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ANTIARRHYTHMIC EFFECTS OF ORG 6001 IN RATS: CORRELATION WITH PLASMA AND TISSUE DRUG CONCENTRATIONS

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1 The antiarrhythmic effects of Org 6001 following oral administration in the rat have been assessed and correlated with plasma, myocardial and skeletal muscle drug concentrations.

2 Arrythmias were induced by coronary artery ligation in anaesthetized rats. Org 6001 (10, 20, 50 and 100 mg/kg given 1 h before ligation) significantly reduced mortality and the incidence of ventricular fibrillation in the 0–30 min post ligation period. Only the highest dose of drug also significantly reduced the number of ventricular ectopic beats following ligation.

3 A linear relationship was observed between the oral dose and the Org 6001 concentrations in plasma and skeletal muscle determined 90 min after drug administration.

4 The Org 6001 concentration in the myocardium was not linearly related to the administered dose and Org 6001 appeared to be concentrated to a higher extent in cardiac than in skeletal muscle at that time.

5 No statistically significant difference in drug levels in the ischaemic left ventricle and normal right ventricle plus septum was observed.

6 The antiarrythmic effect of Org 6001, as measured by changes in the incidence of ventricular fibrillation, correlated with myocardial concentrations of the drug.

Introduction

Org 6001 (3α-amino-5α-androstan-2β-ol-17-one HCl) protects against arrhythmias induced by coronary artery ligation after both acute (Marshall & Parratt, 1975) and prolonged oral pretreatment (Kane, Lepran, McDonald, Parratt & Szekeres, 1980). It may, therefore, be a useful prophylactic agent in preventing sudden cardiac death resulting from reinfarction. A limited study of the relationship between plasma and cardiac tissue concentrations of Org 6001 and its antiarrhythmic action has previously been described (Kane *et al.*, 1980); in the present study this relationship has been further investigated.

Following acute oral administration the effectiveness of Org 6001 against post-infarction arrhythmias has been assessed in the anaesthetized rat and this effectiveness has been correlated with levels of the drug in plasma and in right and left ventricular muscle. Determinations of Org 6001 levels in skeletal muscle have also been made for comparison with cardiac tissue levels.

Methods

Male Sprague-Dawley rats (220–360 g body wt.) were pretreated orally via a stomach tube with water or Org 6001 in doses of 10, 20, 50 or 100 mg/kg in a volume of 0.1 ml/100 g body wt. with the exception of the highest dose which was given in double the volume because of its solubility. Approximately 30 min later, the rats were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.) and artificially ventilated (stroke volume, 2 ml/100 g; 54 strokes/min). Carotid arterial blood pressure and a standard lead I or II electrocardiogram (ECG) were recorded using a mingograph 81 ink-jet recorder (Elema Schönander). A femoral vein was cannulated for further administration of anaesthetic, if necessary. Rectal temperature was maintained at approximately 38°C. The chest was opened between the fourth and fifth ribs, approximately 2 mm to the left of the sternum. After opening the pericardium, the heart was exteriorized and a 6/0 silk suture was placed under the left coronary artery as described by Selye, Bajusz, Grasso & Mendell (1960). The heart was repositioned in the thoracic cavity and the blood pressure and ECG allowed to stabilize for 15 min. The ligature was tied 1 h after the oral dosing.

During the 0–30 min post ligation period, the severity of the arrhythmias was assessed by noting the

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mortality, the incidence and total duration of the episodes of ventricular fibrillation (VF) and ventricular tachycardia (VT, defined as any run of seven or more consecutive ventricular extrasystoles) and by counting the total number of ventricular ectopic beats.

At the end of the 30 min post-ligation period, 8–10 ml of blood was withdrawn, centrifuged, and the plasma separated from the cellular fraction. The heart was removed, rinsed in cold water, and dissected into the left ventricular free wall and the right ventricular free wall plus the septum. The diaphragm muscle was also removed. The tissue and plasma were stored at −15°C until analysed at the Drug Metabolism R & D Laboratories of Organon B.V., Oss, Netherlands.

The assay method to determine Org 6001 in plasma consisted of protein denaturation, ethyl acetate extraction, derivatization with tertbutyldimethylchlorosilane, *n*-hexane extraction and quantitation by gas chromatography-mass spectrometry using trideuteriated Org 6001 as an internal standard. Tissue was homogenized before analysis and the homogenate was processed as described above. The assay procedure is presented in more detail elsewhere (Vink, Van Hal & Timmer, 1980).

Statistical methods

Data are expressed as mean values ± standard error of the mean ($\bar{x} \pm$ s.e.mean). Statistical significance of differences between means was calculated by Student's *t* test whereas a Chi-squared test was used to analyse the statistical significance of differences in the incidence of a given event. The Terpstra two-sided test, a generalization of Kendall's rank correlation test, was used to analyse statistical significance of a monotonic decrease or increase in tissue plasma ratios versus dose.

Results

In animals given a single oral dose of water 1 h before ligation, the mortality during the 0–30 min post-ligation period was 56%. The cause of death was ventricular fibrillation in 6/9 animals and the remainder died in A-V block progressing to asystole. As is shown in Figure 1, all doses of Org 6001 significantly reduced mortality. The reduction in mortality in the drug-treated group was associated with a decrease in the incidence of VF (Table 1). Table 1 also summarizes the effects of the drug on the severity of the dysrhythmias in animals surviving coronary artery ligation. A significant reduction in the number of extrasystoles was observed only with the highest dose used, although the duration of ventricular tachycardia was significantly reduced by doses of Org 6001 of 20 mg/kg and higher.

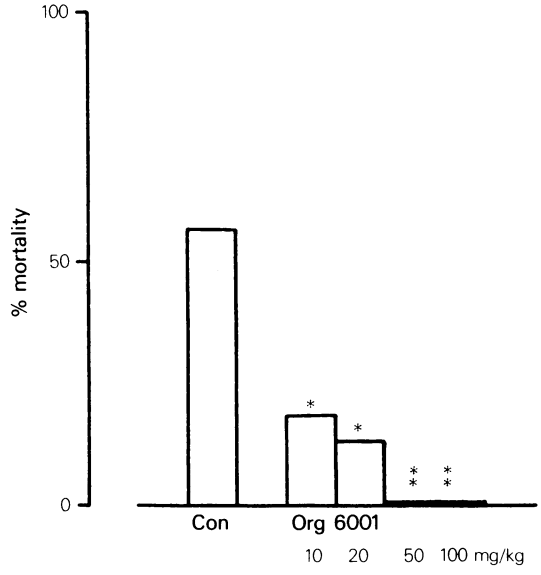

Figure 1 The percentage mortality in rats given a single oral dose of water (Con) or Org 6001 one hour before ligation.
$^{*}P<0.05$; $^{**}P<0.01$.

These doses of Org 6001 did not cause significant changes in either mean arterial blood pressure or heart rate, preligation values of which in the control group were 92 ± 6 mmHg and 459 ± 14 beats/min respectively. The haemodynamic effects of ligation were similar in all groups. No significant change in

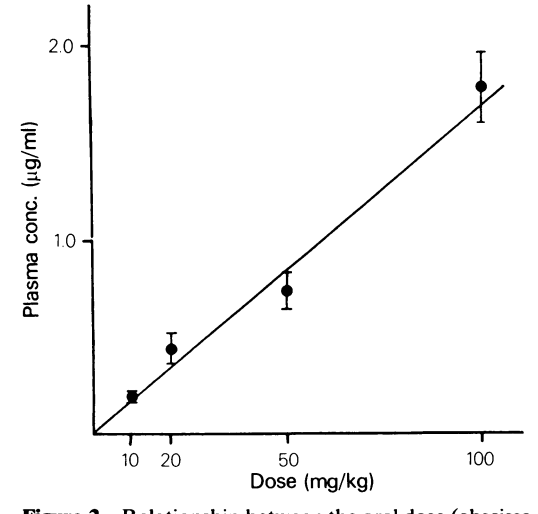

Figure 2 Relationship between the oral dose (abscissa scale) and plasma concentration of Org 6001 (ordinate scale) 90 min after a single oral dose in rats. Plasma levels were determined in 6 animals per dose group.

Table 1 The effects of acute oral pretreatment with Org 6001 on the severity of the arrhythmias (the number of ventricular extrasystoles, and the durations of tachycardia (VT) and of ventricular fibrillation (VF)) in the 0–30 min post-ligation period in animals surviving coronary artery ligation

		n	N	*Ventricular extrasystoles*	*VT duration* (s)	*VF duration* (s)	*VF total*
Control		16	7	737 ± 271 (100)	51 ± 21 (100)	15.2 (14)	44
Org 6001	10 mg/kg	17	14	1008 ± 257 (93)	70 ± 22 (86)	106 ± 38 (29)	24
	20 mg/kg	8	7	205 ± 140 (100)	25 ± 19 (43*)	0 (0)	12
	50 mg/kg	8	8	575 ± 328 (75)	72 ± 38 (50*)	9.0 (12)	12
	100 mg/kg	8	8	*47 ± 34 (62)	0 (0**)	0 (0)	0*

*$P<0.05$; **$P<0.001$.
Values are expressed as mean ± s.e.mean. n = number of animals in group. N = number of survivors.
The percentage incidence of arrhythmias is shown in parentheses and the percentage incidence of VF in all animals (VF total) is also given.

heart rate occurred on ligation and there was only a transient fall in arterial pressure immediately after ligation.

There was a linear relationship between the oral dose and plasma concentrations of Org 6001 (Figure 2). Regression analysis revealed a slope of 0.017 and an intercept of 0.02 μg/ml. The intercept was not significantly different from zero. A plasma level of Org 6001 of 200 ± 20 ng/ml, which was achieved 90 min after a single oral dose of 10 mg/kg, significantly reduced mortality from 56% in the control group to 18% (Figure 1). Over the dose range studied, the ratio of Org 6001 concentration in myocardium:plasma was always greater than the skeletal muscle:plasma ratio. This is illustrated in Figure 3, in which it can also be seen that myocardium:plasma ratio of Org 6001 decreases with increasing dose whereas the skeletal muscle:plasma ratio was constant over the dose range studied. According to the Terpstra test, this decrease was statistically significant at $P=0.05$. The Org 6001 levels in the ischaemic left ventricle correlated well with the levels in the normal right ventricle and septum ($r=0.95$; $P<0.001$). The mean ratio between Org 6001 levels in the left and right ventricle plus septum was calculated to be 1.02. This value was not significantly different from 1.00. Therefore, a statistically significant difference between Org 6001 levels in the ischaemic left ventricle and the normal right ventricle and septum could not be demonstrated. In Figure 4, the percentage incidence of ventricular fibrillation is plotted versus the Org 6001 levels in myocardium

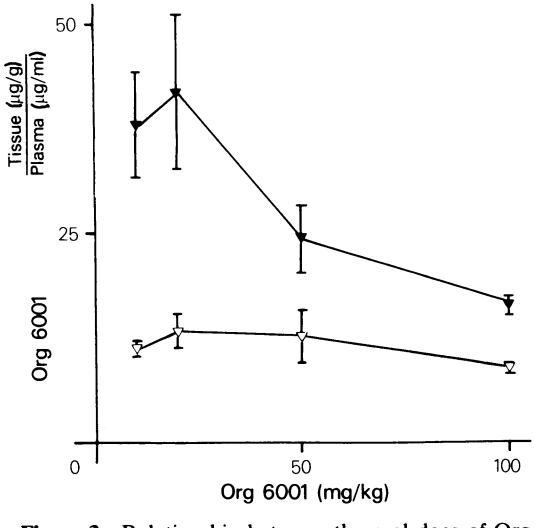

Figure 3 Relationship between the oral does of Org 6001 (abscissa scale) and the myocardial:plasma ratio (▼) and skeletal muscle:plasma (▽) ratio of Org 6001 (ordinate scale) in rats.

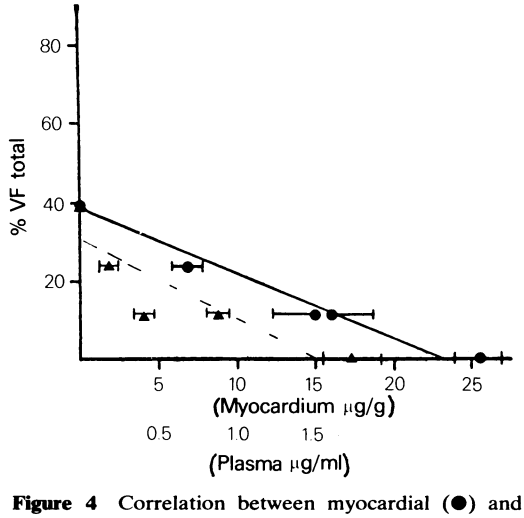

Figure 4 Correlation between myocardial (●) and plasma (▲) Org 6001 concentrations and the percentage incidence of ventricular fibrillation in all animals (% VF total). $r=-0.98$, $P<0.01$ and $r=-0.84$ and $P<0.10$ respectively.

and plasma. The myocardial levels of Org 6001 correlated well with the percentage of ventricular fibrillation, while plasma levels showed a poor correlation.

Discussion

The results of the present study confirm the potent antiarrhythmic activity of Org 6001 following oral administration. All doses of Org 6001 studied significantly reduced mortality and the degree of this protection was dose-related. The reduction in mortality was associated with a reduced incidence of ventricular fibrillation but where a substantial increase in survival was noted (i.e. following doses of 10 and 50 mg/kg) this was also accompanied by an increase in the number of extrasystoles occurring in the survivors (Table 1). This finding is understandable if one accepts that, although animals in which ligation would have proved fatal if untreated survive following drug pretreatment, the effects of ligation are still sufficiently severe to induce a large number of arrhythmias. Thus, in the present study, because of drug-induced changes in mortality, the number of extrasystoles occurring in the survivors could not be used to assess accurately the antiarrhythmic activity of the drug. This has not been the case in previous intravenous studies using this model (Kane *et al.*, 1980; Clark, Foreman, Kane, McDonald & Parratt, 1980) in which mortality in the control group was 16% and rarely changed following drug administration. It appears therefore that, in this model, oral dosing with placebo greatly increases mortality (from ventricular fibrillation) following coronary artery ligation, a finding which has been repeatedly observed in our laboratory. In this more severe model, antiarrhythmic activity is probably therefore best assessed by drug-induced changes in mortality and in the incidence of fibrillation rather than from alterations in the number of ventricular ectopic beats. The reason for the increased severity of the effects of coronary artery ligation in animals subjected to oral dosing is not known but may be the result of the stress to the animal of the dosing itself. Stress has been shown to be associated with increased sympathoadrenal activity and increased output of catecholamines in the rat (McCarty, Gilad, Weise & Kopin, 1979) and this may sensitize the heart to the effects of coronary artery ligation. However, a comparison of the heart rates and arterial blood pressures of animals subjected to oral dosing with those from previous control groups not subject to oral dosing failed to reveal any significant difference.

Oral administration of Org 6001 did not significantly affect pre-ligation values of heart rate or arterial blood pressure and even following the highest dose (100 mg/kg), the haemodynamic effects of ligation were similar to those in the control group. This confirms the potent antiarrhythmic effect of Org 6001 at doses which do not produce significant cardiovascular depression.

A significant reduction in both mortality and in the incidence of ventricular fibrillation was observed following the administration of even the lowest dose of Org 6001 (10 mg/kg). This single oral dose, given 1 h pre-ligation, yielded a mean plasma concentration of 200 ± 20 ng/ml.

Over the dose range used in this study, a linear relationship was observed between the percentage incidence of fibrillation and the myocardial concentrations of Org 6001 (Figure 4). Usually there is a sigmoid relationship between drug concentration and its effect, but presumably with these concentrations we were observing only the linear part of the dose-response curve. An extension of the dose range employed may well have shown the full dose-response curve. Although, over the concentration range studied, there was a linear relationship between the oral dose and the plasma and skeletal muscle concentrations, it was observed that the concentration of the drug in the myocardium was not linearly related to the dose; at high doses the myocardium:plasma ratio of Org 6001 decreased (Figure 3). This would suggest that at high doses, tissue saturation is occurring in the target organ. The negative correlation between the myocardial levels of Org 6001 and the percentage incidence of ventricular fibrillation and the poor linear relationship between plasma levels and percentage incidence of ventricular fibrillation (Figure 4) indicate that plasma levels may not always be a useful guide to the possible antiarrhythmic activity of the drug. Org 6001 appeared to be concentrated in both skeletal and cardiac muscle, the ratio of tissue to plasma levels being 11 in skeletal muscle and between 16 and 40 (depending upon the oral dose) in the heart. However, no difference in the myocardial content of Org 6001 in the ischaemic and normal muscle was seen. The apparent preferential uptake of Org 6001 into the myocardium as compared with skeletal muscle may be due to many factors such as differences in blood flow to the tissues, rapid tissue absorption or slow elimination from the cardiac tissue. As we measured tissue levels only at one time after dosing, no conclusions can be drawn from the present study about the tissue kinetics, nor can a kinetic explanation for the observed differences in tissue level be given. A more detailed pharmacokinetic/ pharmacodynamic study in which it was possible to monitor both Org 6001 concentrations and its pharmacological effects as a function of time (a protocol which was not possible in the present study) would need to be carried out to differentiate between such factors. It is of interest to note however, that the myocardial:plasma ratios quoted for

other antiarrhythmic drugs such as procainamide and disopyramide are 2.3–2.5 (Mark, Kayden, Steele, Cooper, Berlin, Rovenstine & Brodie, 1951; Bagwell, Walle, Drayer, Reidenberg & Pruett, 1976; Strauss, Bache, Masterton, Abou-Donial, McHale & Menzel, 1978) and 2–4 (Karim, Kook, Campion & Doherty, 1977; Patterson, Stetson & Lucchesi, 1979) respectively. Thus Org 6001 would appear to be concentrated in the myocardium to a greater extent than other antiarrhythmic drugs, its accumulation in cardiac muscle being akin to that of certain glycosides (Kuschinsky, Lüllman & van Zwieten, 1968; Kuhlmann, Rietbrock & Schnieders, 1979).

In conclusion, the effectiveness of oral pretreatment with Org 6001 in protecting against coronary artery ligation-induced arrhythmias has been confirmed. It has also been shown that this antiarrhythmic effect of Org 6001 correlates better with myocardial than with plasma concentrations of the drug.

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