Human metabolism of tolfenamic acid. I. Isolation, preliminary characterization and pharmacokinetics of tolfenamic acid and its metabolites

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SUMMARY

The metabolic fate and pharmacokinetics of tolfenamic acid, a new anti-inflammatory agent, was studied after intravenous and oral administration of $^{14}$C-tolfenamic acid to one healthy volunteer. The recovery in urine was 77% of the intravenous dose and 93% of the oral dose. About 11% of the doses was found in faeces after both routes of administration whereas no radioactivity was detected in expired air, saliva or red cells. In plasma 90-99% of the radioactive compounds were bound to proteins whereas in vitro protein-binding of tolfenamic acid was 99.7%. Tolfenamic acid was biotransformed into several metabolites and only less than 10% of the doses was excreted into urine as the glucuronide/sulphate conjugate of unchanged drug. Tolfenamic acid and four metabolites were separated by TLC, together they accounted for 90-100% of urine radioactivity. Two major metabolites with preliminary identification as hydroxylation products of tolfenamic acid were isolated in pure form for further structural analysis. After fast initial decline the total plasma radioactivity decreased slowly with a mean half life of 58 hours. The elimination rate of tolfenamic acid into urine was fast with a half life of 1.9 hours whereas the metabolites were eliminated more slowly. Their elimination showed three phases, a rapid initial phase up to six hours, second phase up to 48 hours with half lives ranging from 9 to 13 hours and a third terminal slow, quantitatively minor, phase thereafter.

In conclusion, tolfenamic acid is practically completely absorbed from the GI tract, probably undergoes considerable enterohepatic circulation, is highly protein bound and extensively metabolized to several metabolites slowly excreted into urine.

INTRODUCTION

Tolfenamic acid, N-(2-methyl-3-chlorophenyl) anthranilic acid, is a new anti-inflammatory agent which structurally closely resembles mefenamic, flufenamic and meclofenamic acids, i.e. other fenamates previously introduced into clinical use (Fig. 1). The pharmacokinetics of tolfenamic acid is characterized

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Fig. 1 : Structure of tolfenamic acid and three other fenamates. Asterisk shows the location of $^{14}$C-label in the tolfenamic acid molecule.
by good gastrointestinal absorption, rather fast elimination of unchanged tolfenamic acid and extensive metabolism: it has been found that only about 10% of the dose is excreted into the urine as conjugates of unchanged drug (1). Our preliminary observations indicated the presence of several metabolites of tolfenamic acid in human urine (2). Other fenamates are known to be extensively metabolized into hydroxylated derivatives with subsequent conjugation to glucuronides (3,4). Since there are a number of striking similarities in the metabolic disposition of fenamates, a fairly similar metabolic pattern for tolfenamic acid was to be expected.

The purpose of the present study was to investigate the metabolism of tolfenamic acid in man using 14C-labeled tolfenamic acid, characterize the urinary metabolites of tolfenamic acid on the basis of their chromatographic behaviour and by other chemical means, and to isolate the metabolites for further structural analyses by mass spectrometry and nuclear magnetic resonance studies. In addition, the aim was to get preliminary information about the pharmacokinetics of the tolfenamic acid metabolites in man. The results of the mass spectrometry and nuclear magnetic resonance analyses will be presented in a separate paper (Khalifah et al., following paper).

**MATERIAL AND METHODS**

**Synthesis of 14C-tolfenamic acid.**

o-Chloro[carboxyl-14C]Benzoic acid (The Radiochemical Centre, Amersham, England) and 3-chloro-4-methylaniline were reacted in dimethylformamide containing copper catalyst and some base. The reaction product was precipitated from alkali solution with hydrochloric acid and chromatographed on preparative silica gel thick-layer plate in the solvent system: ethyl acetate-isopropanol-ammonia (55:35:20). The zone of tolfenamic acid was separated and the product was further purified by recrystallization from ethanol-water. The radiochemical purity was tested by TLC in two solvent systems and by reverse isotope dilution analysis and was found to be > 99%. Sp. activity was 0.69 mCi/mmol.

**Experimental procedures**

14C-Tolfenamic acid (8.8 µCi/100 mg) was administered orally to one healthy female volunteer (age 43, weight 68 kg) after overnight fast. On a separate occasion 5.2 µCi/100 mg of tolfenamic acid dissolved in alkaline physiological saline solution, pH 10.9, was infused over 30 min in the cubital vein of the same volunteer. Timed blood samples were collected into heparinized tubes during two weeks after the drug administration. All urine was collected in fractions during 8 days. Faeces were collected for up to one week. Samples of expired CO2 were collected by trapping into hyamine solution containing phenolphthalein as indicator up to 8 hours after the oral dose. Timed samples of saliva were collected both after oral and intravenous administration.

Blood samples were centrifuged immediately. Plasma was separated and the red blood cells rinsed thrice with cold physiological saline.

For isolation of tolfenamic acid metabolites two healthy male volunteers were given tolfenamic acid (Clotams 100 mg caps, Medica Pharmaceutical Company, Ltd, Helsinki, Finland) 300 mg t.i.d. for one week. All urine excreted during the three last days of the drug intake period was collected.

**Protein binding**

The protein bound fraction of total radioactivity in plasma was determined by using equilibrium dialysis method (5) in heparinized plasma samples obtained 5, 15, and 30 min, and 1 and 3 hours after the i.v. dose. Protein binding of tolfenamic acid in vitro was measured over a concentration range of 0.01 µg/ml - 100 µg/ml by the same technique.

**Radiometry**

Measurement of radioactivity in plasma, red cells, urine, saliva, breath samples, faeces and cuttings from TLC plates was performed with a liquid scintillation counter (Wallac Rack Beta LSC 1215, Turku, Finland). The red cells and samples of faeces we solubilized with Protosol® (NEN, Boston, U.S.A.) and decolourised with H2O2. Autoradiographs of the TLC plates were obtained by exposing the plates against X-ray films for 1-6 months.

**Isolation of tolfenamic acid metabolites**

Urine was treated with β-glucuronidase and sulfatase (G 0751, Type H 1, Sigma, St. Louis, U.S.A.), 500 U/ml at pH 5.0-5.5 overnight at 37°C. After acidification to pH 2.0 the urine was extracted.