Interaction of Chlorpromazine with Bile

A. E. Clarke and M. A. Denborough

Chlorpromazine causes precipitation both of the glycoprotein and protein components of bile in vitro. The reaction depends on an electrostatic interaction between the negatively charged carboxyl groups on the bile components and the positively charged amine groups on the drug molecules in solution. The optimum conditions for the interaction between chlorpromazine and bile components have been established, and the suggestion is made that this precipitation may be responsible in part, for cholestatic jaundice associated with the administration of chlorpromazine.

Additional Keyphrases blood-group specific glycoproteins • cholestatic jaundice • sialic acids • phenothiazines

Cholestatic jaundice occurring during treatment with a range of drugs including phenothiazines and steroids is well documented (1, 2) but the mechanism of obstruction to bile excretion is not understood. Cholestasis associated with the administration of phenothiazines is of particular interest in view of the wide use of these drugs as antidepressants and tranquilizers. Phenothiazine derivatives regularly produce subclinical interference with biliary secretion, and about 1.0% of patients taking these drugs develop jaundice that is not dose dependent (1). The cholestasis is rarely fatal; generally it subsides and recovery is complete (2).

The characteristic histological appearance of cholestatic jaundice is of bile plugs in dilated bile canaliculi. This apparent alteration in the physical properties of bile in cholestatic jaundice led us to examine the interaction between components of bile and one of the phenothiazines, chlorpromazine ("Largactil").

From the University of Melbourne Department of Medicine, The Royal Melbourne Hospital, Victoria, 3050, Australia. Received June 15, 1971; accepted June 28, 1971.

Methods

Bile, obtained at operation from a patient (group B secretor) with obstruction of the common bile duct because of carcinoma of the head of the pancreas, was fractionated by density-gradient ultracentrifugation. The bile was brought to an initial density of 1.42 g/ml with cesium chloride and fractionated after sedimentation in a Spinco Model L ultracentrifuge (rotor 50 Ti) at 40,000 rpm for 40 h at 4°C, as described by Creeth and Denborough (3).

Chlorpromazine hydrochloride was a gift of May and Baker Ltd. An aqueous solution (100 mmol/
liter) of this material has a pH of 5.0; on titration with NaOH the solution becomes an oily suspension at pH 6.2. A chlorpromazine solution (0.1 mol/liter) in phosphate buffer (0.1 mol/liter, pH 5.8) was prepared by adjusting an aqueous solution of chlorpromazine to pH 5.8 with NaOH (0.1 mol/liter) and adding an equal volume of phosphate buffer (0.2 mol/liter, pH 5.8).

The interaction of chlorpromazine and bile fractions was followed by measuring the turbidity at 500 nm of a reaction mixture consisting of 200 µl of the appropriate concentration of chlorpromazine in phosphate buffer (0.1 mol/liter, pH 5.8) and 200 µl of the particular bile fraction dialyzed against the same buffer, after incubation at 25°C for 30 min. At 500 nm there was little interference from absorbance of chlorpromazine. For each determination we ran a blank in which the chlorpromazine was replaced with the phosphate buffer and another in which the bile fraction was replaced with the same buffer.

Total carbohydrate was measured by the phenol-H₂SO₄ method (4); total protein was determined by the method of Lowry et al. (5), with crystalline bovine serum albumin as standard.

Results and Discussion

Fractionation of the bile in a CsCl density gradient is shown in Figure 1. The dense carbohydrate-rich glycoprotein fractions were well separated from the protein fractions of lower density. The fractionation pattern of the carbohydrate-containing material closely followed the blood group substance activity, indicating that blood group substances form a significant component of the total glycoprotein secretion in bile. Cholesterol and bile pigments were concentrated in the two top fractions. Fractions 10 and 3 were taken as representative of the glycoprotein and protein components, respectively.

The interaction of bile fractions 10 and 3 with various concentrations of chlorpromazine is shown in Figure 2. The final glycoprotein concentration was 100 µg of carbohydrate (as galactose) per milliliter; the final protein concentration was 100 µg of protein per milliliter.

The interaction between the glycoprotein and chlorpromazine increased with increasing chlorpromazine concentrations up to 30 mmol/liter; further increase in the chlorpromazine concentration did not appreciably alter the turbidity of the reaction mixture. However, the interaction between the protein fraction and chlorpromazine increased to a maximum at 10 mmol of chlorpromazine per liter and then decreased to 14% of the maximum turbidity at 50 mmol of chlorpromazine per liter. A linear relationship was established between both protein and glycoprotein at concentrations up to 500 µg/ml and turbidity, at chlorpromazine concentrations of 5 and 30 mmol/liter, respectively.

The possibility that negatively charged carboxyl groups of the terminal sialic acid residues on the glycoprotein are involved in the interaction of chlorpromazine with glycoprotein was considered and investigated by splitting off the sialic acid and measuring the interaction of the modified glycoprotein with chlorpromazine.

The sialic acid was removed both by acid hydrolysis and by the action of neuraminidase. After mild acid hydrolysis (1 h at 80°C in H₂SO₄, 1 mol/liter) of glycoprotein (2 mg/ml), the only free reducing sugars detected chromatographically in the hydrolysate were sialic acid and traces of fucose. Incubation of glycoprotein (1.8 mg/ml)

\[ \text{Fig. 1. Fractionation of bile from a group B secretor after centrifuging to equilibrium in a CsCl density gradient} \]

The initial density was 1.42 g/ml. Sedimentation was for 40 h at 40,000 rpm in rotor 50 Ti of a Beckman Model L ultracentrifuge. (a) Separation of protein (\(-\bullet\-\)) and carbohydrate (\(-\circ\-\)); (b) Blood group substance activity: H score \(-\bigtriangledown\-\); B score \(-\bigtriangleup\-\).
The interaction was followed by measuring the turbidity at 500 nm of a reaction mixture consisting of 200 µl of the appropriate concentration of chlorpromazine in phosphate buffer (0.1M, pH 5.8) and 200 µl of the particular bile fraction dialyzed against phosphate buffer (0.1M, pH 5.8), after incubation at 25°C for 30 min. —○—○—, protein (fraction 3) at a final concentration of 100 µg protein/ml; —△—△—, glycoprotein (fraction 3) at a final concentration of 100 µg (as galactose)/ml.

with neuraminidase (Behringwerke, 50 units/ml) in sodium acetate (0.1 mol/liter, pH 5.6) and CaCl₂ (1 mmol/liter) at 37°C for 20 h released sialic acid only. Further addition of enzyme and incubation did not increase the amount of sialic acid released, as estimated by the Warren (θ) method. In each case the free sugars were removed by dialysis and the extent of interaction of the modified glycoprotein with chlorpromazine was measured. The results are shown in Figure 3. After acid hydrolysis the interaction with chlorpromazine was completely abolished and after neuraminidase treatment it was decreased to 20% of the interaction with the untreated bile glycoprotein.

The total amount of sialic acid released by neuraminidase was less than that released on acid hydrolysis, probably because of the inaccessibility of some of the sialic acid residues to the enzyme.

Thus, the presence of sialic acid on the glycoprotein is apparently essential to the interaction with chlorpromazine, which probably depends initially on a loose ionic interaction between the negatively charged carboxyl groups of the sialic acid and the positively charged drug molecules in solution. This type of interaction would account for the shape of the curve shown in Figure 2: interaction and precipitation increase until there has been an effective neutralization of all the sialic acid residues on the glycoprotein molecule, after which further increase in chlorpromazine concentration does not alter the solubility properties of the chlorpromazine–glycoprotein complex.

A similar interaction between phenothiazines in solution and the sialic acid residues on the red blood cell membrane has been proposed to account for the inhibition of red cell agglutination in the ABO blood group system by the presence of these drugs (7).

The form of interaction of the bile protein fraction with increasing concentrations of chlorpromazine shown in Figure 2 can be interpreted on a similar basis.

Electrophoresis of this fraction at pH 8.6 showed bands corresponding to the albumin, α₁, α₂, β, and γ-globulins of normal serum, of which albumin was the major component. At pH 5.8, all the components except the band corresponding to γ-globulin moved to the anode—most proteins of this fraction bore a net negative charge. At low chlorpromazine concentrations there would be an interaction between accessible negatively charged groups on the proteins and the positively charged drug in solution. At the chlorpromazine concentration at which the protein is electrically neutralized by the chlorpromazine, there would be a minimal mutual
repulsion between adjacent molecules, which could allow a clumping together of the protein molecules, possibly mediated by binding between the nonpolar side chains of the protein and the aromatic portion of the chlorpromazine. At higher chlorpromazine concentrations all the accessible negative charges on the protein would be neutralized by the drug so that the chlorpromazine-protein complex would have a net positive charge and, as a result, an increased solubility.

Experimental evidence in support of this type of interaction was obtained by specific modification of the carboxyl groups of the protein with carboxdiimide and glycinemethylester, as described by Hoare and Koshland (8), followed by investigation of the interaction of the product with chlorpromazine.

The reaction mixture contained protein (10 mg) in pyridine-pyridinium buffer (pH 4.75, 0.1 mol/liter, 0.75 ml), glycinemethylester HCl (125.5 mg) in pyridine-pyridinium buffer (pH 4.75, 0.1 ml) and 1-cyclohexyl-3-(2-morpholinoethyl)-carboxdiimide metho-p-toluene sulfonate (43.5 mg) in 0.15 ml of the same buffer. The reaction was allowed to proceed for 2 h at 25°C, then acetate buffer (pH 5.6, 1 mol/liter, 5 ml) was added to the final reaction mixture and dialyzed exhaustively against phosphate buffer (pH 5.8, 0.1 mol/liter).

We determined the extent of reaction of the modified protein with various concentrations of chlorpromazine and of a control treated the same way except for omission of the diimide and the glycinemethylester. The control material gave the same pattern of interaction as the untreated protein (Figure 2), while the interaction with the modified material was abolished. This indicates that free carboxyl groups on the protein are required for the precipitation reaction with chlorpromazine.

The precipitates formed between chlorpromazine and bile proteins, and chlorpromazine and bile glycoproteins, under the optimum conditions for precipitation shown in Figure 2, were collected by centrifuging for 1 h at 37,000 g. The precipitates were suspended in phosphate buffer (pH 5.8, 0.1 mol/liter) and dialyzed exhaustively against the same buffer. The precipitate from the glycoprotein (fraction 10) contained 62% of the total carbohydrate and 60% of the blood-group substance activity. On dialysis the precipitate redissolved with an 85% recovery of the blood-group substance activity. However, although a similar proportion of the protein in bile fraction 3 was precipitated (62%), it did not redissolve when the chlorpromazine was removed by dialysis. Apparently the tertiary structure of at least part of the proteins involved in the interaction with chlorpromazine are irreversibly altered during complex formation.

Possibly a precipitation of the bile protein and glycoprotein components, as has been demonstrated in vitro, could occur in vivo and form the bile plugs that are histologically demonstrable in the bile canaliculi of patients suffering from chlorpromazine jaundice. The site of the initial blockage is unknown, but is probably postmicrosomal, as these patients excrete normally conjugated bilirubin (2).

This suggests that the drug first comes into contact with the glycoprotein and protein manufactured by the liver cell after it has been metabolized by the microsomal enzymes. The known metabolic pathways of chlorpromazine include aromatic hydroxylation, demethylation, sulfoxidation and N-oxidation, followed by conjugation with glucuronic acid (9, 10). The ability of the side-chain nitrogen to form salts is retained after all these reactions (10, 11), and so an interaction between the modified drug and the biliary components would be similar to the interaction demonstrated in vitro with the drug itself.

Wherever the biliary components and the metabolized drug make contact—whether within the cell, at the cell surface, or in the bile canaliculi—the extent of the reaction, and consequently the severity of the jaundice, will depend on many variables, such as the effective concentration of the reactants, which in turn depends on the rates of transport and metabolism of the drug and the rates of biosynthesis of bile components. However, the administration of the usual doses of chlorpromazine (50 mg, three times a day) would be expected to give concentrations in the bile of the order required for a precipitation reaction to occur with the biliary components.

References