon (requiring admission for prostacyclin therapy in patient 5).

There were no significant differences in mortality or dialysis rates between those receiving bosentan, and the control cohort. Renal function in those off or discontinuing dialysis improved in both groups, and although average improvement was greater in the BIRD-1 patients compared with historic comparators, none of these differences were statistically significant.

Vascular marker analysis in the BIRD trial

ET-1 levels in plasma were elevated in SRC compared with normal controls (median healthy controls 0.50 pg/ml; SRC 1.48 pg/ml; $P<0.0005$). The mean level of ET-1 in the BIRD-1 cohort was not significantly different from our cross-sectional SRC comparator cohort. Patients treated with bosentan at the time the sample was taken have a higher level of ET-1 (mean untreated 1.46 pg/ml vs. 3.05; *t*-test $P<0.05$).

Discussion

SRC remains a life-threatening condition and is associated with significant morbidity. We report the first prospective clinical trial that recruits exclusively recent cases of SRC. It is also the first evaluation of ET-RA therapy in SRC. Bosentan is a non-selective ET-RA licensed for the treatment of PAH and cases of SSc with ongoing digital ulcers. It undergoes metabolism in the liver, and hence does not require dose adjustment in renal failure. It is not significantly removed by dialysis, and the dose is not altered in those requiring dialysis.[17] The routine use of ACE inhibitors significantly improved short-term survival, but overall outcome remains poor, especially in cases that remain on long-term dialysis. The need for better treatment together with indirect evidence pointing to increased endothelin bioactivity in SRC provides rationale for this study.

The profile of vascular markers related to endothelial and vessel wall fibroproliferative changes is in line with previous reports and confirms the severe obstructive vasculopathy that is the hallmark of SRC. It is noteworthy that some aspects of the vascular change have not been reported previously especially the development of small intramural vessels that may suggest a degree of vascular adaptation. The degree of interstitial matrix deposition and immune cell infiltrates are consistent with changes that are reported at other sites in SSc. It is likely that these changes are present even in the absence of SRC as has been suggested in previous studies of SRC.

The levels of endothelin ligand and receptor expression are very relevant to the use of endothelin receptor antagonism as a potential therapy for SRC and represent one of the key findings of this study. In other vascular beds, there is also clear evidence of upregulation in SSc especially in lung tissue associated with fibrosis and in PAH. The location of endothelin B (ETB) receptor expression is especially interesting based upon the uncertainty concerning the role of this receptor in mediating the

vasoconstrictor activity or mitogenic activity of ET-1. The high levels of expression in tubular and glomerular structures are notable and relevant in the light of reports that monospecific endothelin antagonists may be used in SRC and in view of data that suggest that ETB may have important physiological effects on renal tubular function. The lack of adverse events noted in the use of bosentan in SRC is encouraging based upon the distribution of ET-1 ligand and receptor expression.

Here was clear evidence of expression of ET-1 ligand and both ETRA and ETRB in SRC. This is in keeping with earlier studies showing increased ET-1 expression in glomeruli and arteriolar lesions in renal biopsy samples of scleroderma patients, in contrast to other renal conditions.[18] There were not sufficient biopsies available for formal quatitation of the expression but the results of the immunostaining provide indirect support for the exploration of endothelin receptor antagonism in SRC and are in line with other studies that have been published.

This study confirms that bosentan is safe and well tolerated when given in addition to ACE inhibitors in the treatment of SRC for 6 months. This is the case regardless of whether dialysis is required or not. No significant drug-related adverse events were reported in this study. Minor side-effect profile for the patients treated with bosentan in this study was consistent with that observed in previous trials and in clinical practice. Commonly occurring side effects include abnormal liver function tests (in ~5% of patients), anaemia (mean drop in treated patients 0.9 g/dl) and nausea. Less commonly leg oedema and headaches may occur.

It is noteworthy that the baseline blood pressure and renal function are very similar for the cases entering BIRD-1 and the larger comparator cohort from our centre. The overall mortality is similar, and although renal function is somewhat better at each follow-up time point for those not requiring dialysis, and the overall frequency of dialysis is lower in the trial patients the number of cases is too small to draw robust conclusions about potential efficacy of bosentan in preventing renal damage or facilitating renal recovery after SRC. It does seem that cessation of bosentan is associated with increases in systemic blood pressure. This is consistent with the known anti-hypertensive effect of ET-RA. It is possible that the elevated levels of endothelin that are observed in these cases during bosentan treatment may contribute to a rebound hypertension at discontinuation. In all cases this was treated adequately by adjusting concurrent anti-hypertensive therapy.

Renal biopsies in SRC show accumulation of mucin in interlobular arteries, and fibrinoid necrosis of arterioles,[4] often with a characteristic 'onion skin' appearance. Immunohistochemical staining has confirmed the upregulation of the endothelin axis including ET-1 and ETRB in renal biopsies at SRC.[19]

The longitudinal analysis of ET-1 serum levels after SRC in cases taking bosentan provides clear evidence that ET-1 levels increase with bosentan. This may reflect reduced clearance of ET-1 through blockade of pulmonary ETB receptors, which are believed to operate as a reuptake mechanism for ET-1 accounting for the rise in ET-1 levels seen after drug treatment was started. It is possible that endothelin A (ETA) receptor blockade might have a different effect from non-selective inhibition. The role of ETB receptors in controlling natriuresis is well described; collecting duct specific ET-1 knockout mice have reduced natriuretic capacity[20] and spontaneous hypertension, ETB receptor collecting duct specific knockout have a similar but less severe phenotype, whereas the ETA receptor knockout causes no alteration in blood pressure or salt and water balance. Conversely, ETB receptor blockade, however, result in an increase in ET-1 levels, and a rise in renal vessel resistance and reduction in GFR (presumably mediated at least in part through increased ETA signalling).[21] Whether this is of importance when ETA receptors are also blocked is unclear.

In conclusion, this study confirms a potential role for endothelin ligand and receptor in mediating the pathology of SRC and provides important confirmation of the safety of using a non-selective endothelin receptor antagonist in the context of SRC in combination with standard therapy. Future studies should address whether this has benefit in terms of short- or long-term outcomes in a larger study cohort. In addition, while the relative importance of the ETRA and ETRB remains uncertain it would be logical and worthwhile to explore the use of an ETRA specific antagonist in this condition. It is well-recognized that recovery in renal function after SRC may occur over several years.[2] This presumably reflects a process of tissue remodelling which may be affected by ET-1; it might be worth treating for longer than 6 months in a future trial. It may also be more efficacious to start treatment rather sooner in the course of the disease. Any future study should be significantly larger to demonstrate efficacy, and will probably need to be multi-centred to recruit sufficient cases. The endothelin axis represents an exciting therapeutic target for vasculopathy in SSc, but further studies need to be performed in renal disease.

Funding

The BIRD-1 clinical trial was an investigator-initiated study sponsored by University College London. This work was supported in part by an unrestricted educational grant from Actelion pharmaceuticals who also provided all study medication. Additional funding came from research grants from the Scleroderma Society (UK), Raynaud's and Scleroderma Association and Arthritis Research UK provided infrastructure support that facilitated this project.

Conflict of interest: None declared.

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