INFLUENCE OF VARIOUS ANTIHYPERTENSIVE AGENTS ON LIFESPAN OF RENAL HYPERTENSIVE RATS

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- 1 Daily treatment of two-kidney clipped renal hypertensive rats with hydralazine, hydrochlorothiazide (HCTZ) and a new orally active inhibitor of the angiotensin-converting enzyme, captopril (SQ14,225), was correlated with survival rates for up to 9 months.
- 2 The groups of rats given captopril alone or captopril plus intermittent or chronic HCTZ had the best survival rate, whereas HCTZ alone or hydralazine did not beneficially affect survival.
- 3 Survival rates correlated well with control of BP in these animals.

Introduction

ALTHOUGH renal hypertensive animals have served as predictive models for certain hypertensive states in man since the early 1930s, there are few reports that document the chronic effects of antihypertensive agents on the BP or lifespan of these hypertensive animals.

We decided to study the effects of a number of different types of antihypertensive agents after chronic daily administration to renal hypertensive rats in the hope of finding an agent that was effective in prolonging the lifespan of these animals. At the time we began this study there was some literature on the use of β -adrenoceptor blocking agents in this model that led us to exclude these agents from our study. Lundgren (1974) has reported that treatment with propranolol for up to 8 weeks in the two-kidney Goldblatt hypertensive rat had no effect on BP although it produced the expected heart rate decrease. Propranolol also did not block the vascular damage characteristic in this model.

Leenen & Ackerman (1976) were not able to prevent the onset of increase in systolic BP by giving propranolol 2 d before clipping the renal artery; and the β -adrenoceptor-blocking agent was also not able to block the increase in plasma renin activity. Niarchos *et al.* (1977) did report that they were able to decrease the incidence of development of hypertension by pretreating with propranolol for 3 d before clipping, but they also confirmed that established renovascular hypertension in rats was not affected by β -adrenoceptor-blocking agents.

We thus chose to compare, in this model, the long-term effects of a vasodilator, hydralazine; a diuretic, hydrochlorothiazide (HCTZ); and a new

experimental antihypertensive agent, captopril (SQ 14,225; D-3-mercapto-2-methylpropanoyl-L-proline), an orally active inhibitor of angiotensin I converting enzyme (ACE), the enzyme responsible for converting angiotensin I (AI) to angiotensin II (AII) (Ondetti *et al.*, 1977; Ferguson *et al.*, 1977).

Captopril has been shown to reduce BP when orally administered for 2 d to two-kidney renal hypertensive rats (RHRs) and spontaneously hypertensive rats. The reductions in BP seem to be directly related to the inhibition of this peptidyl dipeptide hydrolase (ACE, kininase II) by captopril, as at this time no other significant pharmacological effects have been demonstrated which might account for the antihypertensive activity (Rubin *et al.*, 1978a).

Methods

Nine groups of male rats of the normotensive Sprague-Dawley (COBS-CD) strain (NR), 15 rats/group, were used in this study. The rats were about 10 weeks old and ranged from 120-150 g in body weight at the time of surgery. Each rat was anaesthetized with ether, the left renal artery was isolated by a flank incision, and a silver clip, 0.22 mm in diameter, was placed on that artery. The contralateral kidney and renal artery were untouched. The animals were maintained on a normal rat diet of Wayne Lab Blox and tap water *ad libitum*. The rat diet provided 170 μ mol sodium/g and 246 μ mol potassium/g. Each rat was housed in an individual stainless steel wire mesh cage, and subjected to a daily light cycle of 12 h, a room

Figure 1 Effects on indirect SBP in conscious two-kidney RHRs of daily oral doses of H₂O 5 ml/kg (a, group A); SQ14,225 30 mg/kg (b, group D); and hydralazine 6 mg/kg (c, group I). Each point is a mean $\pm$ s.e.; asterisks denote 24 h readings after the preceding daily dose; all other readings are 4 h after the daily dose. (Data derived from Rubin *et al.*, 1978; with permission.)

Table 1 Treatment regimen for groups A–I

Two-kidney RHR group	No. of rats	Daily oral dose gavage 5 mg/kg/d	5 days crossover treatment
A	15	Control (H ₂ O only)	—
B	15	Control (H ₂ O only)	Captopril
C	15	Control (H ₂ O only)	HCTZ
D	15	Captopril 30 mg/kg/d (in H ₂ O)	—
E	15	Captopril 30 mg/kg/d (in H ₂ O)	H ₂ O
F	15	Captopril† 30 mg/kg/d (in H ₂ O)	HCTZ*
G	15	HCTZ 6 mg/kg/d (in 0.25% methylcellulose solution)	—
H	15	HCTZ 6 mg/kg/d (in 0.25% methylcellulose solution)	Captopril
I	15	Hydralazine 6 mg/kg/d (in H ₂ O)	—

*Days 28–33, 91–96 and 182–187, unless otherwise indicated.

†Daily dosage of each drug was maintained from day 109 onwards.

Figure 2 Effects of SBP in conscious two-kidney RHRs of daily oral doses of H₂O 5 ml/kg and intermittent 5-d dosage with SQ14,225 30 mg/kg at 1, 3 and 6 months (a, group B); and of daily oral doses of SQ14,225 30 mg/kg and intermittent 5-d withdrawal of dosage at 1, 3 and 6 months (b, group E). (Data derived from Rubin *et al.*, 1978; with permission.)

temperature of 72–75°F and a relative humidity of 40–45%.

All two-kidney-RHRs were used 6 weeks after unilateral clipping. A total of 135 RHRs with indirect SBP of 160 mmHg or greater were used. Fifteen rats were assigned to each of the following nine treatment groups, identified hereafter by the letters A to I, as shown in Table 1.

Groups of six animals from groups A to I were allowed to acclimate for 6–7 min at 37°C in individual wire mesh restrainer cages. Each rat was fitted with a tail cuff for air inflation and a Korotkoff sound microphone for the detection of arterial pulsation. An oscilloscope was used for the visual appearance and disappearance of the pulse. SBP measurements were made on a Narco Physiograph Manometer. Heart rate was measured using a biotachometer (Narco Bio-Systems). These determinations were made (1) before the initial dose, (2) 4 h after each dose, except as noted, at 3- or 4-d intervals, (3) occasionally 24 h after each dose, and (4) during the 5 d combination dosage, just before the combination dose and generally 4 or 5 h after each dose over the 5-d period.

BP data are reported as mean $\pm$ s.e. Significance of

differences was considered when $P \leq 0.05$. Data reported in this paper show the results of approximately 6 months treatment on BP and 9 months on survival rate.

Results

Daily oral administration of water vehicle for over 6 months had no significant effects on SBP of two-kidney RHRs (Figure 1a). Administration of captopril 30 mg/kg orally caused rapid reductions in SBP of about 30–50 mmHg which were relatively consistent over the 6-month dosing period (Figure 1b). Substitution of captopril with water for 5 d after the 6-month dosage period resulted in a gradual return of SBP toward control; re-administration of captopril again promptly reduced SBP (Figure 1b). Administration of hydralazine 6 mg/kg orally also caused a rapid and marked reduction in SBP in two-kidney RHRs. However, in contrast to captopril-treated rats, there was a rapid tolerance to the antihypertensive effect of hydralazine with SBP returning almost to control levels within 4 weeks of dosing with no further significant effects on SBP despite continued dosing (Figure 1c).

Figure 3 Effects on SBP in conscious two-kidney RHRs of daily oral doses of H₂O 5 ml/kg and intermittent 5-d dosage with HCTZ 6 mg/kg at 1, 3 and 6 months (a, group C); daily oral doses of HCTZ 6 mg/kg alone (b, group G); daily oral doses of SQ14,225 30 mg/kg with intermittent additional dosage with HCTZ 6 mg/kg (c, group F); and daily oral doses of HCTZ 6 mg/kg and intermittent 5-d dosage with SQ14,225 30 mg/kg at 1, 3 and 6 months (d, group H). (Data derived from Rubin *et al.*, 1978; with permission.)

In two-kidney RHRs receiving only water, intermittent 5 d daily dosage with captopril (30 mg/kg orally) at 1, 3 and 6 months caused rapid, consistent reductions in SBP (Figure 2a). When captopril was discontinued, SBP slowly recovered to its usual level in about 1 week. Similarly, in another group of two-kidney RHRs receiving captopril

30 mg/kg orally daily for 6 months, intermittent withdrawal of compound of 5-d periods at 1, 3 and 6 months resulted in a gradual return of SBP toward control levels; re-instatement of captopril treatment consistently reduced SBP after each withdrawal period (Figure 2b).

HCTZ 6 mg/kg orally had no important or

Figure 4 Survival rates of two-kidney RHRs dosed for 9 months. ●, SQ14,225 + HCTZ (group F, $n = 15$); ○, SQ14,225 (groups D and E, $n = 30$); ■, HCTZ (groups G and H, $n = 30$); □, hydralazine (group 1, $n = 15$); △, control, water-vehicle (groups A, B and C, $n = 45$). n is the initial number of rats (see methods section for further details). (Data derived from Rubin *et al.*, 1978; with permission.)

consistent effect on SBP of two-kidney RHRs when given intermittently for 5-d periods at 1, 3 and 6 months (Figure 3a) or when administered daily for 6 months (Figure 3b). However, in two-kidney RHRs maintained on daily dosage of captopril 30 mg/kg orally for 6 months, concomitant administration of HCTZ 6 mg/kg orally for 5-d periods at 1, 3 and 6 months enhanced the already established antihypertensive effect of captopril (Figure 3c). Similarly, in two-kidney RHRs maintained on daily dosage of HCTZ 6 mg/kg orally for 6 months, the additional administration of captopril 30 mg/kg orally for 5-d periods at 1, 3 and 6 months resulted in large reductions in the SBP which were generally greater than those obtained with captopril alone (Figure 3d).

No consistent effects on heart rate were noted in the two-kidney RHRs treated with water, captopril, HCTZ or a combination of captopril plus HCTZ (range: 408–453 beats/min); however, hydralazine caused a consistent tachycardia (range: 450–502 beats/min) despite the tolerance observed for its antihypertensive effects.

The survival rates of the two-kidney RHRs dosed for 9 months are shown in Figure 4. The data for

groups A, B and C (controls), for the two groups maintained chronically on captopril (D and E) have been pooled. The greatest survival rate occurred in group F chronically treated with captopril plus HCTZ, and captopril alone, whereas the HCTZ, hydralazine and vehicle control groups all showed the poorest and virtually identical survival rates.

Discussion

These studies indicate that successful control of BP in the RHRs for 6–9 months is able to significantly, favourably influence survival rates. The best survival rates were the groups given the angiotensin-converting enzyme inhibitor, captopril (SQ 14,225), plus intermittent or chronic HCTZ. The next best survival rate was that for the groups treated chronically with captopril alone. These survival rates were clearly better than the other groups, but clearly worse than the group under captopril–diuretic therapy. There were no significant differences among the survival rates of the water, HCTZ alone or hydralazine groups.

The survival rates seem to be closely correlated with BP control. This seems very obvious in the hydralazine group, in which deaths only appeared after the tachyphylaxis to the BP effect was apparent. Also the fact that HCTZ did not lower BP and did not influence survival substantiates this correlation.

Rubin *et al.* (1978) have reported that captopril produces a significant reduction in heart weights of RHRs during 6 months of chronic treatment and Horovitz *et al.* (1978) have reported the same effect in the spontaneous hypertensive rat. This effect on heart size also seems to be correlated with BP control and may be related to survival rates.

Whether specific interference with the renin–angiotensin system by captopril plays a role in the prolonged survival of RHRs is unknown and must await further animal and clinical experimentation, in which captopril is compared with other agents which effectively reduce high BP. The first hypothesis must be that control of the hypertension is responsible for the improved survival of the captopril group. However, the potential clinical significance of these data for captopril is obvious and supports the well-documented long risk factor of hypertension and the significant benefit of effective antihypertensive therapy in both animals and man (Freis *et al.*, 1972; Pickering, 1972).

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Discussion

DR ROSENTHAL (Ulm): I am sure you do post mortems on your animals, did you find any significant difference between the groups with respect to atherosclerotic lesions? What was your reason for not including a group of animals treated with a β -blocker?

DR HOROVITZ (Princeton): We have not completed all the post mortems yet. There do appear to be differences. We did notice changes in eye and mesenteric vessels that seem to correlate with the hypertensive animals and did not show up in the controls. We do not have data in qualitative or quantitative form on which we can make any inferences.

In answer to the second question, it was because the data at the time we started the study indicated that in at least two of the three months of treatment the β -blockers were not effective in lowering the BP in the renal hypertensive rat. We also found the same situation with hydrochlorothiazide and indeed with

hydralazine after a month of treatment. We were not aware of the latter findings at the time of initiating the study. I'm sorry now we didn't include a β -blocker-treated group.

DR FULTON (Manchester): Could Dr Horovitz speculate on the reason for the difference in the mortality in animals who are given the angiotensin I — converting enzyme inhibitor and the inhibitor plus hydrochlorothiazide?

DR HOROVITZ: Why does the addition of the diuretic change the mortality rate? The lower mortality rate is probably due to a somewhat better control of BP. It would be easy to hypothesize that with the converting enzyme inhibitor we obtain fairly good control of the renin-angiotensin system, but there may still be a small volume component in the hypertension of these animals that we're not controlling and we pick that up by adding the diuretic.