Eur J Clin Pharmacol (1996) 49: 371–375

PHARMACODYNAMICS

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Effect of α_1-adrenoceptor antagonists, prazosin and urapidil, on a finger skin vasoconstrictor response to cold stimulation

Received: 27 December 1994/Accepted in revised form: 21 June 1995

Abstract *Objectives*: Cold stimulation causes a finger skin vasoconstrictor response, which is regulated by stimulation of α-adrenergic receptors and is reduced by administration of prazosin. The purpose of this study was to investigate, using a laser Doppler flowmeter, whether the decrease in the finger skin vasoconstrictor response to cold stimulation produced by administration of two different α_1-adrenoceptor antagonists, prazosin and urapidil, was correlated with the corresponding plasma drug concentration, and whether this method could be used to evaluate the relative potency of these α_1-adrenoceptor antagonists in human subjects.
Method: In thirteen healthy male subjects (20–42 y), finger tip skin blood flow was measured during cold stimulation before and 1, 2, 3, 6, and 9 h after administration of placebo, prazosin (1 mg) or urapidil (60 mg).
Results: Both prazosin and urapidil significantly decreased the vasoconstrictor response to cold stimulation. The degree of the decrement in the response indicated by the reduction ratio was significantly correlated with the plasma concentration of prazosin and urapidil. The α_1-adrenoceptor blocking activity of prazosin estimated by the regression lines was about 130-times more potent than that of urapidil.
Conclusion: These findings suggest that the cold stimulation response of finger skin vasoconstriction may be used to evaluate the relative α_1-adrenoceptor blocking potency of drugs.

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Key words Prazosin, Urapidil; Vasoconstrictor response, laser Doppler flow, finger tip blood flow, cold stimulation, healthy volunteers

Introduction

The inhibitory effect of α_1-adrenoceptor antagonists on the pressor response to phenylephrine and noradrenaline is considered to be an index of their relative α_1-adrenoceptor blocking potency in vivo [1, 2]. However, such a manaeuvre potentially induces an abrupt elevation in blood pressure and may cause cardiovascular events. It is important that a safer method be employed to estimate the α_1-adrenoceptor blocking potency of an agent in vivo.

Cold stimulation induces an elevation in endogenous noradrenaline concentration, which, in turn, causes α-adrenoceptor-mediated vasoconstriction and a reduction in blood flow, especially in finger skin [3].

A periflux laser Doppler flowmeter is an easy and rapid method for detecting changes of finger tip blood flow (FTBF). This method showed that FTBF fell during cold stimulation and that the response was blunted by the α_1-adrenoceptor blocking agent, prazosin [4]. These findings suggest that inhibition of the reduction in FTBF caused by cold stimulation is mediated through the α_1-adrenoceptor blocking action of prazosin. Based on these findings, it was suggested that this method might be applicable to evaluating the α_1-adrenoceptor blocking action of a drug.

In the present study, the effects of prazosin and urapidil, another α_1-adrenoceptor blocking agent, on changes in FTBF during cold stimulation were determined in human subjects. The first purpose was to examine whether there was a correlation between the plasma drug concentration and its inhibitory effect on the change in FTBF. The α_1-adrenoceptor blocking potency of prazosin has been shown to be about 80-times greater than that of urapidil in the rat [5]. The second purpose, therefore, was to examine whether the

Table 1 The effect of placebo, prazosin (1 mg), or urapidil (60 mg) on blood pressure and pulse rate before and during the cold stimulation. (n = 13, Mean ± SD)

Time (h)	Parameter	Placebo		Prazosin		Urapidil	
		before	during	before	during	before	during
0	SBP (mmHg)	116.5 (14.9)	130.3 (15.4)	116.1 (14.6)	130.8 (17.9)	113.1 (12.4)	127.2 (13.5)
	DBP (mmHg)	68.2 (10.8)	74.2 (13.9)	68.5 (11.2)	73.8 (15.5)	68.2 (10.3)	73.0 (10.4)
	PR (beats·min^{-1})	70.6 (12.7)	82.3 (10.3)	68.7 (12.7)	79.6 (13.2)	67.8 (11.6)	72.8 (9.5)
1	SBP (mmHg)	118.1 (14.2)	129.3 (18.9)	108.9 (10.0)	124.0 (12.8)	112.0 (12.3)	120.7 (13.4)
	DBP (mmHg)	70.5 (10.4)	74.9 (11.7)	65.4 (7.0)	68.5 (10.2)	64.0 (10.5)	67.3 (13.1)
	PR (beats·min^{-1})	65.8 (9.6)	75.8 (10.9)	72.9 (10.8)	81.1 (11.7)	67.1 (9.1)	73.4 (9.6)
2	SBP (mmHg)	113.8 (13.4)	127.2 (18.0)	108.2 (12.3)	122.8 (11.5)	106.5 (10.6)	116.8 (10.6)
	DBP (mmHg)	66.6 (12.7)	75.9 (12.0)	65.2 (8.6)	64.5 (11.4)	63.8 (10.1)	67.6 (9.7)
	PR (beats·min^{-1})	62.8 (8.6)	73.4 (9.9)	70.9 (12.1)	79.7 (11.1)	69.5 (11.5)	77.2 (12.9)
3	SBP(mmHg)	110.7 (14.0)	131.6 (17.6)	111.8 (13.7)	126.3 (11.1)	108.6 (9.3)	119.1 (11.5)
	DBP (mmHg)	66.4 (11.2)	75.5 (13.9)	67.1 (9.1)	72.0 (9.5)	65.8 (7.8)	66.1 (7.4)
	PR (beats·min^{-1})	62.4 (8.4)	73.9 (9.9)	71.3 (12.8)	81.4 (9.5)	67.9 (10.4)	78.3 (11.6)
6	SBP (mmHg)	113.3 (15.1)	127.3 (17.0)	111.4 (11.0)	121.8 (13.5)	109.4 (10.8)	122.1 (16.6)
	DBP (mmHg)	66.7 (12.2)	71.1 (16.3)	66.3 (8.6)	68.7 (10.0)	63.7 (10.5)	66.5 (13.4)
	PR (beats·min^{-1})	64.5 (8.9)	75.2 (10.6)	75.4 (12.2)	80.7 (12.6)	71.8 (15.5)	77.1 (12.7)
9	SBP (mmHg)	117.4 (16.0)	131.8 (18.4)	113.3 (9.3)	126.5 (12.0)	113.0 (11.9)	120.8 (13.8)
	DBP (mmHg)	69.5 (11.9)	76.1 (14.0)	66.4 (6.5)	69.8 (9.6)	67.2 (10.3)	67.6 (13.7)
	PR (beats·min^{-1})	68.6 (8.4)	77.2 (11.4)	71.9 (10.9)	81.9 (13.5)	70.1 (8.5)	75.8 (11.1)

dose-response regression lines of prazosin and urapidil were different.

Material and methods

Subjects

The study was performed in 13 healthy male subjects, ranging in age from 20 to 43 years (mean (SD) 29.2 (7.3) y), and of body weight 55 to 81 kg (66.6 (6.3) kg). All subjects gave their informed consent to the study.

Finger tip blood flow (FTBF) measurement

Subjects were seated comfortably, with both arms resting on a table at the heart level. Cutaneus FTBF in the pad of the right midfinger was recorded with a laser Doppler flowmeter (Peri Flux PF3, Perimed Co., Lid., Stockholm, Sweden), using an integrating probe with seven efferent fibers (PF 313 Integrating Probe, Perimed Co., Lid., Stockholm, Sweden). Laser Doppler flow is expressed in arbitrary units. Cold stimulation was performed by immersing one foot in ice water (1–4 °C) for 30 s. Blood pressure and pulse rate in the left arm were also measured using a semi-automated sphygmomanometor (NIHON COLIN BP-103iII, Komaki, Japan) before and during the cold stimulation. The recording of FTBF was started after the subject had rested for more than 10 min, and when the value of FTBP was stable the cold stimulation test was performed. The normal microcirculatory response to cold stimulation is a rapid transient vasoconstriction with a decrease in FTBF, which gradually returns to its pre-stimulation level. A quantitative index of the reduction in FTBF was determined using the following expression:
reduction ratio (RR) = $(FTBF_{bef} - FTBF_{min}/FTBF_{bef}) \times 100(\%)$, where $FTBF_{bef}$ is the blood flow just before the cold stimulation and $FTBF_{min}$ is the minimum blood flow recorded during cold stimulation. In the present study, the variation of reduction ratio at 0 h on the three occasions was less than 7%.

Protocol

The study was carried out on three different occasions at intervals of one week. Subjects received placebo on the first study day. On the second and third study days they received 1 mg prazosin (Minipress®, Pfizer Pharmaceuticals Inc., Tokyo, Japan) or 60 mg urapidil (Ebrantil®, Kaken Pharmaceutical Co., Tokyo, Japan) in a randomised cross-over fashion. After an overnight fast, the drugs were given orally with 100 ml water around 8:30 h. The cold stimulation test was performed before and 1, 2, 3, 6 and 9 hours after drug administration. FTBF, blood pressure and pulse rate were measured on each occasion. Blood samples for plasma drug assay were obtained before and 1, 2, 3, 6 and 9 h after administration of prazosin and urapidil. The plasma samples were immediately separated and stored at −20 °C. Subjects did not smoke or take any caffeine-containing or alcoholic beverages for 12 h before or during the study. They had a light meal 3.5 h after drug administration. The studies were performed in a room with a controlled ambient temperature of 24 – 26 °C.

Measurement of plasma drug concentration

Plasma concentrations of prazosin [6] and urapidil [7] were measured by HPLC.

Statistical analysis

Data are expressed as mean with (standard deviation). Statistical analysis of the differences between the trials was done by analysis of variance (ANOVA) followed by Scheffe's multiple range test. The relationship between plasma drug concentration and Δ RR (the

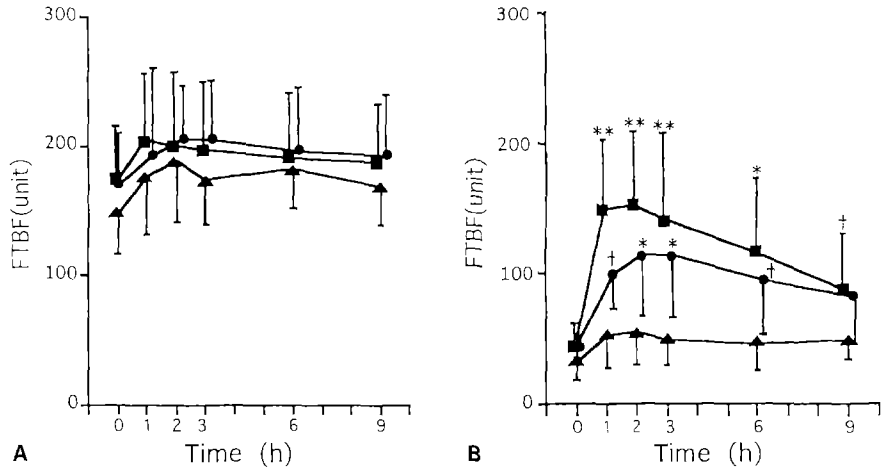


Fig. 1A, B Finger tip blood flow (FTBF) before (**A**) and its minimum value during (**B**) the cold stimulation test after the administration of placebo (▲), prazosin 1 mg (■), or urapidil 60 mg (●). $n = 13$, mean with SD, + $P < 0.05$, * $P < 0.01$, ** $P < 0.001$ compared to placebo

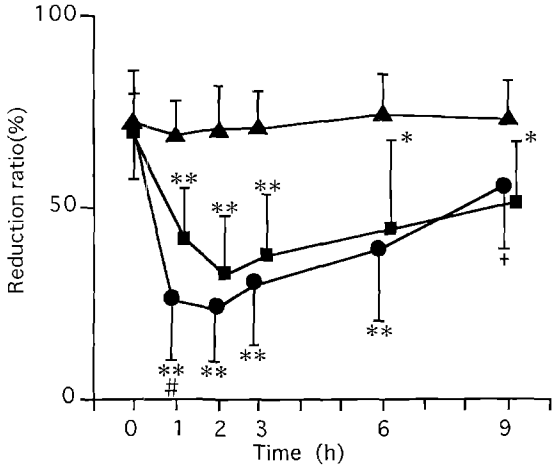


Fig. 2 The reduction ratio after administration of placebo (▲), prazosin 1 mg (■), or urapidil 60 mg (●). $n = 13$, mean with SD, + $P < 0.05$, * $P < 0.01$, ** $P < 0.001$ compared to placebo, # $P < 0.01$ compared to urapidil

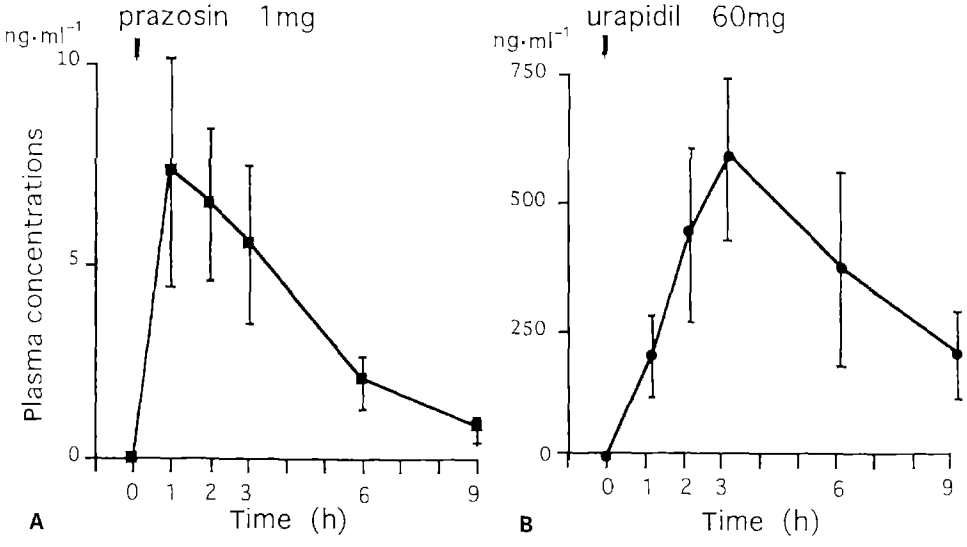
difference between the reduction ratio of placebo and that of prazosin or urapidil) was determined by linear regression analysis.

Results

There were no significant differences in blood pressure or pulse rate between the placebo, prazosin (1 mg), and urapidil (60 mg) trials at any observation point (Table 1). The time course of FTBF before the cold stimulation and its minimum value during the test are shown in Fig. 1. The basal FTBF was not significantly changed by prazosin or urapidil. FTBF during cold stimulation was significantly greater following prazosin and urapidil. The reduction ratio was significantly decreased by prazosin and urapidil (Fig. 2).

Plasma concentration of prazosin and urapidil are shown in Fig. 3. As shown in Fig. 4, a significant positive correlation was found between the plasma drug

Fig. 3A, B Plasma drug concentrations after prazosin 1 mg (**A**) and urapidil 60 mg (**B**)



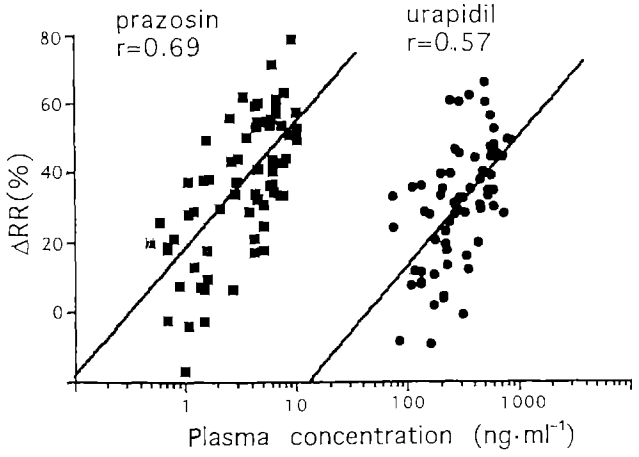


Fig. 4 Relationship between plasma drug concentrations of prazosin (■) and urapidil (●) and the decrement in the reduction ratio (Δ RR) during each treatment. (Δ RR = (RR on prazosin or urapidil)–(RR on placebo))

level and the decrement in the reduction ratio (Δ RR) (prazosin: $y = 36\log x + 17$, $r = 0.69$, $P < 0.001$, urapidil: $y = 37\log x - 62$, $r = 0.57$, $P < 0.001$).The two regression lines obtained were clearly separated. The estimated average concentration of prazosin and urapidil that decreased the reduction ratio by 30% (ED_{30}) was 2.3 ng·ml^{-1} and 301 ng·ml^{-1}, respectively.

Discussion

α_1-Adrenoceptor antagonists are now widely used as antihypertensive drugs. In human subjects, the haemodynamic responses to systemic infusion of increasing doses of phenylephrine or noradrenaline have been used to evaluate the α_1-adrenoceptor blocking potency of drugs [1, 2]. However, this method potentially risks an excessive rise in blood pressure so a safer method is needed.

Laser Doppler analysis is a relatively new method, which allows real-time non-invasive measurement of skin blood circulation. It is based on the fact that moving red blood cells cause a frequency shift (Doppler effect) when they scatter a laser light beam. The size of the Doppler signal is proportional to the flow or flux of erythrocytes (the product of erythrocyte concentration and their average velocity) within a volume of skin which is approximately hemispherical in shape and has a radius of about 1 mm [8]. In this study we used a new integrating probe, with a bundle of seven efferent fibres, which increases the total measuring volume approximately seven-fold [9]. Although measurements obtained with a laser Doppler flowmeter are of a relative nature, the method is particularly appropriate for the study of local skin vasomotor responses in human subjects [10].

The finding that cold stimulation increases skin sympathetic activity was made with a direct, invasive method [11]. Low et al. [12] reported that the skin vasoconstrictor response to cold stimulation could be detected by using a laser Doppler flowmeter. In addition, Khan et al. [4] reported that prazosin markedly decreased such a response, suggesting that post synaptic α_1-adrenoceptors were involved in the rapid skin vasoconstriction seen in response to cold stimulation. Therefore, it was expected that examining this response with a laser Doppler flowmeter could be used to evaluate the efficacy of α_1-adrenoceptor antagonists in vivo.

In the present study, prazosin and urapidil decreased the vasoconstrictor response to cold stimulation, and a significant correlations were observed between the plasma drug concentrations and the decrement in the vasoconstrictor response expressed as the reduction ratio. Judging by the regression lines obtained, prazosin was about 130-times more potent than urapidil in reducing the vasoconstrictor response to cold stimulation. The α_1-adrenoceptor blocking potency of prazosin is reported to be about 80-times greater than that of urapidil in the rat [5], which is not very different to the present finding in humans. Based on these findings, it is suggested that this method, using a laser Doppler flowmeter, which is safe and simple, may be useful in non-invasively evaluating the relative potency of α_1-adrenoceptor blocking drugs in human subjects.

Postjunctional α_1- and α_2-adrenoceptors have been demonstrated in human finger skin vessels, and these different receptors are involved in the vasoconstrictor response to catecholamines [13]. Prazosin blocks α_1- and α_2-adrenoceptors [14], while urapidil blocks α_1-adrenoceptors [15]. Such difference in activity at α_2-adrenoceptors might, at least in part, contribute to the difference in potency of the two drugs.

As the pressor response to catecholamines was not examined in this study, it is not clear whether this cold stimulation method is more precise than the catecholamine infusion method in evaluating the relative α_1-adrenoceptor blocking potency of drugs. In addition, as a trial using cold stimulation of the contralateral hand was not done, the possibility cannot be ruled out that local application of cold to the foot or to the contralateral hand might have a different effect on the finger tip blood flow after α_1- adrenoceptor blocking agents.

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