Metabolism of $[^{14}C]$ Felodipine, a New Vasodilating Drug, in Healthy Volunteers

K.-J. Hoffmann and L. Andersson
Hässle Cardiovascular Research Laboratories, Mölndal

Summary

After oral administration of $[^{14}C]$ felodipine (27.5mg) to 4 healthy volunteers, 6 main urinary metabolites were identified by gas chromatography-mass spectrometry. The compounds were isolated by solvent extraction at pH 2.2 and silylated prior to analysis. They were formed by dehydrogenation of felodipine followed by ester hydrolysis, hydroxylation of the alkyl groups and conjugation. These metabolites were excreted both as free acids and as conjugates accounting on average for 37% of the excreted amount (23% of the dose). A specific liquid chromatographic assay with radioactive detection was developed to determine the acidic metabolites in all collected samples. The urinary excretion rate declined biphasically for the mono-acids III and IV, whereas the excretion rates of metabolites VI, VII and VIII, formed via aliphatic hydroxylation, were better fitted to equations of first-order processes.

Felodipine$^1$ (3,5-pyridinedicarboxylic acid, 4-[2,3-dichlorophenyl]-1,4-dihydro-2,6-dimethyl-, ethyl methyl ester) is a calcium antagonist which lowers blood pressure by reducing peripheral resistance by means of a direct, selective action on smooth muscle in arterial resistance vessels (Ljung 1985). Orally administered felodipine is rapidly and completely absorbed from the gastrointestinal tract in humans (Edgar et al. 1985) with a mean bioavailability of about 16%, indicating extensive first-pass metabolism of the drug. Bäärnhielm et al. (1984, 1986a), using liver microsomal preparations from different species, have described dehydrogenation of the 1,4-dihydropyridine system to the corresponding pyridine as the primary metabolic step in vitro. This metabolite is present in human plasma but the calcium antagonistic properties are lost by aromatisation of the dihydropyridine ring (Ljung, unpublished results; Triggle 1982). Between 54% and 65% of a dose is excreted in urine by healthy subjects over a 3-day period (Edgar et al. 1985). Felodipine and the pyridine analogue are not detectable in urine (Weidolf et al. 1984). These results indicate that further biotransformation reactions are required to get sufficiently polar compounds for renal elimination of the dose. The aim of the present investigation was to study the pattern of urinary metabolites, to identify the main metabolites and to develop analytical methods for quantification of known metabolites in urine formed after oral administration of $[^{14}C]$ felodipine to healthy subjects.

$^1$ Felodipine is a product of original research by Hässle/Astra Cardiovascular. Trademarks: Plendil®, Splendil®, Modip®.
**I. Materials and Methods**

1.1 Urine Samples

The urine samples were taken during a pharmacokinetic study in healthy subjects (Edgar et al. 1985), in which the drug substances, formulations and study design are described in detail. The volunteers were administered 27.5mg of $[^{14}C]$ felodipine as an oral solution and all urine was collected for 72 hours at the following intervals: 0, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 hours. The samples were kept frozen until analysis. Urine from 4 of 8 volunteers participating in the study was taken for identification and quantification of metabolites.

1.2 Methods of Analysis

Liquid chromatography (LC) was performed with two Altex model 110 pumps, controlled by an Altex model 421 microprocessor. A Rheodyne model 71-25 injection valve was fitted with a 162µl stainless steel sample loop. The guard column was slurry-packed with spheri-5 RP-18, 5µm (30 × 4.6mm ID), and the analytical column was stainless steel (15cm × 4.5mm id) home-packed with Polygosil 60-5 C$_18$. A Waters Lambda Max model 480 detector was operated at 280nm.

Gas chromatography-mass spectrometry (GC-MS) was performed on a Varian Model 3700 GC and Finnigan MAT Model 44S quadrupole instrument connected to a Finnigan MAT Model SS 200 data system. The fused silica capillary column (CP Sil 8, 30m × 0.32mm, $d_F = 0.15\mu m$) was connected to the mass spectrometer transfer line by an open split interface equipped for venting of the solvent front.

Determination of radioactivity as performed by liquid scintillation counting in a Mark III (Searle Analytical Instruments) spectrometer with reference to an external standard for quench correction.

Pooled urine (0-4h) was taken to study the extraction of radioactive metabolites from urine at pH 2.2 into diethyl ether after both chemical and enzymatic hydrolysis of potential conjugates. Prior to chemical hydrolysis at room temperature or at 80°C for 1 hour, 1ml of urine was mixed with 0.5ml of phosphate buffer pH 2.2 (1 molar), NaOH (1 molar) or HCL (5 molar), respectively. Enzyme hydrolysis was performed at 37°C for 16 hours with β-glucuronidase and arylsulphatase. The experiments were repeated with D-saccharic acid 1,4 lactone (final concentration 15 mmol). Following the reaction time all samples were extracted at pH 2.2 with diethyl ether (5ml). The organic phase (0.2-0.5 ml) and the extracted aqueous phase (0.2-0.5ml) were analysed for radioactivity and the results were compared with the known concentration of radioactive metabolites in untreated urine. Each sample was analysed in duplicate.

1.3 Structural Determination of Metabolites

Metabolites were extracted from urine which had been subjected to enzyme hydrolysis. They were silylated by dissolving the dry residues in N,O-bis (trimethylsilyl) acetamide and N,N-dimethylformamide (1:1 v/v). The samples were heated at 60°C for 1 hour and analysed without further purification by GC-MS. The samples were injected in splitless mode (1 minute, injector temperature 300°C) with the column oven held at 180°C for 1 minute. The oven temperature was then raised linearly to 300°C by 10°C/minute. Electron-impact mass spectra (70eV) were continuously acquired by the data system. Identification of metabolites was based on identical mass spectra, HPLC and GC retention times of metabolites and synthetic references.

1.4 Metabolic Pattern

A reference solution of metabolites III, IV, VI, VII and VIII was prepared by dissolving the synthetic metabolites in a small volume of methanol. Water was added to give a final concentration (6 × 10$^{-6}$ molar) of each compound.

Pooled urine from the 0-4h collection interval (1ml) was mixed with 0.5ml of the reference solution. After addition of synthetic metabolite XI, the sample was acidified to pH 2.2 with 0.5ml of phosphate buffer (1 molar) and extracted with 5ml