Digital blood flow and 5-hydroxytryptamine receptor blockade after ketanserin in patients with Raynaud's phenomenon

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- 1 The effect of ketanserin on digital blood flow and 5-hydroxytryptamine (5-HT)-induced platelet aggregation was studied in 18 patients with Raynaud's phenomenon.
- 2 Measurements were made before and after a single dose of 20 mg ketanserin and again after 1 month of continuous treatment with 20 mg ketanserin three times a day.
- 3 Digital blood flow was assessed by changes in digital pulse volume; platelet aggregation by changes in light transmission after challenge with 10^{-5} M 5-HT.
- 4 Ketanserin induced a rise in digital blood flow and blocked the platelet response to 5-HT; the two events were not correlated and were independent of subjective improvement.
- 5 These data do not support the suggestion that 5-HT₂-receptor blockade is of major importance in the dilatory effect of ketanserin in Raynaud's phenomenon.

Keywords Raynaud's phenomenon platelet aggregation ketanserin 5-hydroxytryptamine

Introduction

The dual effects of ketanserin, a selective antagonist of 5-hydroxytryptamine (5-HT)₂-receptors with some α_1 -adrenoceptor blocking properties, on vasoconstriction and on platelet aggregation elicited by 5-HT, are the rationale for the use of ketanserin against Raynaud's phenomenon.

However, although acute ketanserin administration significantly increased digital blood flow and skin temperature in patients with Raynaud's phenomenon (Stranden *et al.*, 1982; Tomlinson, 1985), the haemodynamic changes after long-term treatment were disappointing and subjective improvement debatable (Brouwer *et al.*, 1987; Coffman *et al.*, 1989; Longstaff *et al.*, 1985; Marasini *et al.*, 1988).

Furthermore, the recent finding of dissociation between 5-HT₂ platelet receptor inhibition and clinical benefit after chronic ketanserin treatment (Marasini *et al.*, 1990), casts some doubts on the mechanism of the drug against this particular vasospasm.

In this study we investigated whether ketanserin-induced digital vasodilation paralleled the inhibitory effect of the drug on platelet response to 5-HT in patients with Raynaud's phenomenon, to investigate further the importance of 5-HT₂ receptor blockade in the vasodilatory effect of ketanserin in Raynaud's phenomenon.

Methods

Patients

Eighteen patients with Raynaud's phenomenon (16 women and two men, median age 48 years, range 19–61), not taking any medication, entered this study.

Fourteen had primary Raynaud's phenomenon and four had Raynaud's phenomenon associated with systemic sclerosis (SS).

The diagnosis was based on accepted criteria (Allen & Brown, 1932; Subcommittee for Scleroderma, 1980).

The measurements were made between 09.00 h and 11.00 h, during the months of November and December 1989, in a 22°C temperature-controlled room, with patients lying in supine position. A mercury-filled silastic strain gauge was placed around the distal phalanx of the third finger of the dominant arm and the pulsatile volume changes were recorded by a plethysmograph connected to a Gould chart recorder.

Measurements

After a resting period of at least 30 min and establishment of a stable baseline, plethysmographic curves were recorded continuously for 10 min, then 20 mg ketanserin were given and 30 min later recording continued for 60 min. The pulse amplitudes were expressed as percentages of the basal values.

Blood pressure and heart rate were measured before and every 30 min for 90 min after ketanserin.

Immediately before and once vasodilatation was obtained, venous blood was collected without stasis, mixed with 1/10 volume of 3.8% sodium citrate and centrifuged at 130 g for 15 min (platelet-rich plasma). Platelet-rich plasma was diluted with platelet-poor plasma, prepared by higher speed centrifugation (950 g for 20 min), to $3.0 \pm 0.5 \times 10^5$ platelets μL^{-1} . Measurements were made immediately after centrifugation and exposure to air was carefully avoided. Platelet counts were made with a Thrombo-counter C.

Aggregations were measured by a Born-type aggregometer (Elvi 640) and expressed as the maximum increase in optical density 4 min after the addition of 10^{-5}M 5-HT. Eight patients, four with primary and four with SS-associated Raynaud's phenomenon (median age 49 years, range 19–60), were again studied 30 days after chronic administration of 3×20 mg day^{-1} ketanserin, p.o.

Digital pulse volume and platelet aggregation to 5-HT were again measured 12–14 h after the last chronic ketanserin dose and again after an additional 20 mg.

The patients recorded the number and severity of digital vasospasms daily.

In acute studies 20 mg ketanserin have been used to limit the cases of complete blockade of platelet aggregation, as we previously observed with a dose of 40 mg (Marasini *et al.*, 1990), and

to result at the same time in a sufficient vaso-relaxing effect. In chronic studies 60 mg day^{-1} ketanserin were given to ensure a therapeutic dosage, usually in the range of 40–80 mg day^{-1} .

Statistical analyses

The data were analyzed statistically with the Wilcoxon rank-sign test for paired data and the Student's *t*-test (in case of variables normally distributed) and by calculation of linear correlation coefficients. Unless otherwise specified, data were expressed as median values plus the range of observations.

Results

As summarized in Table I, the intake of 20 mg ketanserin was associated in all patients with a rise in digital pulse amplitude and a decrease in platelet response to 5-HT. The extent of digital vasodilatation varied greatly among patients (range 8.4%–69.5%) and was independent of the inhibition of 5-HT₂ platelet receptors, the type of Raynaud's phenomenon or the age of the subject. Ketanserin 20 mg completely blocked 5-HT₂ platelet receptors in 11 of 18 patients; the average inhibition for the remaining seven subjects was approximately 60%. No correlation was found between 5-HT₂ receptor inhibition, type of Raynaud's phenomenon or age of the subject.

The effects of chronic treatment on digital pulse amplitude and 5-HT-induced platelet aggregation are shown in Table 2. Twelve–fourteen hours after the last dose, on the 31st day of continuous 3×20 mg day^{-1} oral ketanserin, platelet responses to 5-HT were within pre-treatment values. An additional 20 mg resulted in a rise in digital pulse amplitude not significantly different from that observed after the first dose (37.1 vs 38.1), and in a decrease of 5-HT induced platelet aggregation (3.0 vs 8.7, $P < 0.02$). Again, no relationship was observed between the extent of vasodilatation and that of 5-HT₂ platelet receptor inhibition.

After 1 month of therapy, three patients (37.5%) reported 50% or more decreases in the number of digital ischaemic attacks per day, and 5 (62.5%) had a subjective benefit of at least a 35% decrease in attacks. However, there were no significant differences in the parameters studied between 'responders' and 'non-responders'.

Blood pressure was significantly reduced after 20 mg ketanserin (systolic pressure: 110.8 ± 3.4

Table 1 Digital pulse volumes and platelet responses to 5-HT in 18 patients with Raynaud's phenomenon. Digital pulse volumes are expressed as % change from baseline, platelet aggregations in cm

Patient	Age (years)	Type of Raynaud's phenomenon	Digital pulse volume	Platelet aggregation to 5-HT	
				A	B
1	52	secondary	28.0	9.1	0.0
2	50	secondary	15.9	7.5	0.0
3	19	primary	37.8	9.8	3.2
4	31	primary	8.4	5.0	0.0
5	35	primary	22.5	5.0	2.0
6	49	secondary	39.9	7.8	3.0
7	52	secondary	33.6	12.5	0.0
8	48	primary	38.4	7.0	4.5
9	26	primary	29.0	5.0	0.0
10	49	primary	13.8	6.8	0.0
11	61	primary	37.1	8.5	0.0
12	43	primary	69.5	8.5	2.0
13	21	primary	52.3	8.5	0.0
14	37	primary	15.6	11.8	4.0
15	60	primary	53.1	7.8	0.0
16	44	primary	12.5	7.5	0.0
17	49	primary	47.6	18.0	8.5
18	51	primary	14.3	10.8	0.0
Median values	48		31.3	8.1	*
Range of observations	(19–61)		(8.4–69.5)	(5.0–18.0)	(0.0–8.5)

A = before ketanserin; B = after 20 mg ketanserin; * = $P < 0.001$ **Table 2** Digital pulse volumes, platelet responses to 5-HT and subjective improvement in eight patients with Raynaud's phenomenon after chronic ketanserin treatment. Digital pulse volumes are expressed as % changes from baseline; platelet aggregations in cm; subjective improvement as % decrease in number of attacks/day.

Patient	Digital pulse volume	Platelet aggregation to 5-HT		Subjective improvement
		A	B	
1	40.4	4.5	0.0	50
2	17.1	10.5	0.0	30
3	66.1	9.0	3.0	70
6	21.9	8.5	3.0	30
7	27.8	18.5	4.0	40
8	52.6	7.0	4.0	40
12	78.3	0.0	0.0	30
15	33.7	13.0	3.0	75
Median values	37.1	8.7	*	3.0
Range of observations	(17.1–78.3)	(0.0–18.5)	(0.0–4.0)	

A = 12–14 h after the last chronic 20 mg ketanserin

B = after an additional 20 mg ketanserin

* = $P < 0.02$

vs 118.6 ± 2.5 ; diastolic pressure: 73.3 ± 1.2 vs 77.5 ± 1.2 mm Hg, mean $\pm$ s.e. mean, post- vs pre-treatment, $P < 0.001$).

The fall in diastolic pressure was correlated with the extent of digital vasodilatation ($P < 0.01$), but not with the inhibition of 5-HT-induced aggregation.

There was no significant correlation between

fall in diastolic pressure and digital vasodilatation after 1 month of therapy, probably because of the smaller number of patients.

Ketanserin induced a significant increase in heart rate (66.7 ± 1.1 vs 64.3 ± 1.2 beats min^{-1} mean $\pm$ s.e. mean, $P < 0.001$), which was independent of blood pressure and of digital pulse volume changes.

Discussion

There have been no data published about the relationship between ketanserin-induced vasodilation and ketanserin-induced 5-HT₂ platelet receptor blockade in patients with Raynaud's phenomenon. This study shows that the two events are independent and confirms our previous finding of discrepancies between subjective improvement and platelet inhibition after chronic ketanserin intake (Marasini *et al.*, 1990).

The dissociation between subjective effectiveness and either vasodilation or platelet inhibition in our patients is intriguing, because the grounds for using ketanserin against Raynaud's phenomenon are its vasorelaxant and platelet inhibitory effects. Even more surprising is the lack of correlation between vasodilation and 5-HT₂ platelet receptor blockade, since the two effects should be mediated by the same 5-HT receptors.

The recent report that platelet deactivation by 5-HT₂ receptor blockade paralleled the hypotensive response to ketanserin, at least in 'responder' hypertensives (Amstein *et al.*, 1989), might indicate different mechanism(s) of ketanserin in these situations.

However, the wide range of vasodilation observed in our patients rather suggests that a number of factors affect vascular response to ketanserin in patients with Raynaud's phenomenon.

Unfortunately, very little information is available concerning the physiologic control of human digital blood flow, nor is it even completely clear to what extent the hypotensive effect of ketanserin is related to 5-HT₂ inhibition (Lijnen *et al.*, 1987).

The α_1 -adrenoceptor blocking properties of ketanserin may indeed be involved. Besides 5-HT receptors, α_1 -adrenoceptors have been found in digital vessels (Arneklo-Nobin & Owman, 1985; Coffman & Cohen, 1988), and ketanserin-induced vasodilation in the human forearm resulted from α_1 -adrenoceptor rather than 5-HT receptor blockade (Blauw *et al.*, 1988a).

Differences in density and/or sensitivity of platelet and vascular smooth muscle 5-HT₂ receptors, or the existence of other than 5-HT₂

types of vascular serotonergic receptors, as recently pointed out (Blauw *et al.*, 1988b), are other possible explanations for our results.

Moreover, since the endothelial cell is involved in 5-HT degradation, and since the human endothelial cell probably bears 5-HT₁ receptors with endothelium-dependent vasodilator activity (Vanhoutte, 1987), an endothelial damage—likely present in some of our patients—might affect digital vascular response.

However, we saw that vasodilation was not different in patients with primary and secondary Raynaud's phenomenon, while microvascular abnormalities should be present only in SS-associated phenomenon (Lewis, 1938; Rodnam *et al.*, 1980).

Finally, we cannot rule out that the discrepancies between vasodilation and platelet inhibition we found may be due, at least partially, to the methods we used. Even though vasodilation was significantly related to the diastolic blood pressure decrease, the plethysmographic technique itself may be too insensitive to detect small changes in blood flow that may have occurred, and, although pulse volume measurements were made after a stable baseline had been established, changes in digital flow might depend on different resting vascular tone.

On the other hand, the high percentage of patients who had platelets completely rather than partially blocked after ketanserin, may have impeded the statistical evaluation.

The finding that platelets were not inhibited 12–14 h after the last chronic dose, but vasodilation and platelet blockade could still be induced by an additional dose during chronic treatment, suggests that more frequent doses or slow-release formulation might be more effective in this condition.

In conclusion, our study showing that in Raynaud's phenomenon digital vasodilation and platelet response to 5-HT after ketanserin were dissociated and independent of subjective improvement demonstrates that the mechanism(s) of ketanserin action against Raynaud's phenomenon is quite complicated and the role of 5-HT far from clear, and suggests that 5-HT₂ receptor blockade may be of only marginal importance in the vasodilatory effects of ketanserin in this situation.

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