Br. J. Pharmac. (1982), **76**, 163–168

RELATION OF PLASMA RENIN ACTIVITY TO THE ANTIHYPERTENSIVE EFFECT OF MK 421 IN THE RAT

DAVID BRADSHAW, PETER H. FRANZ & A. LOUISE SUGDEN

Department of Pharmacology, Roche Products Limited, Welwyn Garden City, Hertfordshire AL7 3AY

1 The effect of the angiotensin converting enzyme inhibitor, MK 421 (N-((S)-1-(ethoxycarbonyl)-3-phenylpropyl)-L-Ala-L-Pro), on the blood pressure of two-kidney Goldblatt hypertensive rats has been investigated in relation to the initial plasma renin activity (PRA) and the initial blood pressure of the individual animals.

2 Blood pressure was monitored by an indirect tail-cuff method at 1, 3, 6 and 24 h after dosing. MK 421 produced a fall in blood pressure in the majority of animals, but the extent of this reduction varied considerably between individuals.

3 The change in blood pressure showed a significant correlation with both the initial PRA and the initial blood pressures of the animals. However, only a modest correlation was found between the initial PRA and the degree of hypertension.

4 MK 421 (10 mg/kg, orally) produced a mean blood pressure change which was statistically significant ($P < 0.001$) at all times tested.

5 It is concluded that the degree of antihypertensive activity of MK 421 is related to the degree of activity of the renin-angiotensin system which, in this model at least, is reflected by the PRA.

Introduction

The measurement of plasma renin activity (PRA) is generally considered to indicate the overall activity of the renin-angiotensin system and the extent of the contribution of this system to the maintenance of blood pressure. On this basis it would be expected that the effect of drugs interfering with the renin-angiotensin system would show some correlation with pre-existing PRA values. The finding that the angiotensin converting enzyme (ACE, E.C.3.4.15.1) inhibitor, captopril, was effective in patients with relatively low renin levels was, therefore, to some extent unexpected (Case, Atlas, Laragh, Sealey, Sullivan & McKinstry, 1978; Gavras, Brunner, Turini, Kershaw, Tifft, Cuttelod, Gavras, Vukovich & McKinstry, 1978; Bravo & Tarazi, 1979; Brunner, Gavras, Waeber, Kershaw, Turini, Vukovich, McKinstry & Gavras, 1979; MacGregor, Markandu, Roulston & Jones, 1979). Nevertheless, several clinical studies have shown a correlation between the base-line PRA and the antihypertensive effect of captopril (Atlas, Case, Sealey, Sullivan & Laragh, 1978; Case, *et al.*, 1978; Brunner *et al.*, 1979, Brunner, Gavras, Waeber, Textor, Turini & Wauters, 1980; Case, Atlas, Laragh, Sullivan & Sealey, 1980) although other workers could detect no such relationship (Gavras *et al.*, 1978; Bravo & Tarazi, 1979).

In animals, several reports have suggested that hypertensive models with high renin levels are more responsive to inhibition of the renin-angiotensin system than are low renin models (Bengis, Coleman, Young & McCaa, 1978; Bengis & Coleman, 1979; McCaa, 1981). There is also some evidence to suggest that base-line PRA in individual animals is a determining factor of the degree of antihypertensive activity associated with such an inhibition (MacDonald, Boyd & Peart, 1975; Satoh, Suzuki & Satoh, 1980; Conway, Hatton & Clough, 1981). Sweet, Gross, Arbegast, Gaul, Britt, Ludden, Weitz & Stone (1981), in describing the properties of the ACE inhibitor MK 421, suggested that the degree of blood pressure lowering produced by this compound may be related to the initial PRA since the compound was more effective in high renin models of hypertension in the rat than in low renin models. In the present study we have investigated the relationship of the initial PRA to the individual antihypertensive responses of two-kidney Goldblatt hypertensive rats treated orally with MK 421.

Methods

Induction of hypertension

Male Alderley Park strain rats (supplied from the colony bred at the Roche Drug Safety Laboratory)

0007-1188/82/050163-06 $01.00

weighing 90–150 g were anaesthetized with pentobarbitone and a silver clip with an internal gap of 0.2 mm was placed on the left renal artery. Animals were given free access to food and water and were used for experiment 7 weeks after clipping.

Blood pressure measurements

The rats were warmed for 45–60 min in an oven maintained at 32°C and the blood pressure was measured by an indirect tail-cuff method using a W + W recorder (Gerold & Tschirky, 1968). MK 421 (10 mg/kg) was then administered orally and blood pressure measurements were repeated at 1, 3, 6 and 24 h after dosing.

Plasma renin activity determinations

On the day before the blood pressure measurements, the animals were lightly anaesthetized with methohexitone and approximately 1 ml of blood was withdrawn from the retro-orbital vascular plexus. The blood was collected in glass pipettes, previously flushed through with 6% (w/v) EDTA as anticoagulant, and transferred to plastic tubes containing 50 μl 6% (w/v) EDTA. The tubes were kept in ice throughout and then centrifuged in a refrigerated centrifuge at 1500 *g* for 10 min. Plasma was transferred to clean plastic tubes and stored at −20°C until assay.

PRA determinations were made within a few days of blood collection. Mixtures containing 200 μl of plasma sample, 10 μl of a 5% (w/v) ethanolic solution of phenylmethylsulphonylfluoride and 5 μl of 2 M Tris maleate buffer (pH 6.5) were incubated at 37°C for 30 min. The angiotensin I formed was detected by radioimmunoassay by taking 25 μl aliquots of the incubate and adding 200 μl of 0.1 M Tris acetate buffer (pH 7.4) containing 0.1% (w/v) bovine serum albumin, 200 μl angiotensin I antiserum and 50 μl ^{125}I-angiotensin I. The concentrations of the latter two substances were adjusted to give approximately 20,000 ct min^{-1} 50 μl^{-1} ^{125}I-angiotensin I and 50% binding of the radioactive ligand to the antibody. The mixture was then incubated at 0°C for 24 h after which the free angiotensin I was separated from the antibody-bound fraction by charcoal-dextran sedimentation. The bound angiotensin I, contained in the supernatant, was determined by liquid scintillation spectrometry and the amount of angiotensin I in the original plasma samples was estimated by comparison with calibration values, obtained using synthetic angiotensin I as standard. The values were corrected for endogenous angiotensin I levels measured in plasma samples incubated at 0°C and PRA was then expressed as ng angiotensin I produced ml^{-1} plasma h^{-1}.

Statistical analyses

Statistical significance testing was based on two-tailed Student's *t* tests using the paired *t* test for examining changes in blood pressure within groups and the unpaired *t* test for assessing differences in blood pressure between groups. The interrelationships between body weight, initial blood pressure, changes in blood pressure and PRA were examined by determining the appropriate correlation coefficients.

Drugs

Drugs and chemicals used were: Asp^1 Ile^5 angiotensin I (Calbiochem), ^{125}I-angiotensin I (New England Nuclear), angiotensin I antiserum (rabbit) (Becton Dickinson), methohexitone sodium (Brietal, Elanco), pentobarbitone sodium (Sagatal, May and Baker) and MK 421 (N-(S)-1-(ethoxycarbonyl)-3-phenylpropyl-L-Ala-L-Pro, donated by Merck, Sharpe and Dohme).

Results

All data used for the statistical analyses are shown in Table 1.

Relation of body weight to initial blood pressure

No significant correlation was found between the body weight of the rats and their blood pressure, measured immediately before drug administration ($r = 0.36$, $n = 29$, $0.1 > P > 0.05$).

Net effect of MK 421 on the blood pressure of the animals taken as a group

Table 2 indicates the group mean blood pressure for the different times of measurement. MK 421 (10 mg/kg, orally) caused a significant fall in blood pressure which was evident 1 h after administration and which was still apparent 24 h after this single dose. A group of animals treated with vehicle in the same dose volume (10 ml/kg) showed no significant change in blood pressure over the 24 h after dosing.

Relation of initial blood pressure to the changes in blood pressure induced by MK 421

There was a large variation in the individual blood pressure responses to MK 421 (10 mg/kg, orally) which varied from a rise in blood pressure of 10 mmHg to a fall of 155 mmHg in different animals. The degree of these changes in blood pressure showed a significant correlation with the initial blood pressure of the individual animals. This correlation

Table 1 Data for individual animals used in statistical analyses

Rat	Body (g) wt.	Initial PRA (ng AI ml^{-1} h^{-1})	Initial BP (mmHg)	Change in blood pressure (mmHg) Interval after dosing (h) 1	3	6	24
1	450	2.3	180	−20	−25	−20	−15
2	305	5.7	140	0	+10	0	+5
3	450	2.4	210	−35	−15	−20	−10
4	400	5.3	270	−50	−50	−60	−45
5	440	1.7	220	−40	−35	−30	0
6	370	3.0	220	−25	−25	−10	−10
7	370	3.2	220	−15	−20	−25	−15
8	310	1.5	200	−15	−20	−20	+10
9	310	13.9	250	−50	−95		−105
10	310	1.7	190	−20	−15	−15	−10
11	385	14.2	245	−35	−125	−115	−155
12	445	5.6	160	−20	−20	−30	−30
13	375		165	−15	−25	−25	+5
14	405	19.8	220	−35	−55	−50	−50
15	405	4.1	200	+10	−15	−20	−20
16	345		220	0	−15	−10	+5
17	345	6.4	240	−20	−10	−10	0
18	340	5.7	280	−55	−65	−50	−40
19	310	4.4	285	−30	−55	−40	−10
20	380	3.1	180	−50	−70	−45	−40
21	410	2.8	180	−25	−25	−30	−25
22	355	2.9	160	−45	−15	−30	−30
23	325		290	−65	−40	−10	−20
24	380		180	+5	−25	−30	−5
25	405		210	−25	−40	−40	−30
26	350	22.2	260	−120	−140	−130	−125
27	395	1.1	160	−15	−35	−20	−15
28	285	11.3	290	−50	−120	−150	−55
29	335		200	−10	−50	−70	−15
Mean ± s.e.	369 ± 9 (n = 29)	6.3 ± 1.2 (n = 23)	215 ± 8 (n = 29)	−30 ± 5* (n = 29)	−43 ± 7* (n = 29)	−40 ± 7* (n = 28)	−29 ± 7* (n = 29)

PRA was measured in samples of blood taken 24 h before dosing with MK 421 (10 mg/kg, orally). Blood pressure was measured, by an indirect method, immediately before dosing and at intervals after dosing.
* Mean changes in blood pressure were all statistically significant ($P < 0.001$) as judged by Student's paired t test, performed using blood pressure measurements for individual animals at the various intervals after dosing, compared with the initial values.

Table 2 Mean blood pressure of the whole group of animals at intervals after administration of MK 421 (10 mg/kg, orally)

Interval after dosing (h)	No. of animals	Mean blood pressure (mmHg ± s.e.)
0	29	215 ± 8
1	29	185 ± 7*
3	29	172 ± 7**
6	28	174 ± 8**
24	29	185 ± 8*

*$P < 0.01$; **$P < 0.001$ compared with initial value.

existed throughout the duration of the observation period and the values obtained for the correlation coefficient (r) at the various times after dosing were 0.56 (1 h, $n = 29$, $P < 0.01$), 0.60 (3 h, $n = 29$, $P < 0.001$), 0.49 (6 h, $n = 28$, $P < 0.01$) and 0.40 (24 h, $n = 29$, $P < 0.05$). The results for the 3 h interval after dosing are shown in Figure 1.

Relation of plasma renin activity to the initial blood pressure and to the changes in blood pressure induced by MK 421

Comparison of the individual PRA values with the initial blood pressure values indicated that there was

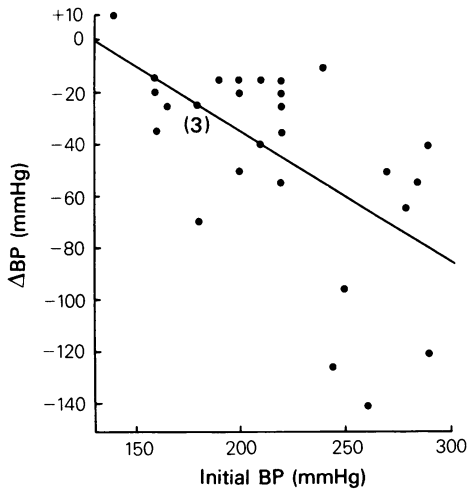

Figure 1 Relation of initial blood pressure to the change in blood pressure (ΔBP) occurring in individual animals 3 h after administration of MK 421 (10 mg/kg, orally). The correlation between the two parameters was highly significant ($r = 0.60$, $P < 0.001$).

some correlation, which was within the 95% confidence limits ($r = 0.45$, $n = 23$). However, a greater degree of correlation existed between the initial PRA values and the degree of change in blood pressure observed after administration of MK 421 (10 mg/kg, orally). The r values obtained at the various times after dosing were 0.61 (1 h, $n = 23$, $P < 0.01$), 0.74 (3 h, $n = 23$, $P < 0.001$), 0.72 (6 h, $n = 22$, $P < 0.001$) and 0.79 (24 h, $n = 23$, $P < 0.001$). The results for the 3 h interval after dosing are shown in Figure 2.

Discussion

The extent to which PRA can be used to predict the antihypertensive effects of ACE inhibitors is not entirely clear. Several studies, particularly in man, have shown that there is a significant correlation between pre-dose PRA and the fall in blood pressure induced by captopril. However, captopril has also been shown to be effective in patients with low renin levels and in animals, such as DOCA-salt hypertensive rats, in which plasma renin levels are below those generally considered to be normal (Miyamori, Brown & Dollery, 1980; Thurston, Bing, Russell & Swales, 1981). We have observed, in studies with two-kidney Goldblatt hypertensive rats, that, on occasion, one or two animals develop a particularly severe hypertension which is associated with a lower than normal body weight and an apparently increased sensitivity to ACE inhibition. In the present study we have investigated whether this phenomenon was confined to a small number of animals, which had perhaps slipped into a malignant phase of hypertension (Möhring, 1975) or whether, on detailed analysis, we could see a more graded relationship between the parameters measured. In addition, the relationship of these various parameters to the initial PRA has been assessed.

Our results indicate that there was no correlation between the body weight of two-kidney Goldblatt hypertensive rats and their initial blood pressure. This would suggest that our previous observations of low body weight/high blood pressure/good responder individuals may simply have been a reflection of isolated incidences of the development of a malignant hypertension. However, a significant correlation was seen between the initial blood pressure and the fall in blood pressure occurring in response to MK 421 (10 mg/kg, orally). This contrasts with the lack of relationship between these two parameters observed in renal hypertensive rats infused with 1-Sar 8-Ala angiotensin II (MacDonald *et al.*, 1975). Our results are similar to theirs in that there was only a modest correlation between the degree of hypertension and the initial PRA. The most striking correlation detected in both studies was that between the initial PRA and the extent of the blood pressure fall.

These relationships suggest that renal artery clipping in rats can increase the involvement of the renin-angiotensin system in maintaining blood pres-

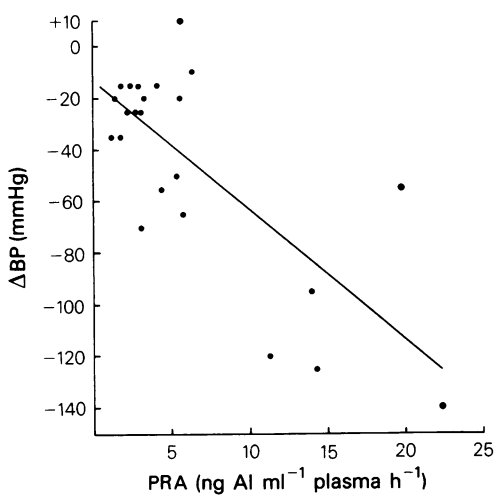

Figure 2 Relation of initial plasma renin activity (PRA) to the change in blood pressure (ΔBP) occurring in individual animals 3 h after administration of (MK 421 (10 mg/kg, orally). The correlation between the two parameters was highly significant ($r = 0.74$, $P < 0.001$).

sure without this necessarily being accompanied by a proportional increase in the blood pressure. This situation could arise from the operation of a compensatory mechanism acting to lower the blood pressure in the face of this increased activity of the renin-angiotensin system.

The renin-dependence of the antihypertensive activity of ACE inhibitors has practical implications regarding the testing of this type of compound in models of renal hypertension. As such it is necessary to ensure that individual animals between groups are matched according to, if possible, their PRA's or, at least, to their initial blood pressures. Lack of matching in this way may have contributed to the lack of clear differentiation between the degree of blood pressure lowering produced by a dose of 3 mg/kg of MK 421, compared with a dose of 10 mg/kg (Sweet *et al.*, 1981). In these experiments the mean blood pressure of the low dose group was greater than that of the high dose group and the antihypertensive effect of the lower dose may therefore have been relatively accentuated.

The clear correlation we have observed, in two-kidney Goldblatt hypertensive rats, between PRA and the antihypertensive response to inhibition of the renin-angiotensin system, contrasts with the lack of such a correlation in DOCA-salt hypertensive rats (Thurston *et al.*, 1981). In this latter model, as in spontaneously hypertensive rats (for references see Rubin, Antonaccio & Horovitz, 1981), ACE inhibition does, however, result in a fall in blood pressure, despite their low plasma renin levels. One possible explanation for these results is that the operation of the renin-angiotensin system within vascular tissue may be a more important determinant of the level of blood pressure than is its operation within plasma (Swales, 1979; Antonaccio, Asaad, Rubin & Horovitz, 1981). It is therefore possible that plasma renin levels are related to changes in blood pressure only when these levels reflect those occurring in vascular tissue. This situation may well exist in the renal hypertensive model we have used but not necessarily in other hypertensive models. If so, then the relationship we have observed between PRA and the antihypertensive effect of MK 421 may not represent a direct cause-effect relationship but may be a reflection of events occurring within vascular smooth muscle. It would therefore be of interest to investigate the relationship between vascular renin levels and the changes in blood pressure induced by compounds known to influence the renin-angiotensin system.

We thank Dr H. Thurston, University of Leicester, for kindly showing us the method for measuring PRA and Merck Sharpe and Dohme for the gift of MK 421.

References

ANTONACCIO, M.J., ASAAD, M., RUBIN, B. & HOROVITZ, Z.P. (1981). Captopril: factors involved in its mechanism of action. In *Angiotensin Converting Enzyme Inhibitors. Mechanism of Action and Clinical Implications.* ed. Horovitz, Z.P. pp.161–180. Baltimore: Urban and Schwarzenberg.

ATLAS, S.A., CASE, D.B., SEALEY, J.E., SULLIVAN, P.M. & LARAGH, J.H. (1978). Involvement of the renin-angiotensin-aldosterone axis in the antihypertensive action of captopril (SQ 14, 225). *Circulation*, **58,** 143.

BENGIS, R.G. & COLEMAN, T.G. (1979). Antihypertensive effect of prolonged blockade of angiotensin formation in benign and malignant, one- and two-kidney Goldblatt hypertensive rats. *Clin. Sci.*, **57,** 53–62.

BENGIS, R.G., COLEMAN, T.G., YOUNG, D.B. & McCAA, R.E. (1978). Long-term blockade of angiotensin formation in various normotensive and hypertensive rat models using converting enzyme inhibitor (SQ 14, 225). *Circulation Res.*, **43,** I45–I53.

BRAVO, E.L. & TARAZI, R.C. (1979). Converting enzyme inhibition with an orally active compound in hypertensive man. *Hypertension*, **1,** 39–46.

BRUNNER, H.R., GAVRAS, H., WAEBER, B., KERSHAW, G.R., TURINI, G.A., VUKOVICH, R.A., McKINSTRY, D.N. & GAVRAS, I. (1979). Oral angiotensin-converting enzyme inhibitor in long-term treatment of hypertensive patients. *Ann. int. Med.*, **90,** 19–23.

BRUNNER, H.R., GAVRAS, H., WAEBER, B., TEXTOR, S.C., TURINI, G.A. & WAUTERS, J.P. (1980). Clinical use of an orally acting converting enzyme inhibitor: captopril. *Hypertension*, **2,** 558–566.

CASE, D.B., ATLAS, S.A., LARAGH, J.H., SEALEY, J.E., SULLIVAN, P.A. & McKINSTRY, D.N. (1978). Clinical experience with blockade of the renin-angiotensin–aldosterone system by an oral converting-enzyme inhibitor (SQ 14, 225, captopril) in hypertensive patients. *Prog. Cardiovasc. Dis.*, **21,** 195–206.

CASE, D.B., ATLAS, S.A., LARAGH, J.H., SULLIVAN, P.A. & SEALEY, J.E. (1980). Use of first-dose response or plasma renin activity to predict the long-term effect of captopril: identification of triphasic pattern of blood pressure response. *J. cardiovasc. Pharmacol.*, **2,** 339–346.

CONWAY, J., HATTON, R. & CLOUGH, D. (1981). Angiotensin, interaction with homeostatic reflexes. In *Angiotensin Converting Enzyme Inhibitors. Mechanisms of Action and Clinical Implications.* ed. Horovitz, Z.P. pp.81–87 Baltimore: Urban and Schwarzenberg.

GAVRAS, H., BRUNNER, H.R., TURINI, G.A., KERSHAW, G.R., TIFFT, C.P., CUTTELOD, S., GAVRAS, I., VUKOVICH, R.A. & McKINSTRY, D.N. (1978). Antihypertensive effect of the oral angiotensin converting-enzyme inhibitor SQ 14,225 in man. *N. Engl. J. Med.*, **298,** 991–995.

GEROLD, M. & TSCHIRKY, H. (1968). Measurement of blood pressure in unanaesthetised rats and mice. *Arzneim. Forsch.*, **18,** 1285–1287.

MACDONALD, G.J., BOYD, G.W. & PEART, W.S. (1975). Effect of the angiotensin II blocker 1-Sar-8-Ala-angiotensin II on renal artery clip hypertension in the rat. *Circulation Res.*, **37,** 640–646.

MACGREGOR, G.A., MARKANDU, N.D., ROULSTON, J.E. & JONES, J.C. (1979). Essential hypertension: effect of an oral inhibitor of angiotensin-converting enzyme. *Br. med. J.*, **2,** 1106–1109.

McCAA, R.E. (1981). Aldosterone and fluid balance, In: *Angiotensin Converting Enzyme Inhibitors. Mechanisms of Action and Clinical Implications*, ed. Horovitz, Z.P. pp. 199–238. Baltimore: Urban and Schwarzenberg.

MIYAMORI, I., BROWN, M.J. & DOLLERY, C.T. (1980). Single-dose captopril administration in DOCA/salt rats: reduction of hypotensive effect by indomethacin. *Clin. exp. Hypertension*, **2,** 935–945.

MÖHRING, J. (1975). Pathogenesis of malignant hypertension: experimental evidence from the renal hypertensive rat. *Clin. Nephrol.*, **5,** 167–174.

RUBIN, B., ANTONACCIO, M.J. & HOROVITZ, Z.P. (1981). The anti-hypertensive effects of captopril in hypertensive animal models, In *Angiotensin Converting Enzyme Inhibitors. Mechanism of Action and Clinical Implications.* ed. Horovitz, Z.P. pp. 27–53 Baltimore: Urban and Schwarzenberg.

SATOH, S., SUZUKI, M. & SATOH, H. (1980). Hypotensive effect of captopril in relation to plasma renin activity in anaesthetised rats. *J. Pharm. Dyn.*, **3,** 258–260.

SWALES, J.D. (1979). Arterial wall or plasma renin in hypertension? *Clin. Sci.*, **56,** 293–298.

SWEET, C.S., GROSS, D.M., ARBEGAST, P.T., GAUL, S.L., BRITT, P.M., LUDDEN, C.T., WEITZ, D. & STONE, C.A. (1981). Antihypertensive activity of N-((S)-1-(ethoxycarbonyl)-3-phenylpropyl)-l-Ala-l-Pro (MK 421), an orally active converting enzyme inhibitor. *J. Pharmac. exp. Ther.*, **216,** 558–566.

THURSTON, H., BING, R.F., RUSSELL, G.I. & SWALES, J.D. (1981). The vasodepressor mechanism of converting enzyme inhibitors, In *Angiotensin Converting Enzyme Inhibitors. Mechanisms of Action and Clinical Implications.* ed. Horovitz, Z.P. pp. 141–159. Baltimore: Urban and Schwarzenberg.

(Received November 2, 1981.)