Absence of potentiation of the skin response to intradermal bradykinin by a long-acting angiotensin converting enzyme inhibitor, trandolapril, at conventional antihypertensive dosage in human volunteers: a double-blind, randomized, cross-over, placebo-controlled trial

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Summary: A double-blind, randomized, cross-over, placebo-controlled study was carried out to determine the extent and duration of potentiation of the action of bradykinin introduced intradermally by a long-acting novel angiotensin converting enzyme (ACE) inhibitor, trandolapril. The investigations were performed in a temperature and humidity-controlled laboratory. Intradermal injections of 1 µg, 2.5 µg and 5 µg of bradykinin and normal saline (as control) were made into the forearm skin of eight healthy normotensive male volunteers aged 21–33 years (mean 28 years) at baseline, 2, 4, 8, 24, 48, 72 and 96 hours after either 2 mg trandolapril or placebo given orally. Skin blood flow outside the induced weal was monitored by laser Doppler flowmetry (mean of recordings at four sites adjacent to the weal within the flare area). Flare area and weal volume were also measured.

Trandolapril reduced the mean arterial pressure. However, there was no evidence that this activity was associated with a potentiation of the cutaneous action of bradykinin.

In conclusion, it would appear that potentiation of the action of bradykinin may not be an important contributing factor to the fall in total peripheral vascular resistance associated with ACE inhibition in humans in the control of hypertension.

Introduction

The administration of angiotensin converting enzyme inhibitors (ACE) is associated with vasodilatation and a reduction in total peripheral vascular resistance. In mild to moderate hypertension there is a fall in blood pressure without an associated tachycardia or fall in cardiac output. Since angiotensin converting enzyme is identical to kininase II, one of the enzymes which catabolizes and inactivates bradykinin, it has been proposed that this effect and the consequent release of prostacyclin (PGI₂) and endothelium-derived relaxing factor(s) (EDRF) from vascular endothelium may be additional to the beneficial effects achieved by the inhibition of formation of angiotensin II alone.¹ Inhibition of the breakdown of bradykinin, introduced intradermally, by cap-

topril and enalapril has been demonstrated at doses which control hypertension.^{2–4}

Trandolapril (RU44570) is a new potent orally active ACE inhibitor characterized by a long duration of action. Bioavailability is good with more rapid onset of action than with enalapril; 100% inhibition of ACE in serum is obtained with a single dose of 2 mg, with 50% inhibition after 8 days. In spontaneously hypertensive rats it was found to be at least three times more potent than enalapril. Inhibition of bradykinin degradation has also been observed.

Characteristic of its class, there is no evidence of stimulation of baroreceptors and reflex tachycardia does not occur. The parent substance and its diacid are both pharmacologically active. Tissue ACE inhibition is 5–12 times as potent as enalapril except in the heart where the ratio of ID₅₀ values is about 45 times higher. In man with repeated daily dosage the steady state appears to be achieved by the second day. Elimination of the radio-labelled

drug occurs in the faeces (65.7%) and in the urine. Tolerability as judged from estimation of hepatic enzymes is good and side effects are uncommon. Cough was reported by 59 of 1959 patients, 458 of whom were treated for longer than a year. Therapy required to be discontinued in 15 cases (unpublished observations).

The purpose of this investigation was to attempt to determine the degree of inhibition of bradykinin degradation associated with administration of a single oral dose of trandolapril known to produce 100% inhibition of ACE.

Materials and methods

The subjects were eight healthy normotensive male volunteers, mean age 28 years (range 21–33 years). Normality was defined on the basis of past medical history, physical examination, resting electrocardiogram and routine haematology, biochemistry and urinalysis. Specific exclusion included cigarette smokers, past or present asthma, anaphylaxis from any cause or a history of angioedema. Subjects were asked to avoid medication of any kind, either prescribed or over the counter, for one week prior to and during the course of the investigation.

The study was double-blind, randomized and placebo controlled. The bradykinin challenge test was performed at baseline and at 2, 4, 8, 24, 48, 72 and 96 hours following a single dose of either placebo or 2 mg trandolapril, administered in random order. The dosage of bradykinin injected intradermally was 0 (saline), 1.0, 2.5 and 5.0 μg in 0.1 ml aliquots obtained from 0, 10, 25 and 50 $\mu\text{g}/\text{ml}$ solutions made up in normal saline, randomized and labelled A, B, C and D, by the pharmacist, to maintain blindness. The injections were made at four widely separated sites on the flexor aspect of both forearms.

The response to bradykinin was assessed by three methods: measuring the blood flow as a voltage output or flux (blood velocity $\times$ RBC number) by the non-invasive technique of laser Doppler flowmetry,² measurement of flare area and weal volume. Measurements were made 15 minutes after each injection. The Doppler probe was placed for 15 seconds at each of four points as close as possible to the point of injection but outside the area of the weal. The data were stored on an IBM desk-top microcomputer and the mean values used in the subsequent analysis. The flare and weal area was measured using transparent acetate sheets and weal volume was obtained by multiplying weal thickness, obtained using modified Holtain skin calipers, by weal area. Accurate measurement of the areas was obtained from the acetate tracings using a digitizing tablet linked to a microcomputer.

The investigation was performed in a temp-

erature and humidity controlled laboratory ($24^\circ\text{C} \pm 1.0^\circ\text{C}$; 20–40%) with the subject seated, lightly clad and with the forearms supported and flexed so that the hands were at mid-sternal level. Except for the tests at 4 and 8 hours post dosing, all the tests were performed in the morning. Subjects were asked to attend having abstained from alcohol and caffeine from the previous evening, and having had a light breakfast of toast and marmalade with fruit juice at least 1 hour beforehand. A salad and fruit juice meal was provided between the 2 and 4 hour and 4 and 8 hour tests.

After 4 weeks the same procedure was repeated using the alternative (randomized) preparation, trandolapril or placebo.

Blood pressure (mean of three readings with a Hawksley random-zero sphygmomanometer) and heart rate were measured during each study period and all adverse events recorded. Blood and urinalysis were performed at the end of each study period.

The District Ethical Committee approved the trial protocol.

Statistical analysis

The data were analysed using the method of summary measures.⁵ A 'summary measure' of the bradykinin response (laser Doppler flowmeter output, flare area or weal volume) for each individual subject at each time point was obtained by calculating the area under the curve (AUC) of the bradykinin log-dose response curve using the trapezium rule and dividing the AUC by the difference in log-dose concentrations. The resulting values can be considered as a type of weighted average of the responses. These values together with the pulse and mean blood pressure data were then used to construct response–time curves for each subject. The AUC of the response–time curves divided by time (to construct a single summary measure for each subject) for placebo and trandolapril days were compared by Student's *t*-test for paired data.

Given the within-subject standard deviation (± 0.40) of the cutaneous responses to bradykinin from our previous study,² it was estimated that eight subjects completing the study will provide 90% power of detecting increases in the mean cutaneous responses of 50% at the 5% significance level.⁶

Results

The baseline blood pressure values before each treatment period were similar: $104.5 \pm 5.7/66.8 \pm 6.0$ mmHg (mean $\pm$ S.D.) prior to placebo and $108.5 \pm 6.6/68.4 \pm 6.6$ mmHg prior to trandolap-

ril. The heart rate values were 67.0 ± 6.5 and 71.6 ± 9.4 beats/minute, respectively. These differences were not significant.

Trandolapril caused a significant fall in mean arterial pressure (MAP) (mean difference -5.6 mmHg, 95% CI -0.1 to -11.1 , $P < 0.05$) with no significant effect on heart rate (Figure 1). No potentiation of the effect of bradykinin injected intradermally was evident on local blood flow as measured by laser Doppler flowmetry (mean difference -0.12 a.u., 95% CI -0.48 to 0.24 , $P = 0.47$), on the flare area (mean difference -42 mm², 95% CI -269 to 185 , $P = 0.67$) nor on weal volume (mean difference 16 mm³, 95% CI -30 to 47 , $P = 0.62$) (Figure 2). There was no treatment effect on the saline response.

No adverse effects were recorded during the course of the study.

Discussion and conclusions

This is the first investigation, using this methodology, in which an ACE inhibitor was shown to fail to potentiate the effect of bradykinin introduced intradermally. This lack of effect may not be unique to trandolapril as many of the newer inhibitors

Figure 1 Mean change ($\pm$ 95% confidence intervals) with time in mean arterial pressure and heart rate following the administration of trandolapril (2 mg) (■) or placebo (●).

Figure 2 Mean response ($\pm$ 95% confidence intervals) with time to bradykinin challenge following the administration of trandolapril (2 mg) (■) or placebo (●).

remain to be tested. However, the results should be interpreted with caution as the study is limited to skin blood flow in the forearm, a single component of the total peripheral vascular resistance. It does not exclude a role for altered bradykinin metabolism in other vascular beds in the blood pressure response to trandolapril.

Though the primary action of converting enzyme inhibitors in producing a decrease in peripheral vascular resistance is prevention of the formation of angiotensin II (ATII), a number of additional possible vasodilatory effects are proposed. These include the potentiation of bradykinin which has a direct relaxant action on vascular smooth muscle, and enhances the endothelial production of the vasodilatory prostanoids and the release of endothelial derived relaxing factor(s).

This substance may in turn inhibit the release of kidney renin by a negative feedback action.¹ Converting enzyme inhibitors may also reduce the release of the vasoconstrictor noradrenaline in the vessel wall, both directly by a prejunctional effect, or indirectly by reducing the production of ATII. They may also reduce the responsiveness of vascular smooth muscle to vasoconstrictor stimuli, for example, alpha-adrenoceptor activation, by a direct action.⁷ From this it is evident that potentiation of bradykinin activity is only one of a number of additional activities of ACE inhibitors. Since our findings indicate that a fall in blood pressure may occur in the absence of bradykinin potentiation in at least one component of peripheral vascular resistance, the blood vessels of the skin of the forearm, this effect may be less important than

previously supposed in the reduction of total peripheral vascular resistance associated with these substances.

It is recognized that hypertensive patients may show qualitative differences in the response to vasoactive drugs when compared to normotensive subjects. Further studies in hypertensives are required before the role of bradykinin in the antihypertensive effect of trandolapril can be defined with confidence.

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