OBSERVATIONS ON THE CLINICAL PHARMACOLOGY AND PLASMA CONCENTRATIONS OF DIACETOLOL, THE MAJOR HUMAN METABOLITE OF ACEBUTOLOL

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1 The pharmacological effects and plasma levels of diacetolol, the major human metabolite of acebutolol, were measured in a double-blind, balanced study in which five healthy men received single oral doses of diacetolol 100, 200, 400 and 800 mg, or placebo, at weekly intervals.

2 Resting and exercise heart rate (HR), forced expiratory volume in 1 second (FEV₁), resting and exercise peak expiratory flow rate (PEFR), and plasma concentrations of diacetolol were determined at 0, 2, 4, 6, 8 and 24 h after each treatment.

3 Diacetolol caused a slight dose-related reduction in resting HR and a substantial dose-related reduction in exercise HR. At the same time it was found that diacetolol had no significant effects on FEV₁ and resting and exercise PEFR.

4 Mean highest observed plasma concentrations (ng/ml) of diacetolol were 177 at a mean of 4.4 h after the 100 mg dose, 243 at 4.0 h after the 200 mg dose, 807 at 5.2 h after the 400 mg dose, and 1,306 at 4.4 h after the 800 mg dose.

5 Using the mean data, there was a strong correlation ($r = 0.90$) between % reduction in exercise HR and the logarithm of the plasma concentration of diacetolol.

6 Diacetolol exhibits marked cardiac β -adrenoceptor blocking activity in man which is still evident 24 h after the administration of the higher doses of the drug. No adverse effects on pulmonary function could be detected.

Introduction

Diacetolol [(±)-1-(2-acetyl-4-acetamidophenoxy)-2-hydroxy-3-isopropylaminopropane] is the major metabolite of acebutolol hydrochloride (Sectral®) in man (Meffin, Harapat & Harrison, 1976; Winkle *et al.*, 1977). During chronic oral acebutolol therapy, the circulating levels of diacetolol exceed those of the parent drug (Winkle *et al.*, 1977; Meffin *et al.*, 1978). Thus it is important to establish what contribution diacetolol may make to the pharmacological activity of oral acebutolol in man; it has previously been demonstrated that acebutolol is a selective β_2 -adrenoceptor antagonist in man after intravenous administration (Kumana *et al.*, 1975). In animal models diacetolol has a similar potency and spectrum of pharmacological activity to that of acebutolol (Basil & Jordan, 1978).

The present study was undertaken in order to determine, under double-blind conditions with placebo control, the cardiac and pulmonary effects of four different single oral doses of diacetolol (administered as the hydrochloride salt) in five

healthy male volunteers. Measurement of the plasma levels of diacetolol was undertaken in order to see whether there was a correlation between these plasma levels and the observed pharmacological activity.

Methods

Volunteers and dosing

Five healthy male volunteers, aged 20–31 years, weight 58–95 kg, took part in the study, having given their informed consent. Each volunteer underwent a complete medical examination and had normal values for the usual laboratory tests of blood, hepatic and renal function. No volunteer had a past history of asthma, diabetes, gastro-intestinal disease or hypersensitivity to β -adrenoceptor antagonists, and each had a normal ECG.

Each volunteer was given single oral doses of dia-

cetolol hydrochloride (containing 100, 200, 400 or 800 mg of diacetolol) and placebo in random order, at least 1 week apart, according to a latin-square design. The study was double-blind, each dose being made up with five tablets of similar appearance, and with each tablet containing 0, 100 or 200 mg of diacetolol.

Clinical measurements

The following variables were measured in each volunteer before and at 2, 4, 6, 8 and 24 h after each dose: resting and exercise HR were measured over 30 s using an ECG, FEV₁ (mean of 3 readings) using a Vitalograph, and resting (mean of 3 readings) and exercise PEFR (mean of the highest 3 of 5 readings) using a Wright peak flow meter. Thus, using exercise as a physiological means of producing sympathetic stimulation, the cardiac β_1 -adrenoceptors and the pulmonary β_2 -adrenoceptors were simultaneously investigated (Kumana *et al.*, 1974).

Experimental procedures

The experimental protocol was as follows: at least 2 h prior to administration of each treatment, volunteers were permitted a light standard breakfast. On arrival at the laboratory (usually around 0930 h) the volunteer rested supine on a couch. After at least 10 min, resting HR was recorded. On standing upright for a few minutes three FEV₁ measurements were recorded about 1 min apart. The volunteer then sat on a Monark bicycle ergometer and three resting PEFR measurements were made about 1 min apart. After these readings had been taken the volunteer exercised for 3 min at a workload which had previously been shown to produce an HR of 150–160 beats/min during the last 30 s of exercise. Exercise HR was recorded in the last 30 s of exercise, and five PEFR readings were taken after about 1, 2, 2.25, 2.5, and 2.75 min of exercise. Shortly after the exercise, venous blood samples (10 ml) were taken into lithium-heparin tubes. The plasma was separated by centrifugation and stored at -20°C . The time between recording the resting HR and taking the blood sample was about 20 min. At about 10.00 h the volunteer was given an oral dose with 100 ml of water.

The entire sequence of events, consisting of the pre- and post-exercise measurements and the blood sampling, was repeated such that the end of each exercise session occurred at 2, 4, 6, 8 and 24 h after the dose. A light lunch was provided after completion of exercise testing at 4 h. Caffeine-containing drinks and smoking were not permitted on the days of the trial.

Measurement of plasma concentrations of diacetolol

The concentration of diacetolol in each plasma sample was determined by a specific high-pressure

liquid chromatographic (h.p.l.c.) method with ultraviolet (u.v.) detection (French, Gulaid & Lewellen, 1981).

Statistical analysis

Statistical analysis of the data was carried out by two-way, cross-classification analysis of variance according to the method of Davies & Goldsmith (1972). The confidence intervals of the differences between each of the pharmacological variables following placebo and drug treatment were calculated using the method of Westlake (1972) and the parameter tables published by Spriet & Beiler (1978).

The significance of the correlation between the observed % reduction in exercise HR and the corresponding plasma concentration of diacetolol was estimated with individual data using the method of least squares for a regression of % reduction in exercise HR on logarithm_e plasma concentration of diacetolol. Also, for ease of presentation, a least squares regression was carried out using % mean reduction in exercise HR and logarithm₁₀ mean plasma concentration of diacetolol.

Results

In one volunteer (subject 2) on his third study day it was not possible to measure any clinical variables at 2 h after the dose (which contained 800 mg diacetolol) due to technical failure. The mean ($\pm$ s.d.) values obtained for the five volunteers on the five separate occasions are given in Table 1 (resting HR), Table 2 (exercise HR), Table 3 (FEV₁), Table 4 (resting PEFR), Table 5 (exercise PEFR) and Table 6 (plasma concentrations of diacetolol). Mean values for % reduction in exercise HR are illustrated in Figure 1. The values for the mean highest observed plasma concentrations (ng/ml) of diacetolol were 177 at a mean of 4.4 h after the 100 mg dose, 243 at 4.0 h after the 200 mg dose, 807 at 5.2 h after the 400 mg dose, and 1,306 at 4.4 h after the 800 mg dose.

Using the individual data, the correlation between % reduction in exercise HR ν logarithm plasma concentration of diacetolol was found to be highly significant having a proportional fit of 51%. For clarity of presentation, Figure 2 depicts the correlation obtained using the mean % reduction in HR ν logarithm₁₀ mean plasma concentration of diacetolol, which has a correlation coefficient of 0.90.

Discussion

The results of this study indicate that the oral administration of a single dose of diacetolol hydrochloride to healthy volunteers has little effect on rest-

Table 1 Mean ($\pm$ s.d., $n=5$) resting heart rate values (beats/min) after oral administration of placebo or diacetolol hydrochloride

Time after dose (h)	Placebo	Diacetolol (mg) (administered as its HCl salt)			
		100	200	400	800
0	68 ($\pm$ 10)	67 ($\pm$ 5)	74 ($\pm$ 11)	75 ($\pm$ 6)	71 ($\pm$ 7)
2	60 ($\pm$ 8)	63 ($\pm$ 7)	59 ($\pm$ 8)	60 ($\pm$ 3)	51 ($\pm$ 5)*
4	66 ($\pm$ 11)	57 ($\pm$ 7)	62 ($\pm$ 15)	59 ($\pm$ 8)	53 ($\pm$ 10)
6	71 ($\pm$ 5)	66 ($\pm$ 7)	68 ($\pm$ 12)	68 ($\pm$ 5)	62 ($\pm$ 6)
8	69 ($\pm$ 8)	65 ($\pm$ 7)	66 ($\pm$ 12)	65 ($\pm$ 6)	58 ($\pm$ 7)
24	69 ($\pm$ 7)	74 ($\pm$ 6)	74 ($\pm$ 6)	66 ($\pm$ 9)	61 ($\pm$ 6)

* = Mean of 4 values

Table 2 Mean ($\pm$ s.d., $n=5$) exercise heart rate values (beats/min) after oral administration of placebo or diacetolol

Time after dose (h)	Placebo	Diacetolol (mg) (administered as its HCl salt)			
		100	200	400	800
0	155 ($\pm$ 9)	154 ($\pm$ 6)	155 ($\pm$ 13)	156 ($\pm$ 6)	152 ($\pm$ 5)
2	152 ($\pm$ 13)	140 ($\pm$ 4)	138 ($\pm$ 11)	131 ($\pm$ 5)	119 ($\pm$ 4)*
4	159 ($\pm$ 16)	141 ($\pm$ 5)	139 ($\pm$ 9)	124 ($\pm$ 5)	117 ($\pm$ 4)
6	156 ($\pm$ 12)	146 ($\pm$ 3)	143 ($\pm$ 8)	129 ($\pm$ 3)	122 ($\pm$ 5)
8	157 ($\pm$ 9)	148 ($\pm$ 3)	143 ($\pm$ 6)	132 ($\pm$ 6)	123 ($\pm$ 5)
24	154 ($\pm$ 6)	158 ($\pm$ 9)	155 ($\pm$ 8)	144 ($\pm$ 7)	137 ($\pm$ 3)

* = Mean of 4 values

Table 3 Mean ($\pm$ s.d., $n=5$) values of forced expiratory volume (l) in 1 s after oral administration of placebo or diacetolol hydrochloride

Time after dose (h)	Placebo	Diacetolol (mg) (administered as its HCl salt)			
		100	200	400	800
0	4.25 ($\pm$ 0.82)	4.22 ($\pm$ 0.80)	4.23 ($\pm$ 0.67)	4.20 ($\pm$ 0.75)	4.41 ($\pm$ 1.04)
2	4.20 ($\pm$ 0.73)	4.27 ($\pm$ 0.80)	4.21 ($\pm$ 0.72)	4.39 ($\pm$ 0.65)	4.32 ($\pm$ 0.91)*
4	4.22 ($\pm$ 0.79)	4.19 ($\pm$ 0.80)	4.27 ($\pm$ 0.71)	4.19 ($\pm$ 0.73)	4.24 ($\pm$ 0.79)
6	4.22 ($\pm$ 0.77)	4.24 ($\pm$ 0.75)	4.23 ($\pm$ 0.67)	4.19 ($\pm$ 0.69)	4.24 ($\pm$ 0.81)
8	4.26 ($\pm$ 0.74)	4.15 ($\pm$ 0.82)	4.22 ($\pm$ 0.75)	4.15 ($\pm$ 0.78)	4.18 ($\pm$ 0.77)
24	4.25 ($\pm$ 0.71)	4.26 ($\pm$ 0.85)	4.26 ($\pm$ 0.75)	4.16 ($\pm$ 0.75)	4.20 ($\pm$ 0.84)

* = Mean of 4 values

Table 4 Mean ($\pm$ s.d., $n=5$) resting peak expiratory flow rate values (l/min) after oral administration of placebo or diacetolol hydrochloride

Time after dose (h)	Placebo	Diacetolol (mg) (administered as its HCl salt)			
		100	200	400	800
0	558 ($\pm$ 83)	557 ($\pm$ 88)	552 ($\pm$ 81)	556 ($\pm$ 81)	552 ($\pm$ 84)
2	562 ($\pm$ 85)	559 ($\pm$ 84)	563 ($\pm$ 83)	554 ($\pm$ 87)	565 ($\pm$ 80)*
4	561 ($\pm$ 89)	560 ($\pm$ 88)	553 ($\pm$ 94)	565 ($\pm$ 98)	556 ($\pm$ 80)
6	553 ($\pm$ 90)	558 ($\pm$ 89)	547 ($\pm$ 87)	560 ($\pm$ 92)	555 ($\pm$ 77)
8	555 ($\pm$ 82)	546 ($\pm$ 81)	553 ($\pm$ 85)	559 ($\pm$ 89)	557 ($\pm$ 87)
24	559 ($\pm$ 75)	559 ($\pm$ 91)	547 ($\pm$ 86)	556 ($\pm$ 95)	560 ($\pm$ 85)

* = Mean of 4 values

Table 5 Mean ($\pm$ s.d., $n=5$) exercise peak expiratory flow rate values (l/min) after oral administration of placebo or diacetolol hydrochloride

Time after dose (h)	Placebo	Diacetolol (mg) (administered as its HCl salt)			
		100	200	400	800
0	582 ($\pm$ 95)	585 ($\pm$ 82)	576 ($\pm$ 86)	581 ($\pm$ 90)	578 ($\pm$ 91)
2	584 ($\pm$ 89)	581 ($\pm$ 90)	579 ($\pm$ 79)	588 ($\pm$ 90)	597 ($\pm$ 78)*
4	585 ($\pm$ 85)	579 ($\pm$ 96)	588 ($\pm$ 84)	587 ($\pm$ 100)	588 ($\pm$ 85)
6	586 ($\pm$ 101)	586 ($\pm$ 92)	576 ($\pm$ 83)	576 ($\pm$ 99)	587 ($\pm$ 83)
8	584 ($\pm$ 92)	573 ($\pm$ 85)	574 ($\pm$ 87)	582 ($\pm$ 97)	583 ($\pm$ 89)
24	585 ($\pm$ 86)	579 ($\pm$ 92)	578 ($\pm$ 75)	581 ($\pm$ 96)	586 ($\pm$ 87)

* = Mean of 4 values

Table 6 Mean ($\pm$ s.d., $n=5$) plasma concentrations of diacetolol (ng/ml) after oral administration of diacetolol hydrochloride

Time after dose (h)	Diacetolol (mg) (administered as its HCl salt)			
	100	200	400	800
0	ND	ND	ND	ND
2	130 ($\pm$ 81)	144 ($\pm$ 84)	344 ($\pm$ 232)	597 ($\pm$ 338)
4	169 ($\pm$ 59)	243 ($\pm$ 117)	697 ($\pm$ 275)	1261 ($\pm$ 838)
6	121 ($\pm$ 34)	166 ($\pm$ 66)	580 ($\pm$ 158)	975 ($\pm$ 445)
8	111 ($\pm$ 50)	153 ($\pm$ 44)	432 ($\pm$ 235)	698 ($\pm$ 302)
24	47 ($\pm$ 54)	89 ($\pm$ 28)*	128 ($\pm$ 75)*	151 ($\pm$ 96)

ND = Not detected; * = Mean of 3 results

ing HR, although some reduction is consistently evident with a dose containing 800 mg of diacetolol (Table 1). Exercise HR, however, is always reduced in the presence of diacetolol and the reduction is most marked with the highest two doses used in this study, where it is still significantly present up to 24 h after the 800 mg dose (Table 2, Figure 1). The large effects of diacetolol on % reduction in exercise HR (Figure 1) indicate that diacetolol hydrochloride exhibits cardiac β -adrenoceptor blocking activity in man when administered orally. The lack of effects of diacetolol on the two tests of pulmonary function used in this study, namely FEV₁ (Table 3) and resting and exercise PEF (Tables 4 and 5), are consistent with data previously obtained in animals suggesting cardioselectivity (Basil & Jordan, 1978).

The strong correlation obtained between the % reduction in exercise HR and the logarithm plasma concentration of diacetolol (Figure 2) is consistent with diacetolol being the major compound responsible for the cardiac β -adrenoceptor blocking activity observed after the oral administration of diacetolol hydrochloride to man.

Overall the results of this study strongly suggest

that diacetolol (formed metabolically from acebutolol) contributes to the pharmacological activity observed when acebutolol hydrochloride is administered orally to man. Since the metabolite (diacetolol) persists in the circulation longer than the parent drug (acebutolol) (Meffin *et al.*, 1976, 1978; Winkle *et al.*, 1977), it is possible that diacetolol may make a major contribution to the long 'pharmacological' half-life of oral acebutolol that has been observed in man (Martin *et al.*, 1978; Watson & Littler, 1979).

It has been previously shown in man that the metabolic production of diacetolol from acebutolol is not under genetic control (Gulaid *et al.*, 1978). The results of this study strongly suggest that the metabolic conversion of acebutolol to diacetolol is not responsible for any inter-patient variation seen in the clinical response to oral acebutolol, since the parent drug and its metabolite have a similar spectrum of pharmacological activity in man.

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Figure 1 Mean % reduction in exercise heart rate after single oral doses of diacetolol hydrochloride (○ 100 mg, ▲ 200 mg, □ 400 mg, ● 800 mg) or placebo (■). * significantly different from placebo $P < 0.05$.

Figure 2 Relationship between % mean reduction in exercise heart rate and $\log_{10}$ mean plasma concentration of diacetolol after single oral doses of diacetolol hydrochloride (○ 100 mg, ▲ 200 mg, □ 400 mg, ● 800 mg). $r = 0.90$.

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