Incorporation of chlorpromazine into bilayer liposomes for protection against microsomal metabolism and liver absorption

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SUMMARY

Chlorpromazine (CPZ) was incorporated into bilayer liposomes carrying negative charges either from phosphatidic acid (PA) or from phosphatidyl inositol (PI). CPZ incorporation was dependent on the amount of negatively charged lipid present in the liposomes. At a concentration of 0.6 mol parts phosphatidic acid as referred to egg phosphatidyl choline (EPC) as matrix lipid, 80 μg CPZ/mg EPC were stably incorporated.

At the saturation concentration a 1:1 molar complex between the phenothiazine drug and the negatively charged lipid is formed. This lipophilic complex retains the CPZ molecules firmly within the lipid bilayer.

In vitro release of CPZ into the medium surrounding the liposomes was found to be a very slow process with release half-times of 30 to 99 hours depending on liposome composition. Microsomal metabolism of liposomally incorporated CPZ was slowed and reduced by 50% as determined by the formation of CPZ-sulfoxide. In single pass liver perfusion experiments it was shown that CPZ absorption is significantly reduced when CPZ is incorporated into liposomes. It is suggested that this protective effect of the liposomes might influence the pharmacological effects of CPZ and reduce its hepatotoxic properties.

INTRODUCTION

The chronic use of the neuroleptic drug chlorpromazine (CPZ) causes hepatotoxic effects like liver function impairment and cholestatic jaundice (1, 2). The amphiphilic properties of CPZ are responsible for its high affinity to lipid cell membranes as well as to artificial phospholipid vesicles (3-5). Among other properties CPZ induces changes in erythrocyte shape and membrane permeability (6, 7). Furthermore, chlorpromazine and related compounds are intercalating into DNA causing inhibitory effects to DNA polymerases (8).

The metabolic degradation of CPZ is a complex process yielding six major metabolites and more than 100 secondary compounds, whereby the degradation is not restricted to the liver, but takes place also in small intestinal mucosa and in whole blood (9-11). Liposomes are used as carriers for various drugs mainly with the aim of improving the pharmacokinetic properties or reducing toxic effects of the encapsulated compounds (12, 13). Especially with cytostatic drugs the potential usefulness of liposomes as drug carriers was demonstrated in various studies (14-17). The stable incorporation of drug molecules into the bilayer membrane of liposomes can only be achieved with lipophilic drugs or prodrugs which intercalate between the phospholipid molecules (14).

The high partitioning of CPZ into lipid membranes which is enhanced by formation of strong complexes with negatively charged lipids was used to prepare liposomes saturated with CPZ. In this study the altered behavior of CPZ in liposomes against metabolic degradation and liver absorption was investigated.
MATERIALS AND METHODS

Chemicals

Egg phosphatidyl choline (EPC), phosphatidyl inositol (PI) and phosphatidic acid (PA) were obtained from Lipid Products Ltd., Nutfield, Surrey, UK. Chlorpromazine hydrochloride (CPZ HCl) was obtained from Serva Feinbiochemica, Heidelberg, FRG and purified by recrystallization from ethyl ether. Chlorpromazine-sulfoxide (CPZ-SO) and other metabolites used as reference compounds were obtained from the US Department of Health and Human Services, Public Health Service, Rockville, Maryland, USA. The cofactors nicotineamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P) and glucose-6-phosphate dehydrogenase (G-6-PD) were from Boehringer, Mannheim, FRG. Sodium-cholate and all other analytical grade chemicals used were from Merck AG, Darmstadt, FRG.

Radiolabeled compounds

The labeled compounds ³H-chlorpromazine hydrochloride (³H-CPZ, ring tritiated, 1.15 GBq/mol specific activity) and ¹⁴C-phosphatidylcholine (di-palmitoyl-¹⁴C, ¹⁴C-PC, 1.8 GBq/nmol specific activity) were obtained from New England Nuclear, Boston, USA and used without further purification.

Preparation of liposomes

The liposomes were basically prepared by the detergent dialysis method described elsewhere (18). Routinely, CPZ base which was obtained from CPZ hydrochloride by precipitation with 20% sodium hydroxide and extracted with chloroform, was mixed with varying amounts of PA (1-3 moles referred to CPZ) in a chloroform solution.

Then EPC (0.15 mmol, in methanol/chloroform 1:1 v/v) as well as the detergent Na-cholate dissolved in methanol were added. The ratio of total lipids to detergent was 0.6 moles. The organic solvents were removed under reduced pressure at 40°C. The dried mixture was solubilized by the addition of phosphate buffered saline, pH 7.4 (PBS) giving a final EPC concentration of 13 mg/ml. The micelle solutions were then dialyzed with a Lipoprep® dialysis instrument during 15-20 hours at room temperature.

Where necessary, labeled ³H-CPZ and/or ¹⁴C-PC were added in trace amounts to the organic solvent mixtures.

Size and homogeneity of the resulting liposome preparations were routinely determined by laser light scattering (18).

Determination of CPZ incorporation

To separate free from liposomally incorporated CPZ, aliquots (1.5 ml) of freshly prepared CPZ liposomes (³H-CPZ and ¹⁴C-PC double labeled) were chromatographed on a Sephadex G 25c column (25 x 1.5 cm) with PBS as eluent at a flowrate of 1 ml/min. The elution was monitored with a LKB Uvicord flowdetector at 254 nm wavelength. Collected fractions of the liposomes were pooled in 20 ± 2 ml and the amount of incorporated CPZ determined by liquid scintillation counting in a Searle Mark III scintillation counter.

In vitro release of CPZ

The stability of CPZ incorporation was tested by re-chromatography of the CPZ-liposomes. Aliquots of CPZ-liposomes were chromatographed under conditions as described above at time intervals of one to 14 days.

Unincorporated CPZ was removed immediately after liposome preparation and the liposomes were kept at 4°C between the chromatography runs.

The release kinetics of CPZ from the liposome membranes of preparations containing various amounts of PA (0.2, 0.4 and 0.6 mol parts referred to EPC) were determined by the use of a steady-state dialysis instrument as described before (3). Briefly, the release of CPZ from the liposomes was measured in a flow compartment which was separated by a semipermeable membrane from a closed compartment containing the CPZ-liposomes. Fractions of the dialysate were collected and analyzed for released ³H-CPZ by scintillation counting. Freshly chromatographed liposomes, devoid of free CPZ, were dialyzed during 50 hours at room temperature.

Microsomal metabolism of CPZ and CPZ-liposomes

Purified rat liver microsomes from Sprague-Dawley rats were prepared according to the method of Gorrod et al. (19). The microsome suspensions were stored at −20°C and used within 24 hours.