Determination of 5,5-Diphenylhydantoin and its Major Metabolites in Biological Specimens by Gas Chromatography and Selected Ion-Monitoring

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Key Words
5,5-Diphenylhydantoin
GC
Metabolites
Selected-ion monitoring

Summary
A specific and sensitive procedure for simultaneous determination of 5,5-diphenylhydantoin, 5-(4-hydroxyphenyl)-5-phenylhydantoin, 5-(3, 4-dihydroxyphenyl)-5-phenylhydantoin and 5-(3-methoxy-4-hydroxyphenyl)-5-phenylhydantoin in biological specimens is described. The method involves formation of butylated or trideuteromethylated derivatives of the drug and its metabolites and analysis by gas chromatography. The lower detectable concentration of these compounds per ml of body fluids or g of wet tissue is either 1 μg or 20 ng, depending on the type of detector used, flame ionization or selected-ion monitoring. Data on the urinary elimination of 5,5-diphenylhydantoin and hydroxylated metabolites in male rats following a single intraperitoneal injection of the drug (25 mg/kg) are also reported.

Introduction
5,5-Diphenylhydantoin (DPH) is a drug widely used for the treatment of epilepsy [1, 2]. The metabolism of DPH has been extensively studied both in laboratory animals and in man. The main pathway of biotransformation involves hydroxylations of the aromatic moiety and formation of catechol and catechol-o-methyl derivatives [3].

So far, DPH and some of its metabolites have been quantitatively evaluated by spectrophotometry, spectrophotofluorimetry, gas chromatography and liquid chromatography [4–10]. These methods lack sensitivity or specificity especially when applied to biological problems. The above limitations have been recently overcome with a mass spectrometric procedure which, however, was only applied to the evaluation of DPH and its p-hydroxy derivative [11]. Interest in the metabolic fate of antiepileptic drugs prompted us to set up a gas chromatographic method with flame ionization detection or selected-ion monitoring for simultaneous determination of DPH and its major metabolites, namely: 5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH), 5-(3, 4-dihydroxyphenyl)-5-phenylhydantoin (DHP), and 5-(3-methoxy-4-hydroxyphenyl)-5-phenylhydantoin (MOPH), in biological specimens. Table I lists these and other abbreviations used to identify the compounds of interests.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Compound</th>
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<tr>
<td>DPH</td>
<td>5,5-Diphenylhydantoin</td>
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<tr>
<td>HPPH</td>
<td>5-(4-hydroxyphenyl)-5-phenylhydantoin</td>
</tr>
<tr>
<td>DHP</td>
<td>5-(3, 4-dihydroxyphenyl)-5-phenylhydantoin</td>
</tr>
<tr>
<td>MOPH</td>
<td>5-(3-methoxy-4-hydroxyphenyl)-5-phenylhydantoin</td>
</tr>
<tr>
<td>MPPH</td>
<td>5-(4-methylphenyl)-5-phenylhydantoin</td>
</tr>
</tbody>
</table>

Material and Methods

Chemicals

DPH, HPPH and 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) were from Aldrich, Beerse, Belgium. DHP and MOPH, for use as reference materials, were extracted with chloroform at pH 4.5 from the urine of DPH treated rats and first purified by preparative thin-layer chromatography on silica gel glass plates developed in a mobile phase of...
chloroform: methanol: acetic acid, 90:10:1. Rf values of DPH and MOPH were 0.37 and 0.56 respectively. Further purification was achieved by high-performance liquid chromatography (HPLC) using a reversed-phase column (25 cm X 4 mm) packed with 10 μm LiChrosorb RP-18 (Merck, Darmstadt, West Germany). The column was eluted with a mixture of acetone and 0.02 M acetate buffer, pH 4.5 (40:60, v/v).

(4-14C)-DPH (specific activity, 54.5 mCi/mol) was from the Radiochemical Centre, Amersham, England. The radiochemical purity of labelled DPH was checked by HPLC as described above. The compound was found to be better than 99 % pure. Trideuteromethyl iodide was from CEA, Gif-sur-Yvette, France; butyl iodide from Merck, and tetrahexylammonium hydrogen sulphate from Håssle, Göteborg, Sweden. Tri-Deuter-8| and n-Butyl-8| were from Pierce, Rockford, Illinois, USA.

All other chemicals and solvents were of the purest grade commercially available.

Animal Experiments

Male rats (200–220 g, body weight) from Charles River Italy (Calco, Como, Italy) were used. Two groups of animals, each comprising five rats, were treated intraperitoneally with a single dose (25 mg/kg in saline) of cold DPH or (4-14C)-DPH. The labelled DPH injected in the animals was diluted with cold DPH to obtain a specific activity of 50 μCi/nmole.

Animals were housed in individual metabolism cages kept in air-conditioned quarters with 12 h light/dark cycles, at a constant room temperature of 22 °C and relative humidity 60 %; they had free access to water and commercial laboratory purine chow (Atromid, Trento, Italy).

Urine samples from both groups were collected in plastic tubes kept on dry ice and then stored at -20 °C until required for analysis.

Extraction Procedure and Derivative Formation

To 0.5 ml urine or plasma or to 0.5 g wet tissue (after homogenization with cold 0.05 M phosphate buffer pH 7.4, 1:4, w/v) either 5 μg or 500 ng MPPH as internal standard were added depending on the expected concentration range and the type of detector used (flame-ionization or selected-ion monitoring).

Hermetically sealable glass tubes bearing a Teflon disk in the inner part of the cap were used and the samples were extracted with 5 ml 0.5 M n-butyl iodide or trideuteromethyl iodide in methylene chloride solution after addition of 100 μl 10 M NaOH and 50 μl 0.1 M tetrahexylammonium hydrogen sulphate solution in 0.1 M NaOH. Extractions were carried out for two hours by shaking at 60 °C in subdued light. The organic phase (4.5 ml) was transferred to conical glass tubes and evaporated to dryness under a nitrogen stream at 35 °C. The residue was dissolved in either n-Butyl-8| or Tri-Deuter-8| solutions and a 1 to 5 aliquot of this solution was injected for either gas chromatography with flame-ionization detection or selected-ion monitoring.

Addition of DPH, HPPH, DHP and MOPH to drug-free urine, plasma and tissue samples at concentrations ranging from nanograms to micrograms per cm³ resulted in the overall recoveries reported in Table II.

Gas Chromatography

Gas chromatography was carried out on a Carlo Erba Fractovap Model G1 instrument equipped with a flame ionization detector. The column was glass, 2 m X 4 mm i.d. packed with 100–120 mesh Gas Chrom Q coated with 3% OV 17 (Applied Science Laboratories, State College, Pennsylvania, USA). All newly prepared columns were conditioned at 280 °C for 1 h without carrier gas flow and the 24 h with a carrier gas flow rate of 30 ml/ min. Air as hydrogen flow rates were adjusted to give maximum detector response. The operating conditions were: column temperature, 280 °C; injection port heater temperature 300 °C; flame-ionization detector temperature, 300 °C.

Mass Spectrometry

Mass spectra were recorded on a LKB instrument model 2091 B, equipped with a Model 2130 computer system for data acquisition, which was used under the following conditions: energy of the ionization beam, 70 eV; ion source temperature, 290 °C; accelerating voltage, 3.5 kV; trap current, 60 μA.

Selected-ion monitoring was carried out at 70 eV by focusing the ion on the ions at m/e 286, 300, 319, 36 and 352, corresponding to the molecular ions in the spectra of the per-trideuteromethylated derivatives of DPH, MPPH, HPPH, MOPH and DHP respectively. The column was described above and the mass chromatographic conditions were: oven temperature, 250 °C; injection port heater temperature, 270 °C; carrier gas (helium) flow rate, 30 ml/min.