The metabolism and excretion of 7-mono-0-(β-hydroxyethyl) rutoside in the dog

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Received for publication: March 14, 1979

Key-words: Flavonoids, hydroxyethylrutoside metabolism, microflora.

SUMMARY

Following i.v. administration of mono-HR to the beagle, plasma levels of both mono-HR and its glucuronide conjugate fell rapidly, neither being detectable 8 h after injection. Following oral administration of 14C-mono-HR, mono-HR-glucuronide was detected in plasma, confirming the absorption of mono-HR, and low levels of 14C were detectable up to 72 h after dosage. Following either oral or i.v. administration of mono-HR, the major route of excretion was fecal elimination of the compound as its aglycone form. Urinary excretion was slight being less than 15% following i.v. dosage and 4% following oral administration.

Metabolism of mono-HR was confined to glucuronidation and hydrolytic cleavage of the glycoside side chain. Ring fission products of mono-HR were not detected.

INTRODUCTION

Pharmaceutical preparations containing O(β-hydroxyethyl) rutosides have been reported effective in the treatment of various peripheral vascular disorders (1-6). The major component of these preparations is 3',4',7-tri-O(β-hydroxyethyl) rutoside, the metabolism and distribution of which have been studied in several species including the primates and man (7-12). A related compound, 7-O(β-hydroxyethyl) rutoside, is also currently undergoing pharmacological investigation. Its metabolism and disposition have been studied in the rat and in the mouse (8,9,11) but not in the larger mammalian species. We present here the results of studies on the metabolism and pharmacokinetics of 7-mono-O(β-hydroxyethyl) rutoside in the dog.

MATERIALS AND METHODS

Materials

7-Mono-0-(β-hydroxyethyl) rutoside (mono-HR) and 7-mono-O(β-hydroxyethyl-[14C2]) rutoside (14C-mono-HR) were made available by Zyma S.A. Nyon, Switzerland. The chromatographic and ultraviolet spectral data for these compounds have been previously reported (13-15).

14C-Mono-HR had a specific activity of 0.06 μCi/mg (32.7 μCi/mmol) and was shown by scintillation counting and radioscanning of chromatograms to be radiochemically pure.

7-Mono-O(β-hydroxyethyl) quercetin (mono-HQ) was prepared from mono-HR by mild acid hydrolysis (16).

Animals and animal experiments

Beagle bitches of approximately 3 years of age and 10-12 kg body weight were maintained in steel metabolism cages which allowed separate collection of urine and faeces but not the trapping of expired gases. 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.

A dose of 250 mg of the non-labelled mono-HR or 250 mg of 14C-mono-HR (14.1 μCi in each of two oral doses and 7 μCi in each of two intravenous doses) was given orally to each animal as a single capsule or by intravenous injection dissolved in 0.9% saline in to a femoral vein.

Blood samples were obtained from a saphenous vein at various times after dosing.
Treatment of biological samples

Blood samples were heparinised and centrifuged, the plasma removed and deproteinised with equal volume of acetone. After centrifuging, the supernatant was dried and either redissolved in methanol for chromatography or in phosphate buffer for enzymic hydrolysis.

Faecal samples were freeze dried in toto and ground to a powder. Weighed amounts of this were continuously extracted with methanol in a Soxhlet system for 24 h. The recovery of metabolites by this method was estimated by comparison of the amounts of 14C with levels obtained by combustion of non-extracted samples. The efficiency of extraction thus measured also provided a measure of the efficiency of extraction of faeces in experiments in which non-labelled mono-HR was administered.

Urine samples were diluted to a standard volume. 20 ml aliquots of these were acidified to pH 1-2 and continuously extracted with diethyl ether for 12 h. ether extracts were evaporated to dryness and redissolved in methanol for chromatography and scintillation counting.

Measurement of 14C in biological fluids and extracts

Urine, methanolic extracts of faeces and ethereal extracts of urine were assayed by addition of duplicate 1 ml aliquots to 10 ml of toluene-Triton-X100 phosphor (17,18). Level of 14C in plasma samples was determined by combustion of 0.5 ml of plasma in a Harvey Biological Material Oxidiser (ICN-Tracerlab Ltd., Hersham, Surrey) and 14C in faeces was determined by combustion of 100-250 mg of freeze dried powdered faeces (11).

Enzymic hydrolysis of conjugates

Samples of urine believed to contain conjugated forms of mono-HR were incubated at 37° for 24 h in the presence of β-glucuronidase (E. coli, type II) or arylsulphatase (Limpet, type III) (Sigma, London) (19). Samples of deproteinised plasma were also incubated with β-glucuronidase prior to assay of mono-HR by scanning densitometry (20). In each case, there were control incubations of mono-HR in buffer, and experimental fluids without enzyme were employed.

Chromatography

Metabolites in urine, faecal extracts and deproteinised plasma samples were separated by chromatography on Whatman 3MM paper developed in butan-2-ol:acetic acid:water 5:1:2, by volume (solvent system 1) or by chromatography on thin layers of silica gel G developed in ethyl acetate:formic acid:water, 70:15:15, by volume (solvent system 2) or ethyl acetate:formic acid:water, 80:6:9 by volume (solvent system 3). The major faecal metabolite of mono-HR was isolated from faecal extracts by band preparative chromatography on Whatman No. 17 paper developed in solvent system 1 and eluted with methanol.

Ether extracts of acid hydrolysed or untreated urine samples were examined by two dimensional chromatography on 20 x 20 cm squares of Whatman No. 1 paper, developed in isopropanol:ammonia:water, 8:1:1, by volume, and the upper phase of benzene:acetic acid:water, 6:7:3, by volume.

Quantification of mono-HR, mono-HR-glucuronide and mono-HQ by scanning densitometry

Non-labelled mono-HR, and mono-HR-glucuronide were quantified in plasma and urine by measuring the fluorescence of complexes of mono-HR with boric and citric acids by scanning densitometry using a Vitatron TLD 100 densitometer. The techniques of thin layer chromatographic separation of these complexes and the optimal conditions for scanning densitometry have been fully reported elsewhere (20).

Non-labelled mono-HQ in faecal extracts was quantified by an analogous method as follows: known amounts of methanolic extract of faeces were spotted on to silica gel. TLC plates impregnated with boric and citric acids along with calibration samples consisting of mono-HQ dissolved in control faecal extract. Plates were developed in solvent system 3 and dried in a stream of air for 2 hours. The plates were then scanned at the Rf of mono-HQ using the following settings: activating filter, UVB, emission filter, 525 nm; Mode, Lin II⁺; Zero C, 4; Level, f. The calibration curves obtained were linear over the range 0-50 μg and reproducible.

Detection of metabolites on chromatograms

The following spray reagents were used for the detection of metabolites: 1% methanolic A1C13 followed by examination under UV light was used to detect mono-HR and its conjugates. Diazotised p-nitroaniline (21) was used to detect phenolic compounds and 2% naphthoresorcinol in 33% trichloroacetic acid was used to detect glucuronide conjugates (22).

Radioactive metabolites were located and assayed by radioscanning of chromatograms using a Packard 7200 radiochromatogram scanner or by liquid scintillation counting of chromatogram segments (15).

Characterization of metabolites by UV spectroscopy

The ultraviolet and visible spectra of mono-HQ was determined over the range 210-450 nm in a Pye SP1800 spectrophotometer in methanol and additionally in the presence of A1C13 and A1C13 + HC1 (8).

RESULTS

1. Plasma levels of mono-HR and its metabolites following i.v. injection.

After injection of mono-HR, plasma levels of mono-HR and its metabolite declined rapidly (Fig. 1). Chromatography of deproteinised plasma samples showed the presence of two metabolites not present in control