LETTERS

Bosentan therapy for patients with severe Raynaud's phenomenon in systemic sclerosis

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Raynaud's phenomenon (RP) is present in more than 95% of patients with systemic sclerosis (SSc). Its frequency and severity in combination with the presence of digital ulcers affect the quality of life of patients.[1] Although the aetiology of RP is multifactorial, endothelin-1 has been suggested to play a role in its pathogenesis.[2] Bosentan, an endothelin-1 receptor antagonist, has been shown to be effective in preventing the occurrence of new digital ulcers when compared with placebo.[3]

Against this background, we hypothesized that bosentan can be a useful and effective strategy in SSc patients with RP. Therefore we assessed the effects of bosentan on RP on subjective and objective outcome parameters. Patients were allowed to continue their oral vasodilating therapy, but parenteral prostanoids had to be stopped one month before study entry.

Fifteen patients (14 women, one man; mean age 52 years, range 34–70) were included. All had limited cutaneous SSc with a median disease duration of four years (range 2–8) and a median RP duration of 10 years (range 4–17). A pretreatment period of four weeks was followed by 16 weeks of bosentan treatment, and a four-week follow-up period. Bosentan was administered as 62.5 mg twice a day for 4 weeks, then 125 mg twice a day for 12 weeks. Patients had to keep a diary of RP attacks during the complete study period, and photoelectric plethysmography (PEP) during cooling and rewarming[4] was assessed at baseline, after eight and 16 weeks of treatment, and at the end of the follow-up period.

Bosentan treatment resulted in a significant reduction in the daily duration, number and severity of RP attacks. The outdoor temperature was, however, significantly higher from week 12 until the end of the study. A significant improvement was already seen after eight weeks of treatment during which the outdoor temperature was stable (table 1).

Despite the reported improvement in RP, blood flow determined by PEP during cooling and rewarming did not improve during treatment with bosentan (fig 1).

In conclusion, treatment with bosentan resulted in an encouraging improvement in the frequency, duration and severity of RP attacks in patients with SSc, but we could not demonstrate an objective improvement in blood flow. The subjective improvement is in contrast with the results of the RAPIDS-1 study, a large randomized placebo-controlled trial in which bosentan was compared with placebo in the prevention of digital ulcers.[3] One might argue that our improvement was caused by seasonal temperature differences or a placebo effect, but no difference in outdoor temperature was seen until week 12. Also, our results show a higher percentage of improvement than the approximately 20–30% found in placebo-controlled trials of RP.[5–7] The absence of effect on blood flow, however, confirms the previously found negative results, but an objective improvement in blood flow was not always found in positive RP trials.[8] Another explanation for the lack of improvement in PEP

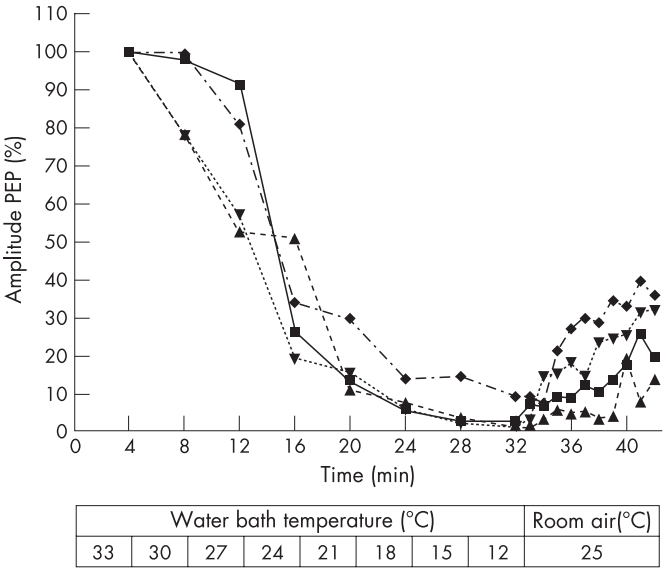

Figure 1 Photoelectric plethysmography (PEP) during cooling and rewarming. Mean values at baseline (■), week 8 (▲), week 16 (▼) and week 20 (◆).

Abbreviations: PEP, Photoelectric plethysmography; RP, Raynaud's phenomenon; SSc, systemic sclerosis

Table 1 Outcome data of the Raynaud's phenomenon diary*

	No. of RP attacks/day	Duration (minutes) RP attacks/day	Raynaud's condition score/day	Outdoor temperature (℃)
Baseline	2.9 ± 1.9	62.3 ± 47.3	4.8 ± 2.6	2.7 ± 1.1
Week 8	1.8 ± 1.5†	30.0 ± 8.7†	3.6 ± 2.5†	1.9 ± 1.9
Week 16	0.9 ± 1.2†	11.6 ± 12.1†	2.7 ± 2.1†	11.9 ± 3.2†
End of follow-up	1.2 ± 1.7†	13.3 ± 13.7†	3.0 ± 2.1†	13.7 ± 3.2†

RP, Raynaud's phenomenon.
*Data are presented as mean ± SD.
†$p<0.05$ compared with baseline.

could be intraindividual variations and the moderate reproducibility of PEP.[9 10]

Therefore, besides the use of a placebo group, a valid and reproducible test is needed for follow-up evaluation of RP trials.

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Unlike ghrelin, obestatin does not exert any relevant activity in chondrocytes

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Obestatin is a 23 amino acid amidated peptide identified as the product of posttranslational cleavage of the preproghrelin,[1] the polypeptide precursor of ghrelin. Obestatin has been reported to have actions opposite to ghrelin,[2] but its ghrelin antagonist properties are still unclear and controversial. As our group has identified ghrelin as a peptide synthesised and secreted by chondrocytes, with significant biological activity on cartilage cell metabolism,[3] it was conceivable that obestatin might exert some physiological action on chondrocytes. These cells are emerging as a local producer and target of several endocrine factors including ghrelin[3] and leptin.[4 5] It is of note that the obestatin co-joined hormone ghrelin has been revealed as a convergent factor between bone metabolism and energy homeostases.[6 7] So, it was plausible to verify the presence of obestatin in chondrocytes and to study the potential physiological effects of this recently discovered peptide.

According to our results, specific enzyme immunoassay (EK03190; Phoenix Pharmaceuticals Inc., Belmont, California, USA, linear range 0.23–2.37 ng/ml) is able to detect immunoreactive obestatin in the supernatants of cultured murine ATDC5 chondrocytes (0.543 $\pm$ 0.032 ng/ml), suggesting that chondrocytes have been equipped with the biochemical machinery in charge of the posttranslational process of the preproghrelin peptide by generating both ghrelin and obestatin. In contrast to ghrelin,[3] obestatin binds weakly to chondrocytes, and quantitative analysis of obestatin binding sites indicates the presence of less than 1000 binding sites per cell. Next, we assessed the effect of obestatin on chondrocyte metabolic activity by means of MTT colorimetric assay. These experiments have been performed in undifferentiated ATDC5 murine cells, but were further confirmed in differentiated mature ATDC5 cells,[8] as well as in the immortalized human chondrocyte cell line, C28-I2 (a kind gift of Dr Mary B. Goldring, Hospital for Special Surgery, New York, USA).[9] Obestatin-stimulated chondrocytes showed a significant decrease in their metabolic activity (fig 1). Intriguingly, this effect was similar, and not opposite, to that exerted by ghrelin,[3] suggesting that obestatin is able to modulate the mitochondrial respiratory chain or oxidative burst as ghrelin did, excluding in this way any kind of antagonism between ghrelin and obestatin.

To exclude any potential cytotoxic effect of obestatin, we analysed the chondrocyte cell cycle by propidium iodide staining flow cytometry and determined lactate dehydrogenase levels in supernatants of human cultured chondrocytes stimulated or not with obestatin. No difference between controls and obestatin-treated cells exists, suggesting that obestatin, per se, exerts no cytotoxic effect.

Obestatin, unlike ghrelin, was unable to modulate significantly either fatty acid or glucose uptake, both macronutrients that serve as precursors for eicosanoids and glycoaminoglycans. Moreover, obestatin had no effects on the quantitative expression of matrix metalloproteases 3 and 9, as well as on enzymes involved in cellular stress such as lactate dehydrogenase, alkaline phosphatase and nitric oxide synthase type 2.

In conclusion, our study suggests that obestatin, unlike ghrelin, plays a marginal role in the regulation of chondrocyte metabolism. We cannot exclude the possibility that the unique indicative response of obestatin as a downmodulator of the chondrocyte respiratory rate might be linked to the induction, or repression, of genes whose roles in chondrocyte physiopathology have yet to be defined. The identification of factors involved in obestatin response may thus provide the basis for future studies on the molecular effects of this novel member obtained by posttranslational processing from the same ancestor peptide that generates ghrelin.