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EFFECT OF CHLORPROMAZINE ON THE FUNCTION OF THE PERFUSED ISOLATED LIVER*

JEAN KENDLER, SUBHASH BOWRY, LEONARD B. SEEFF and HYMAN J. ZIMMERMAN

Medical Service and Liver and Metabolic Research Laboratory,
Veterans Administration Hospital, Boston, Mass. 02130 and
Department of Medicine and Biochemistry, Boston University School of Medicine,
Boston, Mass., U.S.A.

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Abstract—The acute effect of chlorpromazine on the function of the perfused rat liver was evaluated by monitoring the removal of sulfobromophthalein (BSP) from the perfusate, the biliary excretion of the dye and the rate of bile and perfusate flow.

The higher drug concentrations used, $2{\cdot}5 \times 10^{-4}$, 5×10^{-4} and 10^{-3} M/l. of perfusate, led to a decreased rate of removal of BSP from the perfusate and of biliary excretion of the dye, accompanied by a significant reduction of perfusate flow and bile production. These changes were porportional to the concentration of the drug. The lowest dose used, 10^{-4} M/l., resulted in similar but more transient effects.

In accord with other related studies, it is suggested that chlorpromazine may have an intrinsic toxic effect on the liver.

JAUNDICE induced by chlorpromazine (CPZ) has been considered to result from hypersensitivity to drug.[1–4] Despite the strong circumstantial evidence which supports this view, there are clinical and experimental observations that suggest that intrinsic hepatotoxicity of the drug may contribute to the hepatic injury.[5,6] Almost 50% of patients taking CPZ for long periods develop hepatic dysfunction.[4] Studies *in vitro* have demonstrated that CPZ has an adverse effect on liver slices and isolated Chang liver cells.[7,8] The relevance of an adverse effect of the drug on isolated cells or slices to hepatotoxicity *in vivo* is uncertain, particularly in view of the cholestatic nature of CPZ-induced jaundice.

Studies in intact animals have yielded inconsistent and inconclusive evidence of hepatic injury from the agent.[9–11] Accordingly, the present experiments utilizing the perfused liver *ex vivo* were undertaken in an effort to examine the possibility that CPZ might have a cholestatic effect in an experimental system. Such an effect has been demonstrated in this model, for other drugs.[12,13]

MATERIALS AND METHODS

Female rats (Sprague–Dawley, 250–350 g) were kept under standard dietary and maintenance conditions and used as donors of livers. The animals were not fasted prior to experimental procedures.

Livers were perfused following a modified method of Penhos *et al.*[14] Canulations were performed under pentobarbital sodium (Nembutal, Abbot) anesthesia (50 mg/kg

* Reprint requests: Dr. Hyman J. Zimmerman, Chief, Medical Service, Veterans Administration Hospital, Boston, Mass. 02130.

body weight) using a PE-10 tube (i.d. = 0·010 in., o.d. = 0·024 in.) for the common bile duct and a PE-205 (i.d. = 0·062 in., o.d. = 0·082 in., Intramedic, Clay–Adams) for the portal vein. During hepatectomy livers were perfused with oxygenated, heparinized buffer, then transferred to the perfusion chamber (Metaloglass, Boston).

The mean wet weight of the livers were 10·7 ± (S.E.) 0·6 g.

The perfusion chamber was maintained thermostatically at 38° ± 0·5° during the experiment. The flow of the circulating medium was measured through a calibrated bypass. The hydrostatic pressure at portal vein level was maintained at 17 cm water. Oxygenation of the perfusing medium was manometrically controlled by a constant infusion of O_2:CO_2 (95:5 vol. %) gas mixture and continual mixing provided with a magnetic stirrer.

The perfusion medium was wholly synthetic and consisted of Krebs–Henseleit buffer, pH = 7·4, which was checked before each experiment. It contained per 100 ml 2·5 g bovine albumin (35% solution, Pentex Biochemicals), 240 mg of glucose and 3000 USP units of heparin (Liquaemin, Organon). Perfusion volume was maintained at 75–80 ml.

Chlorpromazine was supplied by Smith, Kline, & French Laboratories and added to the perfusion medium after solution in normal saline. For each concentration of CPZ a separate fresh solution was made and 1·0 ml of CPZ/100 ml medium was invariably added after 30 min of perfusion. Sulfobromophthalein (BSP)* was added 1 hr after perfusion was started in a concentration of 10 mg/100 ml of perfusate and the perfusion continued for an additional 45 min. Eleven samples of perfusate (0·2 ml) were obtained at intervals for the determination of the BSP removal rate by the liver. The rate of bile flow was determined by timing collections of bile into 20 μl pipettes.

The BSP concentration in perfusate and bile was measured by colorimetry at 575 nm on a Bausch Spectronic 20. Adequate saline dilutions of perfusate samples (1:50) and bile samples (1:500–5000) were made prior to alkalinization and standards of BSP were simultaneously run in all experiments.

Analysis of variance and Student's *t*-test of statistical significance were carried out as outlined by Snedecor.[15]

A total of 26 perfusions is reported here.

RESULTS

BSP clearance and excretion. Chlorpromazine in concentrations ranging from $2·5 \times 10^{-4}$ to 10^{-3} M significantly decreased the rate of the dye clearance from the perfusate; with the two higher doses ($5·0 \times 10^{-4}$ and 10^{-3} M) the decrease was noted as early as 10 min after it was given (Fig. 1). With a lower concentration of the drug ($2·5 \times 10^{-4}$ M) interference with the rate of dye removal was delayed. The concentration of 10^{-4} M yielded clearance values that to a variable degree were lower but not significantly separated from the control values.

Analysis of the slopes of removal of BSP (Table 1) indicated that the chief effect of CPZ in a concentration of $2·5 \times 10^{-4}$ M was on the early phase (K_1), although at higher concentrations ($5·0 \times 10^{-4}$ M, 10^{-3} M) the effect was on both phases.

Concentration of BSP in bile also was significantly decreased by concentrations of CPZ greater than 10^{-4} M (Fig. 2). Indeed, the degree of decrease in BSP concen-

* Supplied by Hynson, Westcott & Dunning, Inc.

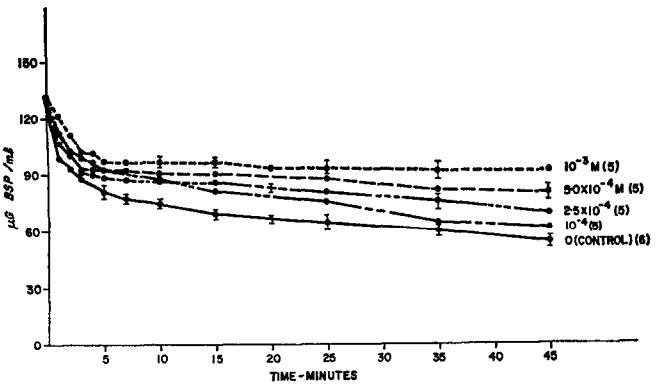

FIG. 1. BSP disappearance from the perfusate in control and CPZ-treated preparations Bars shown are S.E.M. at each point of the curve. Number of experiments is shown in brackets.

tration in bile seemed relatively greater than that of the disappearance from perfusate. In the control and the low-dose (10^{-4} M) CPZ-treated preparations, BSP was excreted in progressively increasing concentrations in the bile, from 0·4 to 0·5 mg/ml at 5 min after being given to a concentration of 6·2 to 6·3 mg/ml at the end of the perfusion. After addition of $2{\cdot}5 \times 10^{-4}$ M of CPZ, there was a significant decrease ($P < 0{\cdot}01$) in the rate of biliary BSP excretion, with concentrations that ranged from 0·3 mg/ml at 5 min to 3·5 mg/ml at the final point tested.

Treatment with $5{\cdot}0 \times 10^{-4}$ M CPZ led to concentrations in the bile below 0·7 mg/ml. No BSP at all could be detected in the scanty bile of preparations perfused with a concentration of 10^{-3} M of CPZ.

TABLE 1. EFFECT OF CHLORPROMAZINE (CPZ) ON BSP REMOVAL SLOPES FROM THE PERFUSATE

CPZ treatment (moles/l.)	N	K_1*	K_2
Controls	6	9·6 ± 0·8	3·1 ± 0·6
10^{-4}	5	8·6 ± 1·8	3·0 ± 1·0
$2{\cdot}5 \times 10^{-4}$	5	5·8 ± 1·3†	2·8 ± 1·3
$5{\cdot}0 \times 10^{-4}$	5	4·8 ± 2·0†	1·4 ± 0·3†
10^{-3}	5	4·9 ± 1·0‡	0·4 ± 0·1‡

* $K \times 10^3$ expressed in μ grams per milliliter per minute ± S.E.M. is calculated at 15 min (K_1) and 45 min (K_2) after BSP administration, by the formula:

$$K = \frac{\log c_a - \log c_b}{t_b - t_a}$$

where c = BSP concentration in perfusate; (μg/ml) at intervals a and b of time (t) in minutes.

† Significantly different from control ($P < 0{\cdot}05$).

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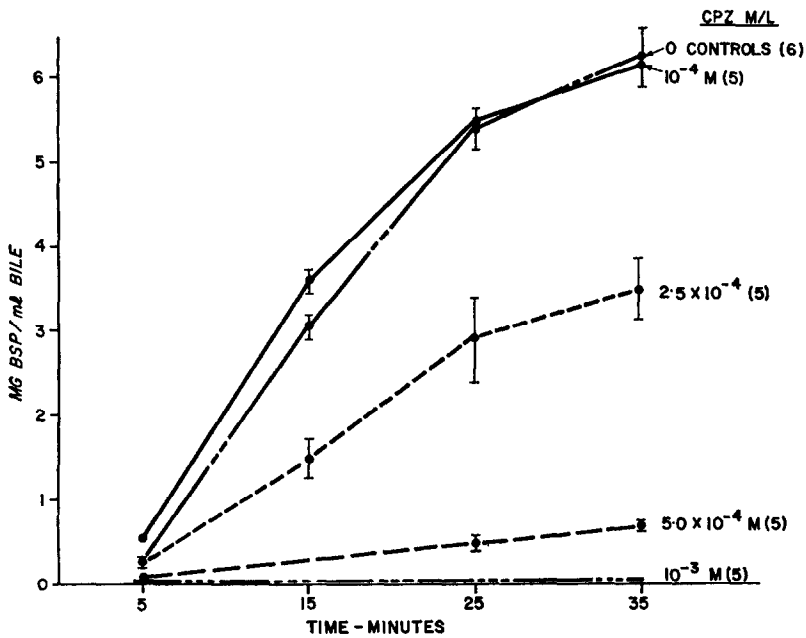

FIG. 2. Biliary concentration of BSP. Number of experiments is shown in brackets.

Bile excretion. The rate of bile flow ranged, in all experiments, from 7·7 to 9·1 μl/min during the first 30 min of equilibration (Fig. 3). The addition of CPZ in all concentrations used induced within the first minute a highly significant ($P < 0{\cdot}005$) and dose-related depression of the rate of bile excretion. With increasing concentrations of CPZ there were proportional rates of diminution of bile flow which ranged

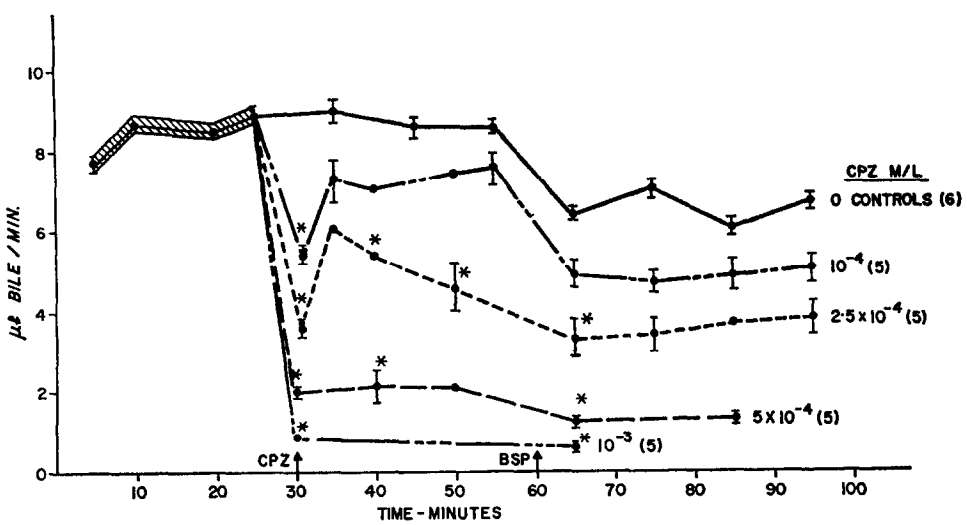

FIG. 3. Rate of bile flow excreted by perfused livers. Number of experiments is shown in brackets. *Significantly different from controls ($P \leq 0{\cdot}05$–$0{\cdot}001$). Number of experiments is shown brackets.

from 60 per cent for the lowest CPZ dose, down to 9 per cent of the initial control values for the highest CPZ concentration. Within 5 min after the initial drop in bile excretion, some restoration of the flow was observed in the livers treated with the lower concentration of CPZ. Livers that were perfused with only a 10^{-4} M concentration of CPZ showed a subsequent bile flow that was not significantly different from controls, while greater concentrations led to persistently subnormal flow. The administration of BSP resulted, as has previously been noted,[16] in a decrease of bile flow for all groups. Indeed, the livers having the lowest rate of bile excretion (after treatment with 10^{-3} M or $5{\cdot}0 \times 10^{-4}$ M of CPZ) stopped excreting bile altogether when the BSP was introduced.

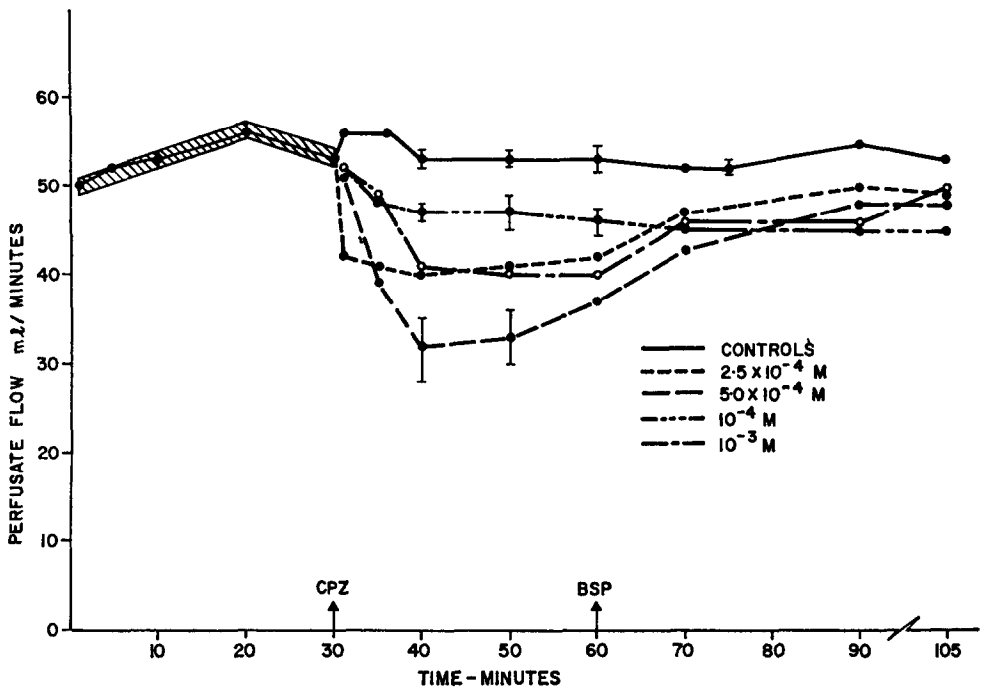

FIG. 4. Effect of CPZ on the rate of perfusate flow. Bars shown are S.E.M. at each point of the curves.

Rate of flow of perfusate. The rate of flow of the circulating perfusion medium ranged from 50 to 55 ml/min in all preparations during the equilibration period. CPZ concentrations of $2{\cdot}5 \times 10^{-4}$, $5{\cdot}0 \times 10^{-4}$ and 10^{-3} M caused significant reductions ($P < 0{\cdot}05$) in the flow of perfusion for the first 20 min after treatment. These changes were subsequently reversed spontaneously, and no dose-relationship of CPZ concentrations to this parameter could be noted (Fig. 4).

DISCUSSION

The results of this study resemble those reported by Eckhardt and Plaa[17] in that CPZ was found to interfere significantly with hepatic circulation and excretory function of the perfused rat liver *ex vivo*. The biliary excretion of BSP and bile flow

were markedly reduced in the CPZ-treated livers as has been reported by others.[9,10,18] The hepatic perfusion flow was depressed temporarily after the addition of CPZ, reflecting a presumed increase in parenchymal resistance to flow.[19,20] Similar data *in vivo* have been reported for mice[10] thus indicating that the isolated rat liver, perfused acutely with CPZ, reacts analogously in this respect to the liver of an intact animal. Eckhardt and Plaa[17] attributed the impairment of excretory function induced by CPZ to the interference with hepatic circulation. While altered perfusate flow induced by CPZ may contribute to impaired excretory ability, the results of the present study do not support the view that the altered flow is presumably responsible for the abnormal excretory function. The decrease in excretory function was related to the concentration of CPZ, while the decrease in perfusate flow was not. Moreover, the effect of CPZ on the rate of bile flow in all experiments preceded the alteration in the flow of hepatic perfusate and persisted after perfusion flow rates had returned toward control levels. These observations are consistent with the reports that the production of bile appears to be largely independent of the hydrostatic perfusion pressure[21] and suggest that the interference by CPZ with bile production and dye clearance does not depend solely on the alteration of hepatic circulation.

The possibility that the model *in vitro* employed may not be relevant to adverse effects of drugs in the whole organism warrants consideration. It has been suggested that the levels of CPZ required to interfere with hepatic function *in vitro* are "far removed" of those observed *in vivo*.[17] Concentrations of $2{\cdot}5 \times 10^{-4}$ M or greater in the present study were found to interfere with excretory function. These concentrations are similar to those that can be achieved in hepatic parenchyma of patients taking CPZ. Levels of 10^{-5} M are found in the blood of patients taking therapeutic doses.[22] Since the hepatic concentration of the drug may be 10-fold that found in the blood[23] concentrations of CPZ used in the present studies *in vitro* seem relevant.

It also is possible that the CPZ effect may be un-specific and one shared by many compounds not necessarily able to produce hepatic injury *in vivo*. However, other agents appear to have different effects in this model. An anabolic steroid (Norbolethone) has been found to interfere with the excretory function of the perfused liver at a concentration one-tenth that of the least effective CPZ concentration.[13] In studies of the effects of erythromycin and its derivatives, and of several phenothiazine derivatives, we have found characteristic and reproducible differences between the effects of various compounds suggesting that the effect *in vitro* has biological significance.

There is controversial evidence regarding the hepatic response to CPZ in intact animals and man. Prolonged administration of the drug to rats has been reported to induce no hepatic injury or jaundice,[24] although some authors have observed fatty metamorphosis to result.[25] The low incidence of jaundice in man after CPZ treatment clearly indicates individual idiosyncratic response.[4] While this idiosyncrasy has been presumed to reflect hypersensitivity to the drug, the high incidence of hepatic dysfunction in patients taking the drug for prolonged periods suggests some degree of intrinsic toxicity of the agent. Furthermore, studies *in vitro* with CPZ indicate possible inhibitory effects at the cellular level. These include depressed activities of some enzymes,[26–28] altered permeability of cell membranes,[29,30] haemolysis of erythrocytes,[31,32] and inhibition of motility of protozoa.[33] Our data suggest that in the isolated rat liver, CPZ is capable, in the given doses, of interfering with the primary excretory function of the liver perhaps by inducing such changes in the hepatic

parenchymal membranes. They also support the hypothesis that cholestatic jaundice, while occurring as an idiosyncratic reaction, may reflect this adverse effect in the hepatocyte, translated by hypersensitivity into frank jaundice.

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