The pharmacokinetics of tetroxopim in the dog

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SUMMARY

The pharmacokinetics and metabolism of the newly developed antibacterial tetroxoprim (TXP) were investigated in four male beagle dogs using a 2-(14C)-labelled drug. TXP was administered intravenously and orally at doses of 5 mg/kg bodyweight. Counting of the total 14C-radioactivity shows that the drug was absorbed completely and eliminated almost exclusively by the kidneys. The quantitative absorption of TXP from the canine gastro-intestinal tract was ascertained by comparison of the AUC-values and the renal recoveries of unchanged drug following i.v. and oral dosage.

The terminal half-lives of non-metabolized compound in plasma were 4.64 and 4.83 for i.v. and oral dosing respectively. Following either i.v. or oral TXP administration, the major route of excretion was renal elimination of the compound as its major metabolite U-I (81-82.2 % of dose) and as unchanged mother substance (11.4-12.3 % of dose).

INTRODUCTION

Tetroxoprim (TXP), -2,4-Diamino-5-(3,5-dimethoxy-4(2-methoxy-ethoxy)benzyl) pyrimidine-, is a new, selective and highly specific inhibitor of bacterial dihydrofolate reductases. (1). The fixed combination of 1 part TXP and 2.5 parts sulfadiazine* has been developed as a powerful chemotherapeutic agent, particularly for the treatment of urinary and respiratory tract infections. (2).

The pharmacokinetics and metabolism of tetroxoprim in the rat, rabbit and in man have been investigated extensively and described in several previous publications. (3,4,5,6).

The aim of the present study was to evaluate the basic pharmacokinetic properties as well as the biotransformation of this new drug in a larger mammalian species, i.e. in the dog.

MATERIALS AND METHODS

Substance

Tetroxoprim, the structure of with is given below, was labelled with carbon-14 at position 2 in the pyrimidine moiety using the method of Buchtel et al, 1980 (7). 2-(14C)-tetroxoprim had a specific activity of 22 k Bq/mg (7.4 MBq/mmmole) i.e. 0.6 μCi/mg (0.2 mCi/mmmole) and was shown by radio-scanning of thin-layer chromatograms and by in-verse isotope dilution analysis to be radiochemically pure.

Animal experiments

Four male beagle dogs (Erkrath/Rodenbach, FRG) of approximately 2 years of age and 13-16.5
kg body weight were maintained in stainless-steel metabolism cages which allowed separate collection of urine and faeces. During the whole investigation period a room-temperature of 22.5 ± 1°C and a relative humidity of 65 ± 5% were guaranteed. Samples of control urine and faeces were collected for 24 h before dosing and a sample of control blood was withdrawn immediately prior to dosing.

Doses of 5 mg/kg 2-(14C)-tetroxoprim (1.43 - 1.82 MBq total) were given orally to each animal as a single capsule or, by intravenous injection (10% dimethylacetamide/0.9 % saline) via a femoral vein.

The drug-formulations were given in a random cross-over design followed by a wash-out period of a fortnight between the oral and parenteral forms. 5 ml blood samples were obtained from the brachial vein at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 48 h after dosing. Urine was collected during the time intervals 0-2, 2-4, 4-6, 6-8, 8-12, 12-24, 24-48, 48-72 and 72-96 h after dosing. Faeces sampling followed every 24 h up to 96 h after medication.

**Preparation of biological samples**

Blood samples were heparinized and centrifuged at 2,700 g. The plasma removed was deproteinized with equal volumes of acetonitrile. After centrifuging, the supernatant was dried and either redissolved in methanol for thin-layer chromatography or in 0.1 M sodium acetate-buffer (pH 4.7) for enzymic hydrolysis.

The total faeces collected every 24 h was diluted with 3-fold volumes of water and homogenized (Ultra-Turrax, Jahnke und Kunkel, Stauffen, FRG) for 10 min.

**Counting of total radioactivity**

Urine and plasma were assayed by addition of duplicate 1 ml (urine) and 0.5 ml (plasma) aliquots to 10 ml Instagel® (Packard Instr., Frankfurt, FRG). The 14C in faeces was determined by combustion of 300-400 mg water-diluted samples in a sample oxidizer, Modell B 306 (Packard Instr., Frankfurt, FRG). Radioactivity was determined in a Packard Tri-Carb, model B 2450 or in a Kontron MR-300 liquid scintillation spectrometer.

**Enzyme treatment of samples**

1 ml samples of urine believed to contain conjugated metabolites were incubated at 37°C for 24 h at pH 4.7 (addition of 1 ml of 0.1 M sodium acetate buffer) in the presence of 25 mg β-glucuronidase (bovine liver, 28.500 Fishman units/g. Serva, Heidelberg, FRG) or a mixture of arylsulfatase (20 U/ml) and β-glucuronidase (30 U/ml) (Helix pomatia, Merck, Darmstadt, FRG). 1 ml samples of deproteinized plasma were also incubated with the enzymes. In each case, control incubations in buffer and biological fluids were made.

**Thin-layer chromatography**

For thin-layer chromatography precoated silica gel plates 60 F 254 (Merck, Darmstadt, FRG) were developed in 3 different solvent systems consisting of chloroform/methanol/methyl ethyl keton/NH4OH (60:22:10:4 v/v/v/v) solvent system 1, chloroform/n-propanol/formic acid (4:4:2 v/v/v = 2) and of n-butanol/water/acetic acid (4:3:1 v/v/v = 3) respectively. Detection and quantification of the separated radioactive spots was carried out with the aid of a radio-t.l.c.-scanner LB 2722-2 equipped with plotter IB 2722-21, ratemeter BF 2304, HV-unit DF 2301 and multi-channel analyzer, model 1024 (all: Berthold, Wildbad, FRG).

**Analysis of data**

Half-lives of unchanged TXP in plasma were calculated by use of the iterating TOPFIT program (8).

**RESULTS**

1. Plasma levels

**a) Total radioactivity (Fig. 1):**

After intravenous injection of 5 mg/kg 2-(14C)-tetroxoprim, the concentration of total radioactivity decreased sharply for about 1 h. This was followed by a gradual increase, culminating 3-4 hours after drug injection with 14C-concentrations of 3.06 µg (eq)/ml and 2.81 µg (eq)/ml respectively.

The same concentrations, i.e. 3.21 µg(eq)/ml at 3 h and 3.17 µg(eq)/ml at 4 h, - were reached at about the same time interval after oral administration of 5 mg/kg of labelled drug. Thereafter, total radioactivity in plasma declined with a mean half-life of 5.9 h in the terminal phase.