An open label study of the endothelin receptor antagonist bosentan in scleroderma renal crisis.

The BIRD-1trial

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ABSTRACT

Objective: The endothelin receptor antagonist (ETRA) bosentan is used to treat scleroderma (SSc) associated pulmonary arterial hypertension. Endothelin-1 is found at high levels in plasma in scleroderma renal crisis (SRC). We have undertaken an open label pilot study of bosentan in SRC (BIRD-1).

Methods: Cases within 6 weeks of confirmed of SRC received bosentan at standard dose for 6 months. Outcome measures included 1 year mortality, renal function, and blood pressure control. These were compared with a cohort of 49 SRC cases managed at our centre between 2000-2004.

Results: Ten cases were screened and 6 enrolled into BIRD-1. Mean (SD) age of the BIRD-1 cohort was 52 (11.5) years. Median duration of SSc at time of SRC was 6 (range 2-72) months. Demographic and baseline clinical variables were not significantly different for the comparator group. All cases were treated with ACEi at full therapeutic doses. Clinical outcomes reflected those observed in other SRC cases managed recently at our centre. Bosentan was well tolerated with no significant drug related serious adverse events occurring. Three subjects clinically worsened after discontinuation of bosentan – two severe Raynaud's phenomenon, and three hypertension requiring additional anti-hypertensive agents. Levels of ET-1 were elevated in all cases at SRC (median healthy controls 0.50pg/ml; SRC 1.48 pg/ml; $p<0.0005$), and increased further with bosentan therapy (3.05 pg/ml; t test $p<0.05$).

Conclusions: Endothelin receptor blockade with bosentan appears safe in SRC but a larger controlled study would be needed to assess therapeutic benefit compared with standard treatment.

Scleroderma renal crisis (SRC) is a life-threatening complication of systemic sclerosis (SSc) that leads to accelerated phase hypertension and acute renal failure. SRC was very often lethal until the advent of routine treatment with angiotensin converting enzyme inhibitors (ACEi), following which the mortality fell appreciably (from 85% at 1 year, to 24% at 1 year in a single centre [1].

There are multiple reports of elevated serum or plasma ET-1 in SSc [2,3]. Vancheeswaran *et al* suggested that plasma ET-1 levels were greatest in patients with limited scleroderma and pulmonary vascular disease or in extensive diffuse scleroderma [4]. Efficacy of endothelin receptor antagonists as treatment for pulmonary arterial hypertension (PAH) (including SSc-associated PAH) provides persuasive evidence for a pathogenic role for ET-1 in SSc-PAH [5,6]. Further support 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 [7]. Renal biopsies in SRC show accumulation of mucin in interlobular arteries, and fibrinoid necrosis of arterioles [8], often with a characteristic 'onion skin' appearance. Immunohistochemical staining has confirmed the upregulation of the endothelin axis including endothelin-1 (ET-1) and endothelin B receptors in renal biopsies at SRC [9].

This pilot study addresses the hypothesis that adding 6 months of bosentan therapy started within 6 weeks of the onset of SRC to ACE inhibitor therapy is safe and well tolerated, and investigates effect on a small number of SRC cases. The rationale for using 6 months of treatment is that SRC occurs as a single episode which resolves over a period of weeks.

METHODS

Study cohort

All cases fulfilled the American College of Rheumatology classification criteria for SSc [10]. Scleroderma renal crisis was defined by new onset of blood pressure >150/85 obtained at least twice over a consecutive 24 hour 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). 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 urinary RBCs (excluding other causes); flash pulmonary oedema; oliguria or anuria; renal biopsy showing characteristic changes.

To be eligible for recruitment, patients had to be over age 18 and have SRC 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 or were receiving glibenclamide, cyclosporin A, or tacrolimus within 1 week of screening, or expected to receive any of these agents during the study. We anticipated recruiting 6-8 patients with SRC per year. Cases were mostly inpatients at trial entry at the Royal Free Hospital, London (a tertiary referral centre), and followed up as outpatients.

BIRD-1 study protocol

This pilot study addressed the hypothesis that adding 6 months of bosentan to standard therapy including ACE inhibitor was safe and well tolerated, and investigated whether outcome in the study cohort reflected that seen in a recent historic comparator cohort at our centre. Patients received bosentan tablets 62.5mg twice daily for 1 month, and then 125mg twice daily for a further 5 months. Outcome measures included mortality, renal function at 6 and 12 months (including need for dialysis); number of maintenance anti-hypertensive therapy at 6 and 12 months. The protocol was approved by the Local Research Ethics Committee of our institution. All trial assessments were performed by the same investigator. Blood pressure was assessed after five minutes of rest whilst 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.

Biochemical assessments

Endothelin-1 was assayed in plasma using a chemoluminescent immunoassay (R&D systems Endothelin-1 Quantiglo; QET00B). Nt-pro BNP was measured by commercial ELISA.

Statistical analysis

Data were analysed using Minitab 16. All significance tests were two sided. Proportions were compared with Fisher's exact test. Non-parametric data were tested with Mann-Whitney U test.

RESULTS

Patient disposition and study cohort

Between January 2006 and April 2007 10 patients with SRC were screened. Three cases were ineligible; one case was referred 8 weeks after scleroderma renal crisis; one case had concurrent metastatic malignant melanoma; one case had accelerated hypertension without sufficient renal decrement for entry. Thus, six patients entered the study. Patients were followed at 1 month, 3 months, 6 months 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.

Patient demographics are summarised in **Table 1**. All 6 subjects 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 20mg prednisolone daily followed by 2 grams of intravenous methylprednisolone at her referring hospital for possible glomerulonephritis, and case 5 with low dose prednisolone (5mg daily). None were treated with ACE inhibitors or angiotensin II blockers (ATIIB) prior to the diagnosis of SRC. All were started on ACE inhibitors at diagnosis, and were commenced on bosentan between 1 and 6 weeks after onset of SRC.

Comparator cohort of recently treated 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 2000-2004 and managed at our centre. This comparator cohort is summarised in **Table 2**. 25/42 had steroid therapy in the month prior to SRC; for 7 records were incomplete. 4/27 received treatment with ACEI or ATII blockers prior to SRC. 12/42 received immunomodulatory therapy prior to or at SRC. All 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 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 ten days later required dialysis for progressing uraemia and hyperkalaemia. She was admitted 10 days after commencing dialysis, with an encephalopathic state, normal brain imaging and a normal brain MRI scan. Bosentan was withdrawn without affecting her clinical condition. The encephalopathy resolved over a two week period without sequelae, and was considered to be related to her scleroderma. At 1 year after SRC she remained well and 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 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 subjects showed signs of clinical deterioration on discontinuing bosentan. Patients 2, 3 and 5 developed further hypertension requiring the addition of one or two further antihypertensive 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 BIRD-1 subjects and the comparator 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.

Circulating vascular markers

ET-1 levels in plasma were elevated in SRC compared with normal controls (median healthy controls 0.50pg/ml; SRC 1.48 pg/ml; $p<0.0005$). 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 $p<0.05$). The change in plasma ET-1 during the BIRD-1 study is shown in **Figure 1**.

NT pro-BNP levels were significantly elevated in cases of SRC. Log values were taken to perform statistical tests, as previous analysis has confirmed log values are normally distributed in

SRC (data not shown). Levels were higher in the three cases requiring dialysis (mean log value 3.61, standard deviation 0.30) than in non-dialysed cases (2.36; 0.45; $p = 0.028$).

DISCUSSION

The routine use of ACE inhibitors has significantly improved short-term survival in SRC, but overall outcomes remain poor, especially in cases that remain on long-term dialysis [1]. We report the first prospective clinical trial exclusively recruiting cases of SRC. It is also the first evaluation of endothelin receptor antagonist (ETRA) therapy in SRC. Bosentan is licensed for the treatment of pulmonary arterial hypertension and in Europe for systemic sclerosis 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.

This study confirms that bosentan is safe and well tolerated when given for six months in addition to ACE inhibitors in SRC. No significant drug-related adverse events occurred in this study. The side-effect profile in this study was consistent with that observed for bosentan in previous trials and in clinical practice. 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. 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 any conclusions about efficacy of bosentan in preventing damage or facilitating recovery after SRC. It appears that cessation of bosentan is sometimes associated with increases in systemic blood pressure. This is consistent with the known anti-hypertensive effect of ETRA. 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.

The association of higher levels of NT pro-BNP with poorer outcome in SRC is a novel observation which needs replication in a larger cohort. It has been associated with poorer outcome in SSc-PAH [11]. It is of note that plasma ET-1 levels are elevated in cases of SRC, to a similar degree to those seen in SSc-PAH (data not shown). Bosentan is known to reduce clearance of ET-1 through blockade of pulmonary ETB receptors, which are believed to operate as a reuptake mechanism for ET1 accounting for the rise in ET-1 levels seen after drug treatment was started. It is possible that 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 [12] 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) [13]. Whether this is of importance when ETA receptors are also blocked is unclear. In healthy volunteers and chronic kidney disease ETB selective blockade has been associated with systemic vasoconstriction in contrast to selective ETA blockade [14].

Our group has recently described that recovery in renal function from SRC occurs over several years [15]. This presumably reflects a process of tissue remodelling which may be modulated by ET-1 antagonism; 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 to determine whether endothelin receptor antagonists have any place in management of scleroderma renal crisis.

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FIGURE LEGEND

Figure 1. Serial endothelin (ET-1) plasma levels in BIRD-1 cohort

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.

Table 1 Clinical and laboratory features of the BIRD-1 study cohort

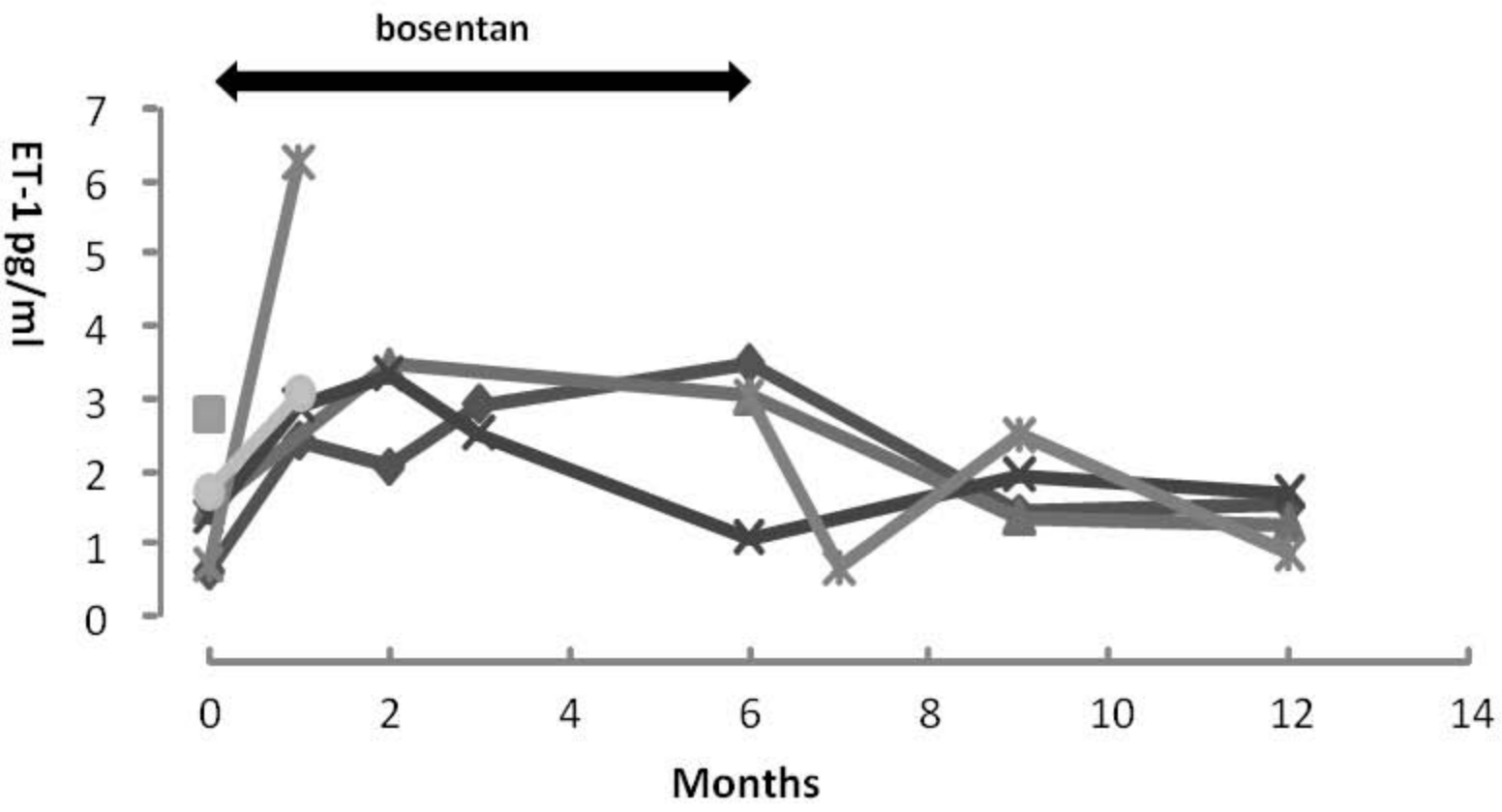
Patient number	1	2	3	4	5	6
Age (y)	59	62	39	64	43	41
Gender	F	F	M	F	F	F
Subset	d	d	l	d	d	d
Autoantibodies	ATA; Ro	ATA	U3RNP	ATA	ARA	ARA
Duration of SSc (months)	4	5	43	2	72	6
Organ involvement		o	o	o,pf	o, lb	o, m
Skin score at SRC	26	23	17	23	23	25
MAHA	y	y	n	y	y	y
Low Platelets	n	y	y	y	y	n
Haematuria	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 (micromol/L)	185	251	94	226	163	184
BP at trial entry	100/50	150/70	140/95	126/56	135/86	110/67

Definition of terms:
ATA anti-topoisomerase-1
ARA anti-RNA polymerase III
o = oesophageal involvement
pf = pulmonary fibrosis
lb = large bowel involvement
m = myositis
MAHA = microangiopathic haemolytic anaemia
d = diffuse
l = limited

Table 2

Summary of renal outcomes for BIRD-1 and recent historic comparator cohort

		BIRD-1 cohort (n=6)	**2000-4 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 (microMol/L)		185 (94-251)	191 (82-1123)
Dialysis	ever	3/6 (50%)	34/49 (69%)
	at 12 mths	2/5 (40%)	25/49 (51%)
eGFR median (range) ml/min for cases not on dialysis	at 3 mths	66 (43-85)	31 (21-83)
	at 6 mths	68 (59-107)	36.5 (19-66)
	at 12 mths	72 (62-107)	41 (23-70)
Mortality at 1 year		1/6 (16%)	6/49 (12%)


Serial plasma ET-1 levels in the BIRD-1 study cohort
bosentan
ET-1 pg/ml
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
Months