METABOLISM IN RAT, DOG AND MAN OF DIFENPIRAMIDE, A NEW ANTI-INFLAMMATORY DRUG

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INTRODUCTION

Difenpiramide (α-pyridylbiphenyl acetamide) (1) synthesized in our research laboratory, is a promising non-steroidal anti-inflammatory drug which is well-tolerated and orally effective (LD$_{50}$ = 1966 p.o., LD$_{50} =$ 74000 in dog; ED = 19.9 mg/Kg in rat carrageneen oedema test). Its metabolism in rat, dog, man and rat tissue homogenate has been studied. The identification of the drug and its metabolites (biphenylacetic acid, p-hydroxybiphenylacetic acid and α-aminopyridine) was carried out using thin layer chromatography (TLC) and UV spectroscopy, gas chromatography-mass spectrometry (GC-MS) and high pressure liquid chromatography (HPLC).

MATERIALS AND METHODS

Reagents and standards. All the chemicals used were of analytical reagent grade and were tested for purity in blank runs. Ketodase was obtained from Warner-Chilcott, Morris Plains, N.J., U.S.A. Biphenylacetic acid was synthesized from biphenyl and acetic chloride by a Friedel-Crafts reaction followed by a Willgerodt-Kindler reaction. p-Hydroxybiphenylacetic acid was prepared in a similar way from p-methoxybiphenyl. The amino acid conjugates were obtained by reaction of biphenylacetyl chloride and p-acetoxybiphenylacetyl chloride with the appropriate amino acid.

Animals. Beagle dogs (10-12 Kg) and Wistar rats (200-250 g) bred in our animal house were used. Animals were maintained on a standard diet and fasted overnight before experiments. Difenpiramide suspended in 1% carboxymethylcellulose was administer-
ed orally to dogs (45 mg/Kg) and rats (50 mg/Kg). Human volunteers received a single oral dose of 250 mg in a gelatin capsule.

Isolation of metabolites. After hydrolysis, serum, urine, bile, stomach and intestine samples were adjusted to pH 2 and extracted with organic solvents. The aqueous layer was made alkaline (pH 9) and reextracted to isolate α-aminopyridine.

For the isolation of the amino acid conjugates the sample was hydrolysed (pH 2) at 60°C for 1 hr. The solution was then extracted with methylisobutylketone and the extract purified by TLC. Drug and metabolites were purified by preparative TLC using plates coated with silica gel (Merck F 254; 2 mm). The plates were developed with cyclohexane:acetone:methanol (65:35:4; system "A") or chloroform :methanol:ammonium hydroxide (20:30:2; system "B").

The band with R_f corresponding to those of the standards (Table 1) were taken and eluted with methanol and the solvent was then evaporated under N_2 at room temperature. The residues were then analysed by mass spectrometry using a direct inlet system (DIS). In all cases the mass spectra resulted identical with those of the authentic compounds.

Ultraviolet spectroscopy (UV). Quantitative analyses were carried out with a Beckman Model 25 Spectrophotometer. The E 1% 1cm and the R_f values for the three standards are shown in Table 1.

Gas liquid chromatography. Analyses were carried out with a Hewlett Packard Model 5750 gas chromatograph equipped with a Flame Ionization Detector. For halogenated derivatives a Perkin Elmer Model F 30 gas chromatograph equipped with a ^63Ni electron capture detector was used. The column consisted of a glass tube (90 cm x 6 mm ID) packed with 100-120 mesh Gas-chrom Q (Applied Science Lab., State College, Pennsylvania, U.S.A.) coated with 3% U.C.W.98.

The column was conditioned for 1 hr at 150°C with a N_2 flow