ANTIHYPERTENSIVE EFFECT OF DIACETOLOL IN ESSENTIAL HYPERTENSION

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- 1 Diacetolol is the *N*-acetylated metabolite of acebutolol and possesses β -adrenergic receptor blocking properties.
- 2 Its antihypertensive action was assessed in accordance with a double-blind randomised cross-over scheme in 17 patients with moderate essential hypertension previously well controlled with acebutolol.
- 3 Significant reductions in lying mean arterial blood pressure were observed with daily doses of 200 mg (– 9%), 400 mg (– 10%) and 800 mg (– 14%), and were associated with significant decreases in heart rate and plasma renin activity.
- 4 The diacetolol mean plasma level measured 8 to 10 h after drug ingestion was proportional to the dose (207 ± 27 , 394 ± 63 and 823 ± 135 ng/ml for respectively 200, 400 and 800 mg/day).

Introduction

Diacetolol [(±)-1-(2-acetyl-4-acetamidophenoxy)-2-hydroxy-3-isopropylaminopropane], 36.903 R.P. or M&B 16.942) is a β -adrenergic receptor blocking agent which, in man, is the principal metabolite of acebutolol, a cardioselective β -adrenoceptor blocking drug (Basil & Jordan, 1981). Pharmacokinetic studies have shown that after oral administration of acebutolol, diacetolol is formed in such substantial quantities that the plasma level of diacetolol usually exceeds that of acebutolol during chronic treatment. Diacetolol plasma half-life after oral administration is about 12 h and is not dose-dependent (Flouvat *et al.*, 1981a). On the basis of experimental data in animals and recent results in man (Flouvat *et al.*, 1981b) it appears that this acetylated acebutolol metabolite has potent β -adrenoceptor blocking activity and is cardio-selective in respect of vascular and bronchial β -adrenoceptors. Diacetolol has slight intrinsic sympathomimetic activity and lacks both significant membrane stabilising activity and local anaesthetic activity.

In a double-blind cross-over study, the efficacy and tolerance of three doses of diacetolol were explored in patients with moderate essential hypertension (WHO stage 1 or 2) previously controlled by acebutolol treatment alone.

As the blood pressure reduction induced by β -adrenoceptor blockers is largely dependent on individual characteristics (Alhenc-Gelas *et al.*, 1978) a series of patients known to be sensitive to β -adreno-

ceptor blockers was selected to study the antihypertensive effect of diacetolol.

Methods

Patients and study design

Eighteen patients gave their informed consent to the study. History, physical examination, laboratory tests and intravenous pyelography failed to reveal any curable cause of hypertension. They had previously been adequately controlled (diastolic blood pressure ≤ 95 mmHg) by acebutolol alone (mean daily dosage and range: 618 mg, 300 to 1200 mg/day). At the first visit the efficacy of acebutolol treatment was checked by measuring blood pressure (BP) and heart rate (HR) three times in the lying and standing position. Then a wash out placebo period was started with outpatient consultations on days 14 and 28 for determination of BP and HR after 10 min in the supine position, then after 1 min in the upright position and lastly after exercise (15 flexions of legs, easy to perform at an outpatient consultation). During this single-blind wash out period, two placebo tablets identical to the diacetolol tablets were given twice a day. At the end of the placebo period only the patients with supine and/or standing diastolic BP higher than 95 mmHg were included in the active phase of the trial. At this point laboratory tests were performed (blood cell

count, plasma sodium and potassium, creatinine, urea, bilirubin, glutamic transaminases, alkaline phosphatase, prothrombin time, cholesterol, glucose, calcium, uric acid, antinuclear antibodies and plasma renin activity after 1 h of walking about) in addition to a complete ophthalmologic examination, fluorescein and rose Bengal stains, Schirmer test.

In accordance with the double-blind cross-over protocol, each patient was randomly allocated to three successive active treatment periods affording 200, 400 or 800 mg of diacetolol daily, two tablets being administered twice a day.

Patients attended outpatient consultations at the midpoint and at the end of each 6 week period. A clinical examination was performed at each visit then BP and HR were measured three times in recumbency after 10 min lying down and after 1 min standing, then once after exercise.

All these measurements were made by the same observer in the same room between 16.00 h and 18.00 h. BP (phases 1 and 5 of Korotkoff sounds) was recorded with a mercury manometer with the cuff placed on the right arm. Patients were systematically checked for side effects at each visit. Moreover, blood was drawn at the end of each period for determination of diacetolol plasma levels by high pressure liquid chromatography (Roux & Flouvat, 1978). Immunofluorescence was used for antinuclear antibody testing (Hamard *et al.*, 1964). Renin was measured by radioimmunoassay of angiotensin I (Ménard *et al.*, 1972).

At the beginning of each period a box containing an adequate number of tablets, prepared by an independent pharmacologist, was given to each patient. A tablet count was made at the end of each period in order to assess compliance with the treatment. At the end of the study, further laboratory tests (including plasma renin activity assay) were performed and the ophthalmological tests were repeated.

Results were expressed as mean $\pm$ s.e.mean. Statistical calculations included variance analysis and a modified *t*-test (Bonferroni method).

As the values noted at the midpoint were very similar to those observed at the end of each period, only the latter are shown in the results section.

Results

Initially 18 patients entered the trial. Their hypertension had been adequately controlled (136/89 mmHg supine and 131/92 standing with an average daily dose of 618 mg acebutolol (300 to 1200 mg/day). At the end of the placebo period one subject was excluded because of persistently normal blood pressure values. Thus six women and eleven men, aged 28 to 69 years (mean 45) completed the entire protocol. Their

hypertension had been detected 4 to 17 years before (mean 7 years) and on the first visit at the clinic, their ocular fundi were in stages 0 (8 cases), 1 (4 cases), 2 (4 cases) and 3 (1 case) according to Keith-Wagener classification. Left ventricular hypertrophy was present in two cases. Plasma creatinine was normal in all cases.

Initially, active treatment periods were scheduled to last 6 weeks each, but for personal reasons (holidays in most cases) it was necessary to prolong some periods for several patients. However the double-blind principle was preserved by the preparation of adequate amounts of tablets by the independent pharmacologist. The mean duration of each period was as follows: 32 ± 2 days for the placebo period, 41 ± 1 days, 46 ± 2 days and 53 ± 3 days for the first, second and third periods respectively. For the eight patients whose last period was extended, the relevant dosages were 200 mg in three cases for an additive period of 25 ± 7 days, 400 mg in three cases for an additive period of 21 ± 4 days, and 800 mg in two cases for an additive period of 28 days. The mean diacetolol doses during the 3 active treatment periods were similar, 435 mg/day (or 6.41 ± 1 mg kg⁻¹ day⁻¹), 494 mg/day (or 6.99 ± 0.80 mg kg⁻¹ day⁻¹) and 471 mg/day (or 6.87 ± 1.01 mg kg⁻¹ day⁻¹) respectively in periods 1, 2 and 3.

Time related BP and HR changes (Table 1)

At the end of the placebo period BP and HR were significantly higher than the corresponding values obtained during acebutolol treatment.

Diacetolol induced a significant drop in BP in all three positions, which was obvious as of the first visit 3 weeks after commencing the active treatment. The BP reduction was maintained throughout the entire active treatment phase. No carry-over effect was observed for systolic and diastolic blood pressure values recorded in the standing position, nor for the systolic blood pressure and the heart rate after effort. Compared to the values noted at the end of the first active treatment period, statistically significant differences were observed for the systolic blood pressure (third period, supine), the diastolic blood pressure (third period, supine and after effort) and the heart rate (second period, supine and standing). These differences occurred despite similar mean daily dosages for the 3 periods of active treatment. This suggests an influence of time on drug efficacy (at least in the supine position), especially if one considers the longer duration of periods 2 and 3, compared to period 1.

Dosage-related BP and HR alterations (Table 2)

In the standing position, where no order effect existed, alterations in BP were dependent on the

Table 1 Variations in blood pressure (mmHg) and heart rate (beats/min) observed with a placebo then with diacetolol in patients previously controlled by acebutolol.

	<i>Acebutolol</i>	<i>Placebo</i>	<i>First period</i>	<i>Diacetolol</i> <i>Second period</i>	<i>Third period</i>
<i>Supine</i>					
Systolic	136 ± 3	149 ± 4***	138 ± 3 ²	136 ± 4 ³	131 ± 3 ^{3,4}
Diastolic	89 ± 2	100 ± 2***	90 ± 2 ³	89 ± 2 ³	83 ± 2 ^{3,5}
Heart rate	68 ± 2	74 ± 2***	71 ± 3	66 ± 2 ^{3,4}	67 ± 2 ³
<i>Standing</i>					
Systolic	131 ± 3	147 ± 4***	131 ± 4 ³	131 ± 4 ³	126 ± 3 ³
Diastolic	92 ± 2	104 ± 2***	93 ± 2 ³	93 ± 3 ³	88 ± 2 ³
Heart rate	76 ± 2	85 ± 2***	81 ± 3 ¹	76 ± 2 ^{3,4}	78 ± 3 ³
<i>Effort</i>					
Systolic	—	165 ± 6	147 ± 6 ²	146 ± 6 ³	142 ± 4 ³
Diastolic	—	101 ± 2	92 ± 2 ³	92 ± 3 ³	85 ± 2 ^{3,5}
Heart rate	—	102 ± 3	95 ± 4	92 ± 3 ²	88 ± 3 ³
Mean daily dose	(mg/kg body weight)	6.41 ± 1	6.99 ± 0.80	6.87 ± 1.01	

*** significantly different from the values observed during acebutolol treatment, $P < 0.001$

¹significantly different from the values observed at the end of the placebo period, $P < 0.05$

²significantly different from the values observed, $P < 0.01$

³significantly different from the values observed, $P < 0.001$

⁴significantly different from the values observed at the end of the first period, $P < 0.05$

⁵significantly different from the values observed at the end of the first period, $P < 0.01$

diacetolol dosage used. Compared with the placebo period, the fall in BP (systolic/diastolic) was 13/9 mmHg with 200 mg/day, 21/15 mmHg with 400 mg/day and 19/14 mmHg with 800 mg/day. Thus, about 60% of the antihypertensive effect was obtained with the lowest dosage of diacetolol (200 mg). 400 mg/day induced a significantly greater drop in BP; no further effect on BP was obtained by in-

creasing the dosage from 400 to 800 mg/day. Similar observations were made for systolic blood pressure after exercise.

Alterations in BP in recumbent posture and after effort were more difficult to interpret because of a possible carry over effect. However a trend similar to that existing in the standing position was observed, i.e. a significant fall in BP with 200 mg/day and

Table 2 Variations in blood pressure and heart rate induced by the three doses of diacetolol

	<i>Acebutolol</i>	<i>Placebo</i>	<i>200 mg</i>	<i>Diacetolol</i> <i>400 mg</i>	<i>800 mg</i>
<i>Supine</i>					
Systolic	136 ± 3	149 ± 4	138 ± 3**	135 ± 3***	131 ± 3*** ¹
Diastolic	89 ± 2	100 ± 2	89 ± 2***	88 ± 2***	85 ± 1*** ¹
Heart rate	68 ± 2	74 ± 2	72 ± 3	67 ± 2*** ¹	65 ± 2*** ¹
<i>Standing</i>					
Systolic	131 ± 3	147 ± 4	134 ± 4***	126 ± 4*** ¹	128 ± 3*** ¹
Diastolic	92 ± 2	104 ± 2	95 ± 2***	89 ± 3*** ¹	90 ± 2***
Heart rate	76 ± 2	85 ± 2	83 ± 3	78 ± 2*** ¹	74 ± 2*** ^{1,2}
<i>Effort</i>					
Systolic	—	165 ± 6	152 ± 6**	143 ± 5*** ¹	139 ± 4*** ¹
Diastolic	—	101 ± 3	93 ± 3***	88 ± 3**	88 ± 3***
Heart rate	—	102 ± 3	94 ± 4*	94 ± 3**	86 ± 3*** ^{1,2}

* significantly different from the placebo period, $P < 0.05$

** significantly different from the placebo period, $P < 0.01$

*** significantly different from the placebo period, $P < 0.001$

¹significantly different from the 200 mg period, $P < 0.05$

²significantly different from the 400 mg period, $P < 0.05$

greater but similar drops in BP with 400 or 800 mg/day. The decrease in BP observed in all three positions with 200 mg/day occurred in the absence of a significant reduction in HR which took place only with the higher doses.

Plasma renin activity

According to the normal range of values in our laboratory (0.7 to 3 ng A1 ml⁻¹ h⁻¹) plasma renin activity values determined in 16 patients at the end of the placebo period could be classified as follows: four values in the low renin subgroup (0.46 ± 0.09 ng A1 ml⁻¹ h⁻¹), eight values in the normal renin subgroup (1.34 ± 0.11 ng A1 ml⁻¹ h⁻¹), four values in the high renin subgroup (3.80 ± 0.27 ng A1 ml⁻¹ h⁻¹).

At the end of diacetolol treatment, a significant reduction in PRA values (0.55 ± 0.13 ng A1 ml⁻¹ h⁻¹ v 1.76 ± 0.35 ng A1 ml⁻¹ h⁻¹, $P < 0.01$) was observed. The decrease in PRA was significantly correlated to the diacetolol dose used during the last period ($r = 0.49$, $P = 0.05$).

Plasma levels of diacetolol

Wide interindividual variations were observed for plasma diacetolol levels at the end of each active treatment phase but a positive correlation ($r = 0.76$, $P < 0.0001$) was found between the doses ingested (expressed in mg/kg of body weight) and the plasma levels. Compared to the 200 mg period plasma level (207 ± 27 ng/ml) the value noted during the 400 mg period (394 ± 63 ng/ml) was significantly greater ($P < 0.01$). The 800 mg period value (823 ± 135 ng/ml) was significantly greater ($P < 0.01$) than both the preceding values. There was no relationship between plasma levels of diacetolol on the one hand and degrees of reduction in blood pressure, in heart rate and in plasma renin activity on the other hand.

Compliance and side-effects

All patients were compliant and none were lost to follow-up. The tablet count at the end of the trial revealed that the patients forgot to take $3 \pm 0.62\%$ of the tablets; this corresponded to an average of 16 ± 3 tablets forgotten by each patient when the total number of tablets to be ingested during the trial varied from 504 to 644.

Clinical tolerance was good. The side effects were minor and did not necessitate withdrawal of treatment in any case. They were as frequent with diacetolol as with placebo. They are those usually encountered with other β -adrenoceptor blocking drugs and do not deserve special comment. The ophthalmological parameters did not change during diacetolol treatment.

In one subject who had a previous history of

jaundice 50 years ago and with a pretreatment bilirubin value of 17 μ mol/l alterations in several hepatic parameters were observed at the end of the study: bilirubin 27 μ mol/l (normal range: < 16), SGPT 59 IU/l (normal range 5 to 41) and prothrombin 65% (normal range: 80 to 100%). Fifteen days after diacetolol withdrawal, the changes in laboratory values were even more marked: bilirubin 19 μ mol/l, SGOT 139 IU/l (normal range: 8 to 38), SGPT 274 and γ -glutamyltranspeptidase 90 IU (normal range: 6 to 28). In this subject, tests for anti-Hbs antibody and antigen, and for antimitochondrial and antismooth muscle antibodies were negative. No hepatic biopsy was performed. All laboratory values remained unchanged in the other patients. Tests for antinuclear antibodies were negative during acebutolol treatment, at the beginning of the trial, and remained negative during diacetolol treatment in all patients, including the one with discrete hepatic abnormalities.

Discussion

During chronic oral therapy with acebutolol in man, the plasma concentration of the acetyl metabolite of this drug exceeds that of the parent compound (Winkle *et al.*, 1977). This finding has important clinical applications when one begins to consider the contribution of this metabolite to the effects of acebutolol. Moreover genetically dependent inter-subject differences in acetylation rates contribute to the toxicity and possibly the therapeutic action of drugs undergoing acetylation. For instance hydralazine-induced lupus reactions supervene more frequently in slow acetylators of the drug (Perry *et al.*, 1970). However a report from Gulaid *et al.* (1978) suggests that the overall process of acetyl metabolite production from acebutolol in man does not appear to be under genetic control.

Preliminary studies in animals and in man of the acetyl metabolite of acebutolol, diacetolol (Flouvat *et al.*, 1981a) suggest that this drug is pharmacologically active, possesses β -adrenoceptor antagonist properties and acts with relative cardioselectivity.

Our study demonstrates that diacetolol possesses significant antihypertensive action against moderate essential hypertension in man. The antihypertensive effect of diacetolol exists at a daily dosage of 200 mg, both systolic and diastolic pressures being significantly reduced 8 to 10 h after the ingestion of 100 mg of diacetolol. This antihypertensive effect was observed at a time when no significant reduction in the heart rate was present in recumbency and orthostatism. The reduction blood pressure was greater for daily doses of 400 and 800 mg. With the dosage range used in this study, it is not possible to know if the plateau phase of the dose-response curve

for this drug is reached. In order to define the magnitude and duration of blood pressure reduction, a study using larger doses of diacetolol has to be performed, including measurements of blood pressure 24 h after drug ingestion. Hansson *et al.* (1977) reported only a moderate decrease in blood pressure when acebutolol was raised from 600 to 1200 mg daily. In our own experience (Alhenc-Gelas *et al.*, 1978) acebutolol doses higher than 800 mg per day do not produce any clear additional drop in blood pressure.

The β -adrenoceptor blocking properties of diacetolol are well illustrated by significant reductions in the heart rate after effort and in plasma renin activity, the drop of the latter seems to be related to the dosage used.

We observed wide variations in diacetolol plasma levels from one subject to another but these plasma levels were related to the doses used. In this study, as in others concerning acebutolol (Martin *et al.*, 1978), the clinical efficacy of diacetolol could not be related to the plasma levels of the drug.

The side effects encountered are those known to occur with β -adrenoceptor blocking agents

(Kellaway, 1976) but in no case were they severe enough to require drug withdrawal.

The doses used in this study did not produce any positive tests for antinuclear antibodies. This specific point was carefully checked because of the recent report from Cody *et al.* (1979) describing antinuclear antibodies (at least with one of 3 different techniques of measurement) during acebutolol therapy at doses of 800 to 1600 mg/day for at least 12 weeks. No such abnormality was detected in this study of patients previously treated with acebutolol (mean duration of treatment = 26 months, range 7 to 60 months) either before inclusion in the trial or after 18 weeks of diacetolol treatment.

Diacetolol, the *N*-acetylated metabolite of acebutolol, is a potent β -adrenoceptor blocking agent in treating hypertension in man when used at a daily dosage of 200 to 800 mg. Its antihypertensive effect is associated with a reduction in the heart rate and a decrease in plasma renin activity.

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