Drug-Induced Phospholipidosis

II. Alterations in the Phospholipid Pattern of Organs from Mice, Rats and Guinea-Pigs after Chronic Treatment with Chlorphentermine

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Summary. In three species chronic treatment with the anorectic drug chlorphentermine causes a profound alteration of the phospholipid/lipid metabolism in the organism, resulting in an increase of the fractions of phospholipids and lipids, e.g. in lungs, livers and adrenals. The results are interpreted as drug-induced generalized phospholipidosis, which is caused by amphiphilic drugs, like chlorphentermine and others. Its extent depends on several factors, like content, pattern and turnover rate of phospholipids in different organs, and on the species.

Key words: Chlorphentermine — Lipids — Lung — Liver — Adrenals.

Studying the influence of chronic application of anorectic drugs, like phentermine, amphetamine, phenmetrazine, aminorex and chlorphentermine on the pulmonary pressure we have observed that chlorphentermine caused an extensive infiltration of the rat lung alveoli by foam cells (Franken et al., 1970; Lüllmann-Rauch et al., 1972; Parwaresch et al., 1973). This phenomenon, however, is not causally related to the pulmonary hypertension induced by chlorphentermine (Lüllmann et al., 1972; Mielke et al., 1973).

Preliminary studies revealed a remarkable increase of the phospholipid content of lungs from rats chronically treated with chlorphentermine. Further evidence for the importance of phospholipids in this context is given by ultrastructural studies (Lüllmann et al., 1973b). These studies disclosed the existence of inclusion bodies (myelin figures) in the foam cells with a periodicity of about 50 Å, typical for phospholipids (Stoeckelius, 1962). According to histochemical analysis, the content of these cells consists mainly of phospholipids (positive Baker-reaction, Lüllmann-Rauch et al., 1972).

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Chlorphentermine also induced the formation of inclusion bodies in other organs (e.g. liver, spleen, adrenals etc.) of different species (mouse, rat, guinea-pig, rabbit; Parwaresch et al., 1973). The present paper describes the extraction and thin layer chromatography of phospholipids and lipids from tissues of mice, rats and guinea-pigs after chronic treatment with chlorphentermine.

**Materials and Methods**

Mice (NMRI), rats (Sprague-Dawley, Bäumler, Wolfratshausen) and guinea-pigs (Heine, Bad Bramstedt) were used in these experiments. Food (Legehennen-Alleinkorn) and tap water was given ad libitum.

Rats and guinea-pigs were kept in groups of 5 and 10 animals, respectively, the mice were placed in individual cages. Chlorphentermine was dissolved in the drinking water (Table 1).

At the end of the treatment period (Table 1) the animals were bled from the carotid arteries in light ether anesthesia. The organs (lungs, liver and adrenals) were removed and homogenized in distilled water. The homogenate was centrifuged, and the weight of the lyophilized precipitate was determined. Similar to Gil and Reiss (1973) the lyophilized precipitate was refluxed for 2 hrs with the solvent diethyl ether: ethanol 1:1 (v:v), filtered, and the residue was once more extracted with chloroform. The combined solvents were evaporated in vacuo and dried under nitrogen. The residue was weighed and dissolved (20 mg/ml) in chloroform : methanol 1:1 (v:v). In order to check the influence of this procedure on the lipid composition, a mixture of pure phospholipids was extracted under identical conditions. The method applied did not alter the chromatographic properties of the test mixture. The extracts were analyzed by thin layer chromatography (TLC) according to the method of Walker (1971). In extracts from adrenals the separation of the different lipid classes was achieved using a technique described by Egge et al., 1970. The different spots were identified by comparison with pure phospholipids and lipids (Serva, Heidelberg). The lipids were visualized as dark spots by heating the plates briefly at 200°C. For a semiquantitative determination the chromatograms were analysed by means of a densitometer (Vitatron) and of planimetry. This procedure was calibrated using a lipid mixture of known composition. The single fractions were expressed as percentage of the total lipids. Using these values the amounts of the extracted lipid fractions were calculated.

<table>
<thead>
<tr>
<th>Chlorphentermine (g/l drinking-water)</th>
<th>Duration of treatment (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mice 0.2</td>
<td>6</td>
</tr>
<tr>
<td>rats 0.5</td>
<td>10</td>
</tr>
<tr>
<td>guinea-pigs 0.5</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1. Chlorphentermine concentrations of the drinking-water, duration of treatment, and number of animals. According to the daily consumption of water the dosage was between 50–60 mg chlorphentermine/kg b.w.