The Effects of Acebutolol Alone and in Combination with Furosemide or Hydralazine in Experimentally Induced Hypertensive G-minipigs

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Experimentally induced hypertensive G-minipigs were used for assessing the antihypertensive effects of acebutolol, a cardioselective β -adrenergic blocking agent. In the acute experiment, six females were used. Acebutolol (3 mg/kg, i.v.) alone or in combination with furosemide (1 mg/kg, i.v.) or hydralazine (1 mg/kg, i.v.) was administered through an implanted catheter. In the chronic experiments, five females received oral acebutolol (100–200 mg/day). The blood pressure, heart rate, plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were used as parameters. In the acute experiment, there were no marked changes in the blood pressure or heart rate during the nondosing period. Acebutolol alone caused a marked decrease in the blood pressure and heart rate. In the two combination tests, combined administration with acebutolol and furosemide had a greater effect on the blood pressure and heart rate than did acebutolol alone. A combined acebutolol and hydralazine regimen caused a slight reduction not only in the blood pressure, but also in the heart rate compared with acebutolol alone. PRA and PAC remained essentially constant, with minor fluctuations, throughout the nondosing period. Following the injection of acebutolol alone, PRA showed an elevation with a significant rise after three hours and PAC showed a tendency to increase. PRA and PAC generally tended to increase in the case of combined administration with furosemide or hydralazine, but these tendencies were less conspicuous than with acebutolol alone. On the other hand, chronic treatment with acebutolol produced a significant decrease in the heart rate from two weeks after the administration and in the blood pressure from four weeks. When subsequent doses were changed, no dose-related changes in systolic or diastolic pressure occurred two weeks after medication and only systolic pressure decreased significantly four weeks after treatment. The heart rate showed no further response to an increase in acebutolol dose. As mentioned above, hypertensive G-minipigs receiving acebutolol alone or in combination with furosemide or hydralazine showed antihypertensive effects.

In a previous paper [12], the authors have reported experimentally induced hypertension in Göttingen miniature pigs (G-minipigs) with unilateral nephrectomy and high sodium chloride loading. It is necessary to confirm whether or not an antihypertensive effect is actually

produced when the hypertensive G-minipigs are given antihypertensive agents. We have reported a pharmacological comparison of angiotensin converting enzyme inhibitor using G-minipigs in *in vivo* and *in vitro* experimental systems [6]. No report has been made

concerning the evaluation of antihypertensive agents using G-minipigs. In this study, the acute and chronic antihypertensive effects of acebutolol, a selective β -adrenergic agent, were evaluated in conscious G-minipigs with experimentally induced hypertension.

Materials and Methods

Six (Group A) and five (Group B) female G-minipigs, ranging from six to eight months of age and 19 to 24 kg in body weight, were unilaterally nephrectomized and loaded with 1 % physiological saline (2,000 ml/day) [12] and deoxycorticosterone acetate (100 mg/week, i.m.) [5]. A catheter was implanted in the jugular vein [11] and blood was drawn through the catheter. The blood was transferred to a test tube containing EDTA, and the plasma was

separated with a cooled centrifuge. To measure the blood pressure and heart rate under non-anesthetized conditions, the animals were suspended using a chinning apparatus. The blood pressure and heart rate were measured using an oscillometric sphygmomanometer (Nippon Colin Co., Ltd.). Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were determined using a radioimmunoassay kit (Dainabot Inc.).

Group A was used for experiments on the acute antihypertensive effects. The diurnal variation in the blood pressure and heart rate, as well as PRA and PAC levels were observed in each hypertensive G-minipig. A solution of the drugs was administered through a catheter. Acebutolol hydrochloride (Acebutolol®;

—○— Mean $\pm$ S.D. (N=6)

Significant differences were not observed as compared with the predosing (0 hr) period.

Fig. 1. Acute changes in the blood pressure and heart rate in the nondosing period.

—○— Mean $\pm$ S.D. (N=6)

Significant differences were evaluated as compared with predosing (0 hr) period.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Fig. 2. Acute changes in the blood pressure and heart rate following the administration of acebutolol alone.

Rhone-Poulenc) was given in a dose of 3 mg/kg, alone or concomitantly with 1 mg/kg of furosemide (Lasix®; Hoechst, a hypotensive diuretic) or 1 mg/kg of hydralazine hydrochloride (Apresolin®; Ciba, a vasodilator). Each hypertensive G-minipig was given the regimens of these single and combination tests three times at 10-day intervals. Control experiments, i.e., the nondosing period, were performed before dosing.

Group B was used for experiments on the chronic antihypertensive effect and received oral acebutolol doses of 100, 200 and 100 mg/day for the first and second four weeks and the next two weeks, respectively. Measurement of the blood pressure and heart rate and also blood sampling for measurement of PRA and

PAC were performed.

Statistical analysis was conducted by means of analysis of variance and Student's *t*-test for predosing observations. The 95% limits of probability were accepted as significant.

Results

Concerning the acute effects of acebutolol alone or in combination with furosemide or hydralazine on the heart rate and blood pressure, the hypertensive G-minipigs exhibited a virtually constant blood pressure and heart rate with no noticeable diurnal variation during the nondosing period (Fig. 1). In response to an injection of acebutolol alone, both the blood pressure and heart rate decreased significantly

—○— Mean $\pm$ S.D. (N=6)

Significant differences were evaluated as compared with the predosing (0 hr) period.

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Fig. 3. Acute changes in the blood pressure and heart rate following the combined administration of acebutolol and furosemide.

—○— Mean $\pm$ S.D. (N=6)

Significant differences were evaluated as compared with the predosing (0 hr) period.

* $P < 0.05$ ** $P < 0.01$

Fig. 4. Acute changes in the blood pressure and heart rate following the combined administration of acebutolol and hydralazine.

Table 1. Changes in the plasma renin activity and plasma aldosterone concentration after the administration of the agents in the acute experiment.

	Hours after administration					
	0	1	3	5	8	24
Renin activity (ng/ml/hr)						
Non dosing	1.20±0.49	0.98±0.37	0.72±0.29	0.75±0.37	0.83±0.42	1.05±0.47
Acebutolol ¹⁾	0.77±0.34	1.28±1.20	1.67±0.66*	3.80±2.67	2.92±1.91	0.98±0.20
Furocemide ²⁾	1.02±0.44	1.44±0.93	0.90±0.44	1.85±1.10	2.03±1.35	1.23±0.93
Hydralazine ³⁾	1.62±0.49	1.48±1.08	1.96±1.45	2.80±2.50	1.80±0.96	1.48±0.49
Aldosterone level (pg/ml)						
Non dosing	13.7±6.9	14.8±4.4	12.3±4.4	13.2±4.2	12.8±4.2	15.8±2.7
Acebutolol ¹⁾	15.9±5.1	16.0±4.2	17.3±1.7	20.0±6.6	24.9±8.3	14.5±2.7
Frocemide ²⁾	15.3±4.4	15.0±5.6	10.5±3.2	18.5±5.9	18.0±8.1	13.2±2.0
Hydralazine ³⁾	11.2±1.2	12.7±3.7	17.8±10.0	15.1±4.2	17.8±4.9	12.8±3.4

Each value represents a mean±S.D. (N=6). * Significantly different at the 5% level compared with predosing (0 hr) period.

- 1) Acebutolol alone
- 2) Combined administration of acebutolol and furocemide.
- 3) Combined administration of acebutolol and hydralazine.

compared with the predosing level (Fig. 2). Combined injection of acebutolol and furosemide also produced a significant decrease in blood pressure and heart rate, and the effect was sustained longer than that after the administration of acebutolol alone (Fig. 3). A combined acebutolol and hydralazine regimen caused a slight reduction in heart rate and blood pressure compared with acebutolol alone (Fig. 4).

Both PRA and PAC remained essentially constant, with minor diurnal changes throughout the nondosing period (Table 1). Following administration of acebutolol alone, both parameters showed elevations, with a significant rise in PRA after three hours and a tendency to increase in PAC after eight hours. PRA and PAC generally tended to increase following the administration of acebutolol in combination with furosemide or hydralazine, but the elevations were less conspicuous than those seen with acebutolol alone.

Concerning the chronic effect of acebutolol, Fig. 5 shows the time-course of the blood

←Fig. 5. Time course of the blood pressure and heart rate after the oral administration of acebutolol. G-minipigs were given doses of 100, 200 and 100 mg/day for the first and second four weeks and the next two weeks, respectively.

pressure and heart rate throughout the experimental period. The heart rate decreased significantly from two weeks after the administration, but significant decreases in blood pressure were observed from four weeks after medication. When subsequent doses were changed, no dose-related changes in systolic or diastolic pressures occurred two weeks after medication and only systolic pressure was significantly decreased four weeks after treatment. The heart rate showed no further response to an increase in acebutolol dose.

PRA and PAC were measured before and after acebutolol treatment. Chronic treatment with acebutolol tended to increase PRA and PAC (data not shown).

Discussion

In a study conducted using various strains of rats, Takami *et al.* [9] observed that acebutolol suppressed the blood pressure when administered by the intraperitoneal route. On the other hand, it has been shown that intravenous injection of β -blockers produced an elevation of blood pressure in rats [4, 10]. In the present study of treatment with acebutolol alone, the decline in systolic and diastolic pressures tended to correlate well with the decrease in heart rate. This may be interpreted as a direct manifestation of decreased cardiac output by the cardioselective action of the β -blocker acebutolol causing only a slight constriction of peripheral blood vessels.

Single oral doses of 400 mg of acebutolol brought about a significant, prolonged decrease in the heart rate and blood pressure in normal subjects and hypertensive patients [1, 3]. In this study, the cardiovascular system of hypertensive G-minipigs showed a good response acutely and chronically to acebutolol dosages. The present data on acebutolol in hypertensive G-minipigs, unlike those obtained in rats [4, 9, 10], were in good agreement with the results in humans [1, 3]. This implies that G-minipigs exhibit similar cardiovascular responses to the drugs in humans.

Brunner *et al.* [2] and Kubo *et al.* [7] have reported their observations regarding the effects of β -blocker predosing on hydralazine-

induced blood pressure and heart rate changes. In this study, the animals showed a slight decrease of heart rate and blood pressure in response to the combined administration of acebutolol and hydralazine as opposed to with acebutolol alone, thus indicating a decrease in the hypertensive effect of acebutolol with hydralazine. The increase in the heart rate that occurs following the administration of hydralazine is interpreted as resulting from a reflexive cardiostimulating effect of vasodilation. The suppression of the antihypertensive effect of acebutolol by the concomitant administration of hydralazine seen in this study seems to suggest that the tachycardiac effect of hydralazine is due to cardiac stimulation by this vasodilator.

The combined use of hypotensive-diuretics and β -blockers is widely seen clinically. It has been noted that enhancement of the antihypertensive effects of hypotensive-diuretics by the concomitant administration of a β -blocker is attributable to inhibition by the hypotensive diuretic-induced increase of renin secretion [4]. Nielsen and coworkers [8] observed a marked enhancement of the effect in dogs and rats receiving both drugs, and the enhancement became evident two days after the administration and thereafter. In this study, the animals given combined acebutolol-furosemide regimens also responded with a greater, longer-sustained decline in blood pressure when compared with acebutolol alone.

It is known that β -blockers generally decrease PRA. However, an increase of PRA occurred following the administration of acebutolol in the present study. G-minipigs with high sodium chloride loading showed suppression of the renin aldosterone system [12]. Accordingly, it is suggested that the administration of acebutolol did not cause a decrease in PRA. It can also be assumed that an intrinsic sympathomimetic effect of acebutolol was manifested as stimulated renin secretion, not associated with its effects on the blood pressure and heart rate.

As mentioned above, hypertensive G-minipigs receiving acebutolol alone or in combination with furosemide or hydralazine exhibited antihypertensive effects. These findings imply that G-minipigs exhibited cardiovascular

responses to the drugs similar to those in humans.

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高血圧ミニブタGにおけるアセプトロール単独ならびに フロセミドあるいはヒドララジンとの併用投与の影響

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実験的に高血圧症を発症させたミニブタGにアセプトロール(心臓選択性アドレナリン性受容体の遮断剤)を投与した場合の降圧効果を検討した。急性実験では、メス6頭を用い、アセプトロール(3mg/kg, i.v.)の単独投与およびアセプトロールとフロセミド(1mg/kg, i.v.)あるいはヒドララジン(1mg/kg, i.v.)の併用投与試験を行なった。慢性実験では、メス5頭を用い、アセプトロール(100-200mg/頭/日, p.o.)の単独投与を行なった。

指標として、血圧、心拍数および血漿レニン活性(PRA)、血漿アルドステロン濃度(PAC)を用いた。急性実験における薬物投与前の試験では、血圧、心拍数ともにほぼ一定した値で推移した。アセプトロール単独投与試験では、血圧、心拍数ともに顕著な低下がみられた。併用投与試験のうち、アセプトロールとフロセミドとの併用投与では、アセプトロール単独投与の場合よりも血圧、心拍数に対する抑制効果が大きかった。また、アセプトロ

ールとヒドララジンとの併用投与では、アセブトロール単独投与の場合よりも血圧、心拍数の低下は顕著でなく降圧効果は小さかった。PRA および PAC については、薬物投与前の試験では大きな変化はみられなかった。アセブトロールの単独投与では、PRA は投与後3時間で有意な上昇がみられ、PAC は上昇傾向がみられた。併用投与試験では、いずれの試験においても PRA および PAC の上昇傾向がみられたが、アセブトロール単独投与の場合と異なり、上昇は小さかった。一方、アセブトロール単独投与の慢性実験においては、心拍数は

投与2週目以降で、血圧は4週目以降で有意な低下がみられた。アセブトロールの増量投与後、収縮期圧、拡張期圧のいずれも2週目までは降圧効果は顕著ではなく、4週目に収縮期圧の抑制効果がみられた。心拍数については顕著な抑制効果はみられなかった。また、PRA および PAC は投与後上昇する傾向がみられた。以上より、実験的に高血圧症を発症させたミニブタGにアセブトロールを投与した場合に顕著な降圧効果が得られた。とくに、アセブトロールとフロセミドとの併用投与では、降圧効果はさらに顕著であった。