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Short Communication

Identification of two new suprofen metabolites in human urine

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Introduction

Suprofen is a nonsteroidal anti-inflammatory drug (NSAID) used for the treatment of mild to moderate pain and for primary dysmenorrhea [1–3]. Previous metabolism studies conducted in humans and other animal species (mouse, rat, guinea pig, dog and monkey) [4–8] resulted in the identification of seven metabolites. In human urine, unchanged suprofen and five metabolites were identified [8, 9]. Our previous studies elucidated the structures of four suprofen isomeric acyl glucuronides using FABMS and NMR techniques [9, 10]. The present work describes the characterization of two new suprofen metabolites in our previous human urine.

Experimental

A single 200 mg solution dose of tritium-labelled suprofen (100 μCi) was administered orally to four male healthy volunteers, and urine samples were collected in 24 h intervals for 2 days. A 5% NaOH-hydrolysed urine pool (100 ml, 0–48) was acidified with 1 N HCl to pH 2.0 and extracted with ethyl acetate. The extract was evaporated to dryness to yield a residue which was reconstituted in methanol and applied to LC and TLC (Fig. 1). Metabolites I and II were collected from the LC and the residues from the collected fractions were individually treated with ethereal diazomethane and analysed by MS and NMR. The

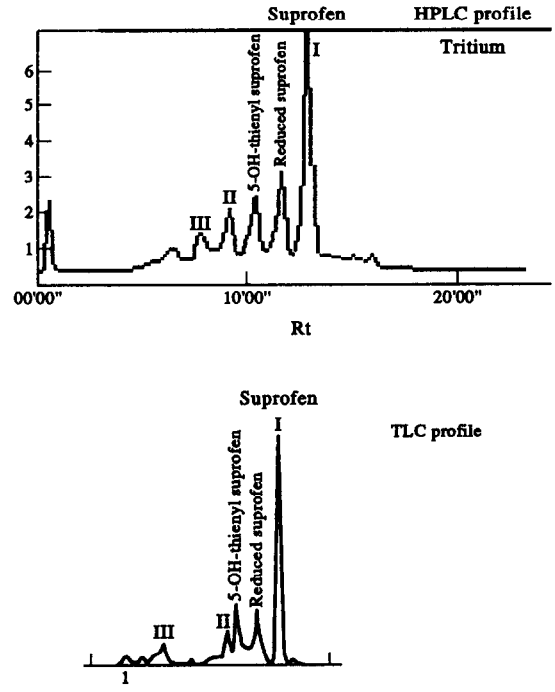

Figure 1
HPLC and TLC metabolic profiles of the base-hydrolysed human urine.

recovery of radioactivity in urine samples was determined by liquid scintillation counting. The LC used was CRT-based gradient liquid chromatograph (Beckman Model 345) with UV detection (Model 165, UV 254 nm, Beckman Instrument, CA) connected to a radioactive flow detector (RAMONOA, IN/US Service Corp., Fairfield, NJ). LiChrosorb

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RP-18 MPLC guard and analytical columns (5 μm, 130 × 4.6 mm) were used for sample analysis. The gradient program was carried out in 20 min with acetonitrile (mobile phase A) and water containing 0.01% acetic acid (mobile phase B). TLC analysis of samples was conducted (chloroform–MeOH–AcOH, 90:10:1, v/v/v) on silica gel GF plates (5 cm × 20 cm; 250 μm; Analtech, Newark, DE).

The plates were analysed using a BID System 100 Radiochromatogram Imaging System (Bioscan, Washington, DC). Electron-impact mass spectra were obtained in a VG 7035 (VG Micromass, Manchester, UK). Chemical-ionization mass spectra were obtained in a Finnigan Model 3300/6100 (Finnigan, Sunnyvale, CA). NMR spectra were determined in deuteroacetonitrile in a Bruker Model WM 360 (Bruker Instruments, Billerica, MA).

Results and Discussion

Cumulative urinary recovery of radioactivity was 85.6% of the dose during the 0–2 day collection period. Suprofen (I) and seven metabolites, five previously reported [8, 9] and two new metabolites, hydroxymethyl suprofen (II) and carboxy suprofen (III), were isolated from base-treated urine and identified (Fig. 2). Putative hydroxymethyl suprofen was isolated in minor amounts from urine (<5% of sample radioactivity), and derivatized as a methyl ester using ethereal diazomethane. The CI–MS analysis of the methyl ester provided an apparent protonated molecular ion at *m/z* 291 (25%) together with two adducted molecular ions at *m/z* 319 ($MC_2H_5^+$) and 331 ($MC_3H_5^+$). The prominent fragment ions at *m/z* 259 (100%, relative scale), 231, 207, 179, 111 and 83 supported a hydroxy group attached to the methyl group of the suprofen molecule (Fig. 2). The proton NMR spectrum displayed resonance signals for a C-methyleneoxy group at δ3.35, an O–Me group at δ3.60, a methine proton at δ3.78 and seven aromatic protons at δ6.95–7.40. Putative carboxy suprofen (III) was present in minor quantities in urine (<5% of sample radioactivity), and was also derivatized as a dimethyl ester by ethereal diazomethane. The EI–MS of the diester exhibited an intense molecular ion at *m/z* 318 (72%), along with key fragment ion at *m/z* 274 ($M^{+\cdot}$-CO_2), 259, 231, 199, 188 (100%, relative scale), 111, and 83, which supported identification of the methyl derivative of the metabolite as a dicarboxylic ester (Fig. 2). The proton NMR spectrum revealed resonance peaks for two O–Me groups at δ3.60, a methine proton at δ5.25 and seven aromatic protons at δ6.20–7.35. Structures of metabolites II and III were elucidated on the basis of spectroscopic data. Formation of these two metabolites can be explained by the oxidation of the methyl group of suprofen to form hydroxymethyl suprofen, which may be further oxidized to form carboxy suprofen. Metabolites I and II further formed glucuronides. Formation of these metabolites represent minor metabolic pathways. Acyl glucuronidation of suprofen is its major metabolic pathway, resulting in urinary excretion of suprofen acyl glucuronides as ~60% of the dose [8–10].

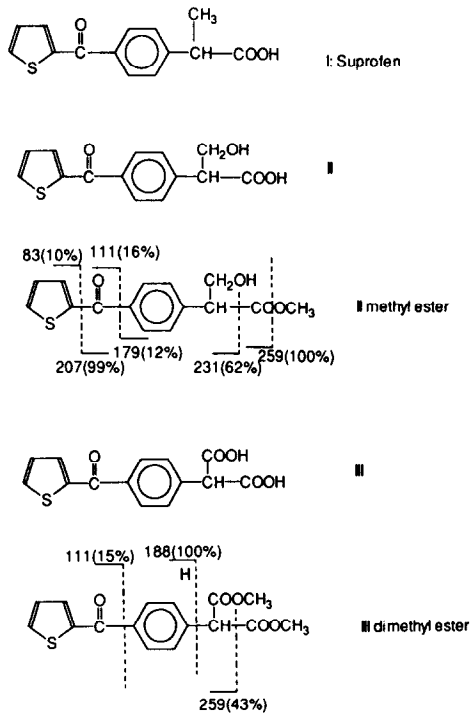

Figure 2
Structures of suprofen, two metabolites and their methyl esters.

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