ANTIHYPERTENSIVE EFFECTS OF A COMBINATION OF A DIURETIC AND A β-ADRENOCEPTOR BLOCKING AGENT IN CONSCIOUS, RENAL HYPERTENSIVE DOGS

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Abstract—The influence of a diuretic, clorexolone and a β-adrenoceptor blocking agent, alprenolol, alone and in combination, on blood pressure, heart rate and serum angiotensin II and aldosterone levels were examined in 5 conscious renal hypertensive dogs. Clorexolone (10 mg/kg p.o., twice a day) caused a slight increase in heart rate, and a gradual decrease in blood pressure which became significant after the second day of treatment (p<0.05). The mean reduction in systolic blood pressure by clorexolone alone was about 15 mm Hg. Addition of alprenolol (10 mg/kg p.o., twice a day) induced a further rapid decrease in arterial blood pressure and there was a tendency toward decrease in the increased heart rate. The mean decrease in systolic blood pressure was about 26 mm Hg and such was highly significant in comparison with that exerted by clorexolone alone (p<0.025). After withdrawal of clorexolone and final replacement of alprenolol with lactose, the decreased blood pressure gradually returned to the initial value. Increases in serum angiotensin II and aldosterone levels seen after clorexolone alone declined to the initial value when alprenolol was given in combination with clorexolone. The antihypertensive effect of this combination is attributed mainly to the inhibition of renin-angiotensin-aldosterone system, to decrease in circulating blood volume, and in part to decrease in the heart rate.

β-Adrenoceptor blocking agents are now widely and effectively used in the treatment of hypertension (1-3). The precise mechanism of the antihypertensive action remains, however, to be elucidated. Diuretics and vasodilators produce an increase in plasma renin activity and often there is a tendency toward increase in heart rate (4-8). It is well known that many β-adrenoceptor blocking agents suppress renin release (9-13) and inhibit a reflex increase in heart rate (5, 14, 15) in both man and animals. The combined use of a β-adrenoceptor blocking agent and a diuretic or a vasodilator would seem, therefore, to be a potentially promising approach to the treatment of hypertensive patients (5, 6, 8, 16).

We gave a combination of a β-adrenoceptor blocking agent, alprenolol and a diuretic, clorexolone to conscious renal hypertensive dogs and our observations are reported herein.

MATERIALS AND METHODS

Measurements of the arterial blood pressure and heart rate in conscious renal hypertensive dogs

Experiments were carried out on 5 conscious renal hypertensive mongrel dogs of either
sex, weighing between 9.5-15 kg. Renal hypertensive dogs were prepared as follows: One kidney was ligated tightly in the shape of figure eight using surgical silk thread (Shineido Co. JIS No. 10) and the contralateral one was excised. Two to three months later when the systolic blood pressure of 150 mm Hg or over was maintained, the animal was used as a renal hypertensive specimen.

Details of the preparation for measurement of the arterial blood pressure under a conscious condition were as described elsewhere (17, 18). Briefly, under pentobarbital anesthesia, an arterial catheter (INTRAMEDIC®, PE50-60) which was filled with sterile physiological saline solution containing sodium heparin (200 units/ml) for recording the blood pressure, was introduced into the aorta via a muscular branch of the femoral artery. The other end of the catheter was exteriorized through the skin at the back of the neck. The experiments were started at the 5th day after implantation of the catheter. The chronically implanted arterial catheter was connected to a pressure transducer (Nihon Kohden, RP-2) and the systolic and diastolic blood pressure was recorded directly in mm Hg; the heart rate was counted from the blood pressure pulse waves for about 10 sec. Measurements of the arterial blood pressure and heart rate were performed in a sound-proof room; the animal, which was fully conscious and unrestrained, was sitting on the floor. Intravenous injection of a drug (isoprenaline, 1 μg/kg) and venous blood sampling (about 8 ml) were performed through a catheter acutely inserted into the cephalic vein of either foreleg. The blood which was collected in chilled tubes containing EDTA-2Na (1 ml blood/1 mg EDTA-2Na) for angiotensin II and aldosterone estimation, was separated immediately and the serum was deep frozen until all samples were analysed.

Measurement of serum angiotensin II and aldosterone levels

Serum angiotensin II level was quantified by making efficient use of the idea of the double antibody method which was first used for the radioimmunoassay of insulin by Morgan and Lazarow (19). Frozen serum samples were thawed on ice and incubated for 24 hr at 4°C with 125I-angiotensin II and antiserum which was produced in rabbits with Ile 2-angiotensin II, and then in the presence of goat anti-rabbit IgG serum, the samples were incubated at 4°C for 4 days. Finally the angiotensin II level in serum was measured on the basis of the value obtained by counting the radioactivity of precipitates and the standard curve constructed by use of standard angiotensin II (Protein Research Foundation, Minoh, Japan).

Serum aldosterone was measured by radioimmunoassay using a modification of the technique of Ito et al. (20). Antiserum was produced in rabbits with aldosterone-3-O-carboxymethyl-oxim-BSA which was prepared according to the procedure of Erlanger et al. (21). Purification of serum aldosterone was performed using microcolumn chromatography (Sephadex LH-20). Standard curve was constructed by use of aldosterone-1,2,6,7- 3H (82 Ci/mM, New England Nuclear).

Experimental protocol

Fig. 1 shows the experimental protocol. In the experimental courses of alprenolol
alone or a combination with clorexolone, the $\beta$-adrenoceptor blocking activity was checked on the basis of the antagonism of tachycardia induced by isoprenaline ($1 \mu$g/kg i.v.) at the decided time (at 0, 1, 2, 3 or 6 hr) after the first administration. For comparison, the response to isoprenaline ($1 \mu$g/kg i.v.) was also examined on the first day of lactose administration (Figs. 1 and 3). Prior to these series of experiments, basal blood pressure and heart rate were measured periodically (at 1:00 p.m.) during 21 days (Fig. 2).

During the series of experiments for more than 60 days, all 5 renal hypertensive dogs were in good health, and general behaviour and appearance were quite normal.

The drugs used were $dl$-alprenolol hydrochloride (Teikoku Hormone Co.), clorexolone (6-Chloro-2-cyclohexyl-3-oxo-5-isoindoline sulfonamide, May & Baker Research Labs.), $l$-isoprenaline hydrochloride (Nikken Kagaku) and lactose (Wako).

Significance of the difference in the paired mean values was evaluated using Student's $t$-test.
RESULTS

Arterial blood pressure and heart rate in conscious renal hypertensive dogs

The average arterial blood pressure and heart rate of 5 dogs used were 156±3.8 (systolic)/113±2.8 (diastolic) mm Hg and 112±6.8 beats/min, respectively, in the conscious state on the 5th day after implantation of an arterial catheter (0 day in Fig. 2). These values were almost the same as those observed in our previous study (17, 22). As clearly shown in Fig. 2, changes in blood pressure and heart rate were not significant for 21 days (p>0.1).

Effects of clorexolone, clorexolone plus alprenolol and alprenolol on blood pressure and heart rate in conscious renal hypertensive dogs

Clorexolone (2×10 mg/kg/day) caused a gradual decrease in blood pressure which became significant after the second day of treatment (p<0.05). The systolic blood pressure value (142±2.9 mm Hg) at 3 hr after the first administration on the 4th day, was reduced by an average 15.2±2.8 mm Hg from the initial value (158±2.9 mm Hg) (p<0.01). A decrease in the diastolic blood pressure was similar to that in the systolic value (Fig. 3). Clorexolone produced an increase in heart rate by about 12 beats/min. In the combination phase of clorexolone and alprenolol, the heart rate was prone to decrease. With a simultaneous administration of clorexolone (2×10 mg/kg/day) and alprenolol (2×10 mg/kg/day), the arterial blood pressure decreased markedly even on the first day of the treatment. On the 4th day of the combination dosing, the systolic blood pressure value (at 3 hr after the first administration) decreased by 26.2±2.1 mm Hg. This decrease was more significant.

![Graph showing changes in arterial blood pressure and heart rate](image-url)

**Fig. 3.** Changes in arterial blood pressure and heart rate after p.o. administration of clorexolone, alprenolol or their combination. β-Adrenoceptor blocking activity of alprenolol was also checked on the basis of the antagonism of tachycardia induced by the i.v. administration of isoprenaline (1 μg/kg).
(p<0.025) than that (15.2±2.8 mm Hg) observed at 3 hr after the first administration on the 4th day of the treatment with clorexolone alone. In further successive experiments with alprenolol (2×10 mg/kg/day) alone and finally with lactose (2×100 mg/head/day) alone, the decreased blood pressure gradually returned to the initial value. On the 14th day of lactose administration, the average value (159±4.6/118±2.8 mm Hg) was almost the same as that (158±2.9/118±1.6 mm Hg) at the beginning of the experiments. In checking responsiveness of the heart to i.v. administration of isoprenaline (1 μg/kg), alprenolol inhibited completely the isoprenaline-induced tachycardia at 1-6 hr, and even at 16.5 hr, the inhibition was about 50%. At 24–30 hr after the cessation of alprenolol administration, however, the β-adrenoceptor blocking activity had vanished.

Effects of clorexolone, clorexolone plus alprenolol and alprenolol on serum angiotensin II and aldosterone levels in conscious renal hypertensive dogs

In the course of experiments, serum angiotensin II and aldosterone levels were also measured (Fig. 4). Clorexolone alone produced an increase in the serum angiotensin II level and this level declined to the initial value when alprenolol was concomitantly administered. Changes in the aldosterone level with clorexolone alone and in combination with alprenolol paralleled those seen in cases of angiotensin II.

DISCUSSION

Diuretics serve frequently as the basal drugs in the clinical management of hypertension and in most cases, they are used effectively in combination with other antihypertensive drugs, because their antihypertensive activities are relatively mild (3, 5, 23, 24). In our conscious renal hypertensive dogs, clorexolone, a diuretic which is about 300 times as potent as chlorothiazide in rats and about 50 times in dogs in terms of the doses producing an equivalent diuretic response (25), exerted only a weak antihypertensive action which became significant.
after the second day of treatment even with the dose level of 10 mg/kg p.o. twice daily. In preliminary trials, 3 mg/kg p.o. of clorexolone produced no significant changes in blood pressure and heart rate in conscious renal hypertensive dogs, for 48 hr. Thus, in our experiments the antihypertensive action of clorexolone was also mild and developed gradually as do other diuretics in cases of experimentally induced hypertension (26, 27).

Recently, it has been claimed that combined therapy with a $\beta$-adrenoceptor blocking agent and a diuretic is an effective regimen for the treatment of hypertension and there are relatively few untoward side effects (5, 6, 8, 16). In our study, herein, the combination of clorexolone and alprenolol exerted more prominent antihypertensive action than did the administration of the individual drug alone. The favorable results of the combined administration of alprenolol and clorexolone inevitably raise the question of what circumstances are responsible. We assumed that the greater fall in arterial blood pressure with administration of clorexolone plus alprenolol is mainly due to the inhibition of renin release and thus to the decrease in angiotensin II. Our reasons are thus: it has been well documented that most $\beta$-adrenoceptor blocking agents including alprenolol lower the resting renin levels or inhibit diuretics- or vasodilators-induced renin release (6, 8, 11, 13, 15, 16). In our experiments, the increased angiotensin II and aldosterone levels during the period of clorexolone treatment were definitely inhibited by the additional administration of alprenolol. The arterial blood pressure decreased to a lesser extent when clorexolone was given alone and it may be that the vasoconstrictor angiotensin II formed, antagonized the antihypertensive action of clorexolone. Thus, the decrease in the vasoconstrictor substance in blood as the result of alprenolol dosing would be expected to lower the arterial blood pressure to an even greater extent. In addition, alprenolol would induce a fall in arterial blood pressure through a mechanism independent of the inhibitory action on the renin release (22, 28). A decrease in blood volume may also play a role in the effective antihypertensive action seen with a combination of clorexolone and alprenolol. Chronic treatment with diuretics undoubtedly decreases the circulating blood volume and clinical experience supports the suggestion that the diuresis augments the hypotensive action of most agents and at least the decrease in extracellular fluid partly contributes to the marked hypotension (12, 29–31). Slight increases in heart rate of dogs treated with clorexolone may indicate a partly increased sympathetic tone, as suggested by Zacest et al. (5) and Waal-Manning and Simpson (7) during diuretic therapy. This is supported by the fact that the slight increase in heart rate is inhibited by alprenolol, and this attenuation may in part potentiate the fall in blood pressure.

From our studies using conscious renal hypertensive dogs, it would seem that the combination therapy of $\beta$-adrenoceptor blocking agents and diuretics is appropriate for the treatment of the hypertension, as others have also observed in man (5, 11) or in dogs and rats (16).

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