Pharmacokinetics of Phenprocoumon in Man Investigated Using a Gas Chromatographic Method of Drug Analysis* **

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Summary. A gas chromatographic method for the determination of phenprocoumon (Marcumar®) in serum and urine is described, which facilitates accurate values down to 0.5 μg phenprocoumon/ml serum. After the i.v. administration of 20 mg phenprocoumon in a single dose to 4 healthy volunteers the following pharmacokinetic data were obtained:

After the initial fast decrease (phase 1) of the serum level of phenprocoumon, probably due to the distribution into the different compartments is followed by a subsequent slower fall (phase 2) which occurs with a serum half-life of 157 h. The apparent distribution volume was 6.5 l. Analysis of the urine demonstrated that 90% of the excreted phenprocoumon detected was in the glucuronide form.

Key words: Phenprocoumon (Marcumar®) — Gas chromatography — Pharmacokinetics.

INTRODUCTION

The coumarin derivative phenprocoumon (Marcumar®) has been commercially available since 1953. In Germany it occupies a leading position amongst anticoagulants, but as for many years no suitable method of drug analysis was available, pharmacokinetic data on the drug were lacking.

In 1968 Seiler and Duckert reported on a fluorometric method of analysis, and were able to determine a serum half life for phenprocoumon of 150 h after a single dose. Fluorometric techniques, nevertheless, show only a limited specificity, and are frequently unsuitable or impracticable for urine studies.

In this paper we describe a gas chromatographic method of drug analysis and present the pharmacokinetic data derived from studies in man.

Substances

The chlorophenprocoumon [3-(ethyl-p-chlorobenzene)-4-hydroxycoumarin, Ro 1-7627] and the tritium labelled phenprocoumon were synthesized and donated by Hoffman-La Roche, Basle, Switzerland. The structural formulae are shown in Figure 1. Using gas chromatography and radio thin layer chromatography both substances appeared as single peaks. The specific activity of the tritium labelled phenprocoumon was 110 μCi/0.1 mg.

METHODS

a) Serum Extraction. 2 ml of serum were mixed with 20 μg of chloro-phenprocoumon dissolved in 40 μl dist. water, containing 1% ethanol, the chlorinated drug acting as an internal standard.

Phenprocoumon

\[ \text{3-\{\text{w-Aethylbenzyi}\}-4-hydroxycoumarin} \]

RO1-7627

\[ \text{3-\{\text{w-Aethyl-p-chlorbenzyi}\}-4-hydroxycoumarin} \]

Fig. 1. Chemical formulae of phenprocoumon and chloro-phenprocoumon
The mixture was acidified with 1 N HCl to pH 1, and extracted twice with 10 ml chloroform. The organic phases were pooled, centrifuged and then passed through a silicon filter. The residue remaining after evaporation to dryness was redissolved in 0.2 ml chloroform, and thin layer chromatography was performed under the following conditions:

Thin layer plates: MN-Polygram Sil G N-HR UV 254 or Polygram Sil G (pre-coated plastic sheets, Machery-Nagel & Co., Düren). Solvent: 80 ml benzene, saturated with 99% formic acid + 4 ml of ethyl-methyl-ketone. Rf-values for phenprocoumon and the internal standard being 0.32.

The bands corresponding to the bands in the reference chromatogram (blue fluorescence with ammonia) were removed and extracted with 10 ml chloroform. The extract was subsequently evaporated at 200 Torr.

b) Urine Extraction. 20–50 ml urine were mixed with 50 μg of chloro-phenprocoumon and acidified to pH 1 with concentrated HCl. The solution was passed over an XAD-2 amberlite adsorption resin (300–1000 g, p.A., Serva GmbH & Co., Heidelberg) which was rinsed with 200 ml distilled water. Phenprocoumon and the internal standard were extracted from the column with 50 ml methanol or after drying with chloroform (infusion speed approx. 1 ml/min).

Following thin layer chromatography of the evaporated extract, the material was processed in the same way as the serum sample. To measure the amounts of phenprocoumon and its glucuronide derivative, the urine was hydrolyzed. The enzymatic hydrolysis was achieved by incubation of the urine for 24 h in a water bath at 37°C with 1000 Fishman-units of Ketodase® (Gödecke AG, Freiburg, FRG)/ml urine at pH 4.5 (1 Fishman-unit = 0.0524 mU glucuronidase) or with a mixture of 0.2 U β-glucuronidase and 0.1 U arylsulfatase/ml (Boehringer Mannheim, Germany). Under both these conditions a complete hydrolysis of phenprocoumon-glucuronide was obtained, as shown by control studies with acid hydrolysis. The acid hydrolysis was performed by adding concentrated HCl to pH 1 and allowing the reaction to proceed at 90°C for 60 min.

c) Gas Chromatography. The evaporated extracts from the serum and/or urine samples were mixed with 100 μl of a combination of BSA (N,O-bis-trimethylsilylacetamide, Serva GmbH & Co.) and TMCS (trimethylchlorosilane, Machery-Nagel & Co.) in a ratio 9:1, in a closed microtube, and incubated for 30 min in a water bath at 70°C. A complete silylation was achieved under these conditions. Usually 1 μl was injected into the gas chromatograph.

d) Requirements for Gas Chromatography. Varian gas chromatograph type 1740. Flame ionisation detector. Glass column 6 ft long, 2 mm diameter. Column filling: 3% OV 1, Gaschrom-Q 100–120 mesh. Injector temperature 250°C, column temperature 235°C, detector temperature 275°C.

Sensitivity 2–32×10⁻¹¹ V. Gas flow: pure nitrogen (carrier gas) 30 ml/min, pure hydrogen 30 ml/min and synthetic air 300 ml/min. The areas of the peaks were calculated according to the formula (height × width at 1/2 height) or by planimetry.

RESULTS

Two well-separated peaks representing phenprocoumon and the internal standard (chloro-phenprocoumon) were obtained using the OV 1 column. Figure 2 shows a typical gas chromatogram. Quantitatively, both substances always show a constant relationship to one another. In Figure 3 it can be seen that the peaks for phenprocoumon and the internal standard show a constant relationship when extracted from urine in various concentrations. By these means a correction for the extraction loss is ensured. The same applies to the extraction of the much smaller amounts found in serum.

The actual amounts extracted from serum and urine could be determined by use of tritium labelled phenprocoumon. The extraction rate after in vitro addition amounted to 64% (serum) and 51.5% (urine), respectively (see Table 1). In some cases more than 75% of the tritium labelled phenprocoumon