Table 1. Comparison of GC retention times of the R_f 0.67 zone and androst-4-ene-3,17-dione standard (methylene units)

	Standard androstenedione	R_f 0.67 zone
Underivatised MO	25.17	25.16
syn form	26.28	26.26
anti form	26.32	26.34

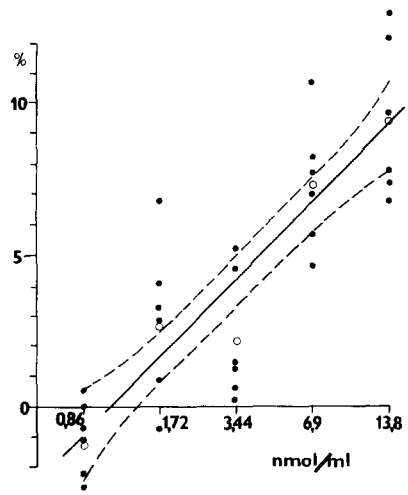

Fig. 1. Androstenedione stimulation of $^{86}Rb^-$ influx into RBC. Individual values (small full dots), means (open dots); computed regression line ($y = 3.6693 \ln x - 10.1875$, $r = 0.8459$, $P < 0.01$); 95% confidence limits (broken lines). x = concn of androst-4-ene-3,17-dione (nmoles/ml) and y = % of stimulation of $^{86}Rb^+$ influx into RBC *in vitro*.

impurity was identified by GC-mass spectrometry (GC-MS) as androst-4-ene-3,17-dione; authentic androstenedione actually stimulates (Na^+–K^+)ATPase activity *in situ*.

23.6 mg commercial testosterone (Merck, batch No. 70152927) was dissolved in methanol, applied to a thin-layer plate (Merck Kieselgel 60 F_{254}, 0.25 mm) and chromatographed in chloroform–ethanol–water (92:8:0.5). The R_f 0.67 zone (detected in u.v. light, 254 nm) was eluted with methanol and the extract was dried. About 0.2 mg of the impurity was used for GC-MS. GC-MS was performed on an OV-1-coated open tubular column (temp programme 2.5°/min from 200 to 280°) connected to a Varian MAT 731 mass spectrometer with a Varian SpectroSystem 200 data system. The accelerating voltage was 8 kV and the ionising voltage 70 eV. Retention times, expressed as methylene unit values were obtained by co-injection with a mixture of *n*-alkanes into a Becker 410 gas chromatograph with column and operating conditions as earlier. The mass spectra of the free and derivatised form match those of the androst-4-ene-3,17-dione standard; the same applies to the GC retention times [methylene units (Table 1)].

Androstenedione puriss. CHR (Koch-Light) was dissolved in methanol and serially diluted samples were prepared with physiological saline at final concns of 0.36–13.6 nmoles/ml incubation mixture. (Na^-–K^-)ATPase activity *in situ* was determined by measuring $^{86}Rb^-$ uptake by human RBC *in vitro* by Lowenstein's method [3, 4]; the results are shown in Fig. 1. Androstenedione produced dose-dependent stimulation of influx, but when influx was inhibited by ouabain (200 nmoles/ml) (up to 35% inhibition), none of the given androstenedione concns stimulated it (data not shown).

The physiological significance of this action of androstenedione is unknown. Androstenedione is present in human blood, however, and $^{86}Rb^-$ influx/stimulating activity was recently observed in the blood of patients with low-renin essential hypertension [5]. This type of hypertension is presumably caused or modulated by an unidentified steroid hormone. It should be added, however, that the concns of androstenedione used in our *in vitro* studies are much higher than those in human blood.

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Biochemical Pharmacology, Vol. 32, No. 6, pp. 1122–1125, 1983.
Printed in Great Britain.

0006-2952/83/061122-04 $03.00/0

Enhanced serum binding of propranolol and oxprenolol and microsomal enzyme induction by rifampicin in the dog

(*Received* 1 *July* 1982; *accepted* 25 *October* 1982)

There have recently been a number of reports concerning the increased binding of drugs to α_1-acid glycoprotein (α_1-AGP) when microsomal enzymes are induced by anti-epileptic drugs such as phenobarbital and phenytoin in man [1], the dog [2, 3] and the rat [4].

Rifampicin stimulates drug metabolism in man and in

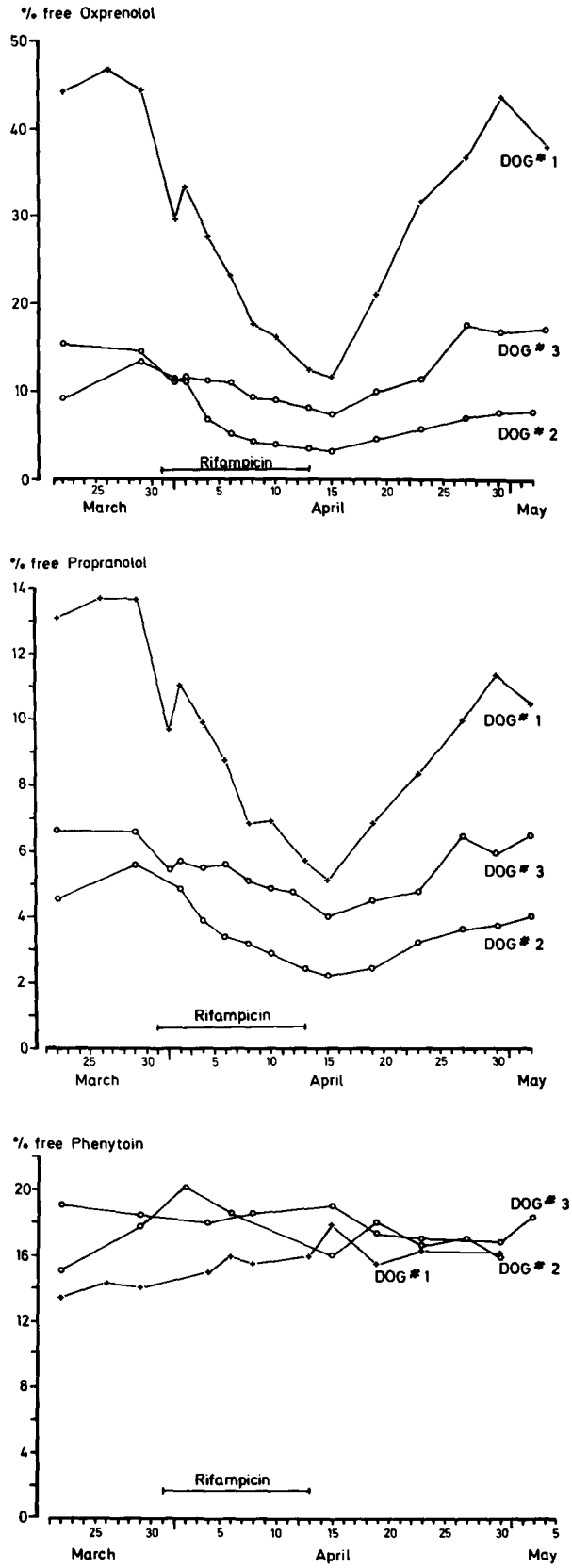

Fig. 1. Percentage free propranolol, oxprenolol and phenytoin before, during and after oral rifampicin treatment (300 mg, twice daily for 10 days) in three dogs.

some animal species (rabbit, mouse, pig) but not in others (rat, guinea-pig) [5]. No data are available on the inductive properties of rifampicin in the dog. The present study was designed to evaluate whether in the dog rifampicin is able to induce drug metabolism, and whether this is accompanied by an increased serum binding of drugs bound to α_1-AGP.

In vitro serum binding of oxprenolol and propranolol, drugs mainly bound in humans to α_1-AGP [6, 7], and of phenytoin, a drug bound to albumin, was measured before, during and after rifampicin treatment in the dog. Hexobarbital and antipyrine clearances were used as indexes of the activity of the drug-metabolizing enzymes.

Materials and methods

DL-[4-^{3}H]Propranolol hydrochloride was obtained from Amersham, [^{3}H]phenytoin from New England Nuclear. [^{14}C]Oxprenolol and rifampicin capsules were kindly donated by Ciba-Geigy.

Three healthy dogs (dog No. 1, female, 26 kg; dog No. 2, female, 25 kg and dog No. 3, male, 25 kg) were studied. They had normal liver and renal function and no infectious or inflammatory diseases as judged by a number of biochemical and hematological tests. During rifampicin treatment, increased serum alkaline phosphatase concns were observed as expected.

The dogs were treated orally with rifampicin, 300 mg twice daily for 10 days. On the days before rifampicin treatment, the dogs were given antipyrine (40 mg/kg in an intravenous injection) and hexobarbital (20 mg/kg in an intravenous infusion over 10 min). On the first two days and the 18th and 19th day after stopping rifampicin treatment, hexobarbital and antipyrine administrations were repeated. Hexobarbital and antipyrine were always given on two different days. Blood samples of 5 ml were taken at various times for hexobarbital and antipyrine determinations.

Blood samples were also taken before, during and after rifampicin treatment for determination of the protein binding of oxprenolol, propranolol and phenytoin. *In vitro* serum binding of [^{14}C]oxprenolol (sp. act. 5.03 nCi/μmole), [^{3}H]propranolol (sp. act. 3.3 nCi/μmole) and [^{3}H]phenytoin (sp. act. 6.3 nCi/μmole) was measured by equilibrium dialysis. Dialysis was performed in duplicate at 25° for 4 hr in Teflon half-cells separated by a cellophane membrane (Visking). One compartment contained phosphate buffer (0.3 ml, 0.15 M, pH 7.4) in which the drugs were dissolved and the other compartment contained 0.3 ml serum. After dialysis, 100-μl aliquots from both compartments were counted in a Tri-Carb Liquid Scintillation Counter 3380 after addition of Picofluor-15 (3 ml). The initial concn of the drugs in the buffer compartment was 1.84 μg/ml for oxprenolol, 20 ng/ml for propranolol and 10 μg/ml for phenytoin.

Concns of antipyrine and hexobarbital were measured gas chromatographically. The method for antipyrine has been described previously [8]. For hexobarbital, methohexital (2 μg) was used as the internal standard and 0.5 ml plasma was extracted with 2 ml of methylene chloride. After evaporation of the organic phase under nitrogen, the residue was dissolved in 10 μl of ethyl acetate. A Hewlett–Packard 5880 A gas chromatograph was used equipped with a N-PD detector. GLC was performed on a 1.80 m × 2 mm i.d. glass column containing 2% OV-17 on 80–100-mesh gas chrom Q with a helium flow of 30 ml/min. Temperatures were: injector, 210°; column, 180°; detector, 300°. Detector conditions were set on the highest response.

The antipyrine and hexobarbital plasma data were assumed to fit a one-compartment open model. The elimination constants were determined by linear regression analysis. Half-life, clearance and volume of distribution were calculated by the usual equations [9].

Results

Figure 1 shows the percentage free oxprenolol, propranolol and phenytoin in the three dogs as a function of time before, during and after rifampicin treatment. The percentage free propranolol and oxprenolol decreased markedly during rifampicin treatment. After stopping rifampicin, the percentage free drug increased again and attained the control values around the 18th day. The percentage free phenytoin did not change during rifampicin treatment. In dog No. 1 the initial percentage free oxprenolol and propranolol was much higher than in the others and the changes in binding during rifampicin treatment were much more pronounced.

Fig. 2 shows as an example the elimination curves for hexobarbital and antipyrine before, immediately after and

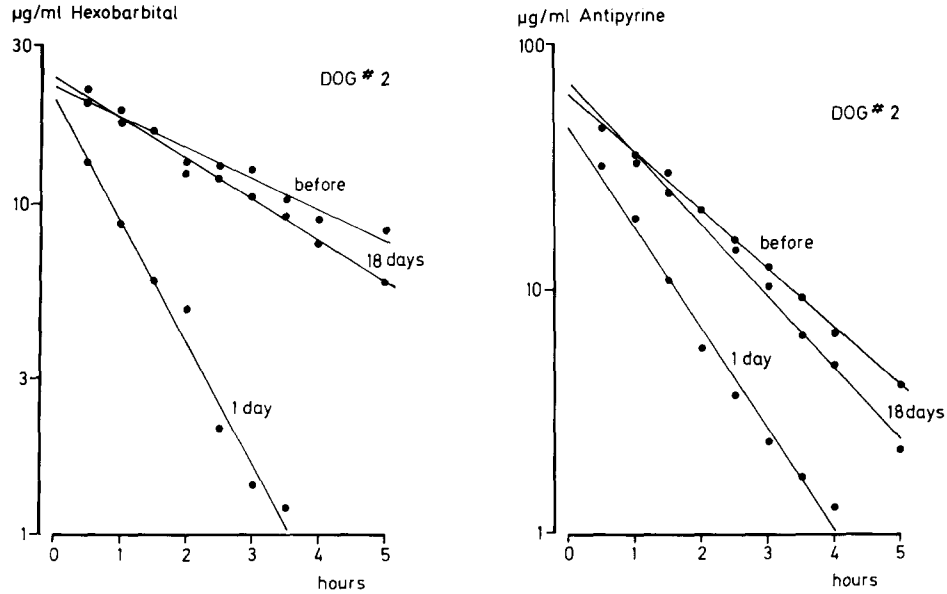

Fig. 2. Hexobarbital and antipyrine plasma concns in dog No. 2, before, 1 and 18 days after oral rifampicin treatment (300 mg, twice daily for 10 days).

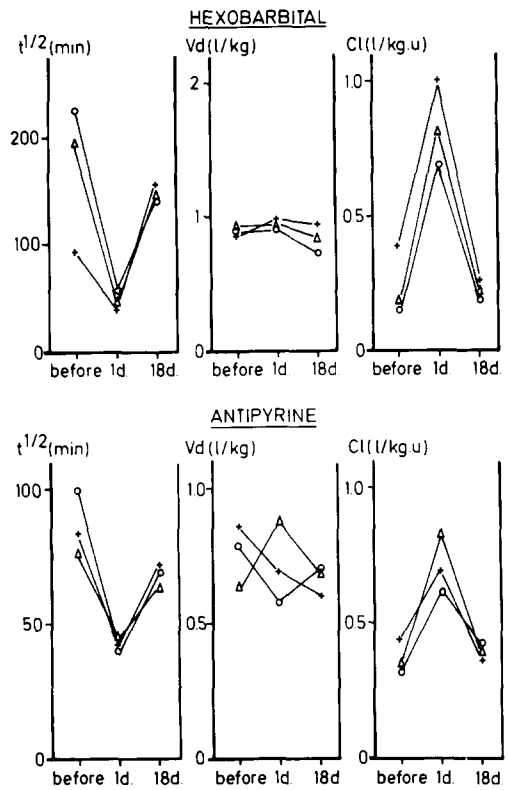

Fig. 3. Pharmacokinetic parameters for hexobarbital and antipyrine before, 1 and 18 days after rifampicin treatment (300 mg, twice daily for 10 days) in three dogs.

18 or 19 days after stopping rifampicin in dog No. 2. Fig. 3 shows the pharmacokinetic parameters obtained for hexobarbital and antipyrine in the three dogs. Rifampicin treatment shortened the elimination half-life of hexobarbital by approximately 70% and that of antipyrine by approximately 50%. No important changes in distribution volumes of both drugs were observed and the metabolic clearance of hexobarbital increased by 350% and that of antipyrine by 100%.

Discussion

Rifampicin is known to be an inducer of microsomal drug metabolism in man, the pig, mouse and rabbit but not in the rat and guinea-pig [5]. Our results show clearly that rifampicin induces drug metabolism in the dog. The mean metabolic clearance of hexobarbital increased more than three-fold and that of antipyrine two-fold; 18 days after stopping rifampicin, the clearances had returned to control values.

Pretreatment with rifampicin for 10 days enhanced binding of propranolol and oxprenolol, two drugs mainly bound to α_1-AGP in man [6, 7], whereas binding of phenytoin, a drug mainly bound to albumin, was unaffected. Similar results were found after treatment of patients or animals with other inducing agents. Routledge *et al.* [1] found an increase in α_1-AGP concns in plasma and an enhanced binding of lidocaine in patients treated with antiepileptics. Abramson *et al.* [3] demonstrated that chronic administration of phenobarbital increases the plasma protein binding of propranolol in beagle dogs and Brinkschulte and Breyer-Pfaff [4] found that a 5-day oral treatment of phenobarbital in rats increased the binding of desmethylimipramine.

It is not proven that enhanced serum binding after treatment with rifampicin in the dog is related to an increase in α_1-AGP binding. If this was so, an increased concn or a chemical alteration of α_1-AGP could be involved; according to Abramson *et al.* [3], the plasma concn of non-precipitable glycoproteins is enhanced in the dog after phenobarbital treatment; on the other hand, α_1-AGP isolated from phenobarbital-treated rats contained a higher percentage of *N*-acetylneuraminic acid [4].

Our results show that in the dog rifampicin treatment induces drug-metabolizing enzymes and enhances the binding of drugs bound to α_1-AGP. As rifampicin is also an inducer in man, similar changes might occur in patients treated with this tuberculostatic agent.

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