



Effects of the cimetidine on the biliary excretion of rosebengal in rats

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Abstract

Effect of the cimetidine on the biliary excretion of Rosebengal (RBG) was determined in rat after treatment with two different doses of cimetidine 80 mg/kg and/or 10mg/kg in aqueous solution administered intraperitoneally. Rosebengal (4mg/kg) was administered i.p. After treatment the excretion pattern of the effect of cimetidine on bile flow, plasma and liver were studied. The study showed that cimetidine at 80mg/kg increased the plasma concentration of RBG excreted up to 45min after administration while bile flow continued to increase even after 120 mini of treatment. At both concentrations of cimetidine there was a tendency in the direction of an increase in bile flow, plasma, liver weight and RBG liver concentrations, while there was decrease in RBG biliary excretion and RBG bile concentration. The excretion of RBG from hepatic cells in bile suggests that cimetidine has negative effect on the liver cells.

Key words: Cimetidine, biliary excretion, bile flow, rosebengal

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INTRODUCTION

Metabolism of environmental contaminants occurs in multiple organs and the site of metabolism is an important determinant of a compound's toxicity. Cimetidine is a drug used in the treatment of peptic ulcer and as a prophylactic in gastric hyperactivity in man^{1,2}. The metabolism of cimetidine occurs by sulphoxidation, which can be induced by phenobarbitone³. Drug interactions with cimetidine found to be associated with a number of adverse reactions have been reported in patients undergoing therapy with cimetidine co-administered with other medications⁴. Cimetidine is an H₂ Receptor affects cytochrome P.450 of the microsomes of hepatocytes^{5,6}.

Studies on the effect of cimetidine after a single dose showed a clear relationship between blood concentration and extent of inhibition of basal gastric acid⁷. When cimetidine was co-administered with other drugs clinically significant drug-drug interactions were observed and reported^{8,9}. The excretion rate of cimetidine may invariably determine the duration of action. In view of the occurrence of drug-related adverse reactions and their possible relationship with differential metabolism and accumulation of derivatives, the present study is designed to investigate the effect of cimetidine on hepatobiliary function in rat. Biliary excretion of rosebengal, a cholephilic organic anion was used as an index for liver functions. The dye was chosen because it is completely and rapidly excreted into the bile by an active process¹⁰ and to study its effect on liver function.

MATERIAL AND METHODS

Cimetidine [N'-cyano-N-methyl N'-[2]- (5-methylimidazol 4-yl) methyl-thio-ethyl-guanidine)] was obtained from pharmacology Department, faculty of pharmacy, Obafemi Awolowo University, Ile-Ife. Rosebengal (RBG) was obtained from British Drug House.

Animals and Treatment

Twenty male Wister albino rats weighing between 200-300g were used throughout. Two doses of cimetidine (80mg/kg

and or 10 mg/kg) in aqueous solution were administered i.p. The control received an equivalent of the aqueous solution. Following the administration of cimetidine, freshly prepared RBG 4 mg/kg in 0.9% saline solution was administered into the femoral vein (Hynson, Westcott and Dunning Inc.). The saline solutions were made up to deliver the proper dosage in a final volume of 8ml/kg.

Biliary Flow

The rats were anesthetized with Phenobarbital sodium (50mg/kg i.p) supplemental doses being administered as needed. The common bile duct was surgically exposed by an abdominal incision (25mm) and cannulated with a sterile 25 gauge (5 x 8) hypodermic needle shaft attached to 20mm pieces of PE-10 tubing. The rectal temperature of the anesthetized rat was maintained at 37°C with a heat lamp to prevent hypothermic alteration in biliary flow¹¹. Four bile samples of 15min duration each, were collected and the volume measured by weighing¹². At the end of the experiment, blood was withdrawn by cardiac puncture and the liver was removed, blotted free of excess blood and weighed. The RBG concentrations in bile, plasma and liver were determined by measuring its absorption with Cecil spectrophotometer (CE 37.3) at 545nm. The change in RBG concentrations ratio of plasma-to-liver, bile-to-plasma and bile-to-liver were calculated¹³. The results were expressed as means \pm SE. the significance was assessed by student's t-test.

RESULTS

Table 1 shows the effect of cimetidine on volume and the rate of bile flow. The concentrations of RBG in the volume of bile per 100g of animal were observed to correlate with the rate of which cimetidine influenced bile flow.

A single intraperitoneal injection of cimetidine in aqueous solution given to Wistar rats resulted in marked increase ($P < 0.05$) in RBG plasma concentration with a maximal response at 45min (Fig. 1a). However the bile flow continued to increase through the 120min (Fig 1b).

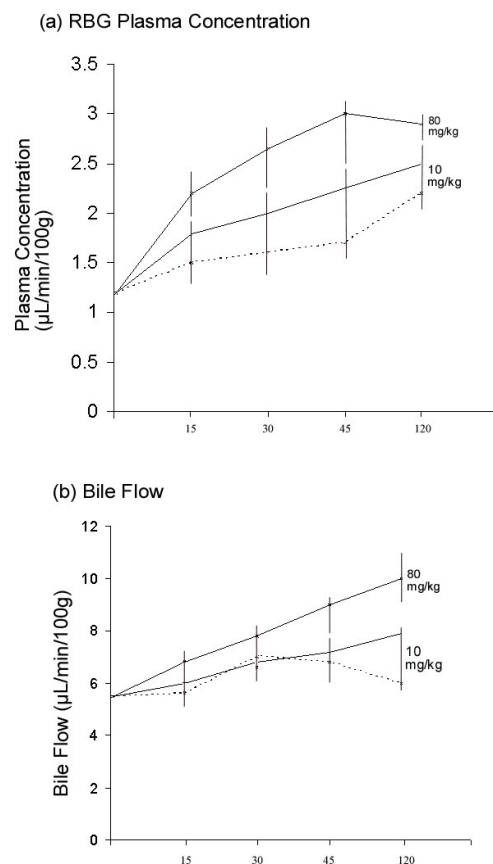


Fig 1. EFFECT OF CIMETIDINE ON PLASMA CONCENTRATION OF RBG

Table 1:Effect of cimetidine on bile flow

Time (min)	Volume of bile collected per 100g of animals' (ml)		Rate of bile flow ml/Min	
	Test	Control	Test	Control
0	0.082 ±0.05	0.133 ±0.03	5.67 x10 ⁻³	5.9 x10 ⁻³
15	0.095 ±0.12	0.118 ±0.05	6.33 x10 ⁻³	6.0 x10 ⁻³
30	0.119 ±0.03	0.110 ±0.04	7.93 x10 ⁻³	6.2 x10 ⁻³
45	0.110 ±0.10	0.101 ±0.05	7.33 x10 ⁻³	6.0 x10 ⁻³
120	0.105 ±0.11	0.095 ±0.01	7.00 x10 ⁻³	6.3 x10 ⁻³

Temporal accumulation of hepatic bile and the rate of flow after cimetidine administration. The mean ± S. E of the volume of the bile of five animals

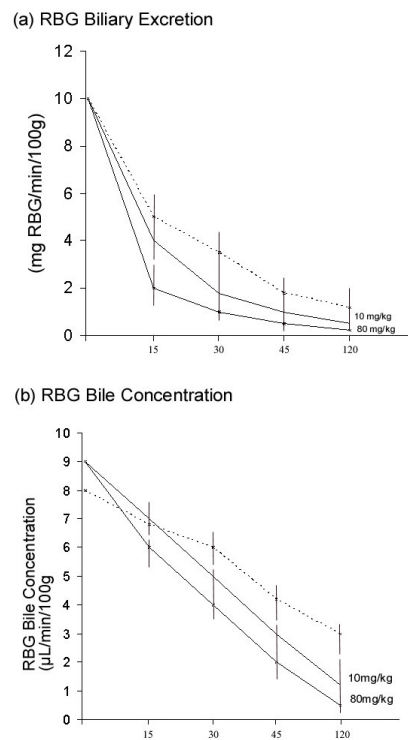
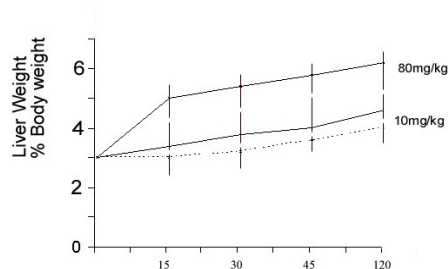


Fig 2. EFFECT OF CIMETIDINE ON BILIARY EXCRETION OF RBG

(a) Liver Weight



(b) RBG Liver Concentration

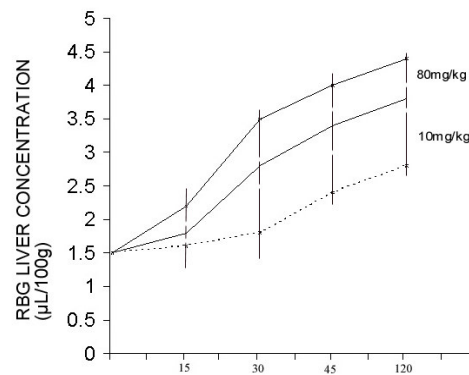


Fig 3. EFFECT OF CIMETIDINE ON LIVER WEIGHT AFTER TREATMENT

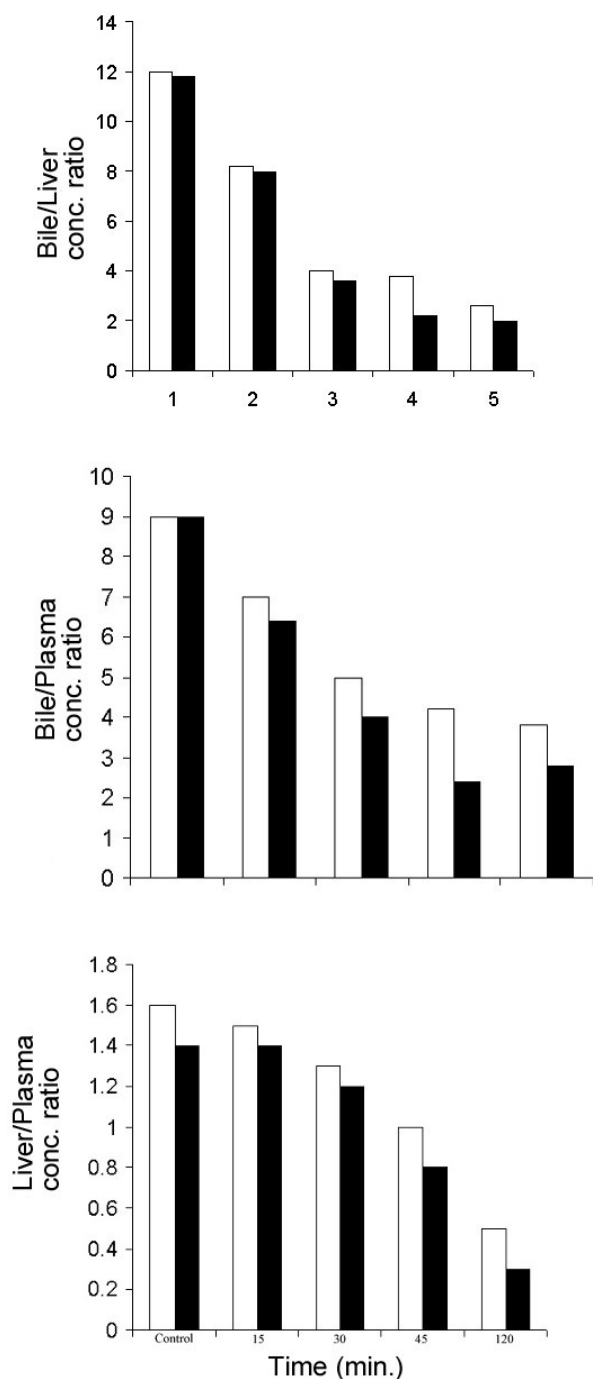


Fig 4. Change in RBG concrete ratio after vivetidine treatment

The rats receiving 80mg/kg cimetidine had higher flow rates than those receiving 10mg/kg cimetidine. However, treatment of the animals with the same concentrations of cimetidine decreased the RBG biliary excretion (Fig. 2a) and RBG bile concentration (Fig. 2b).

The decrease continued throughout the 120min experimental time limit.

Cimetidine caused marked increase in liver weight through not significant as shown in Figure 3a. The increase concentration in RBG in the liver as expressed in microlitre per 100g-body weight was also dose related since the higher dose caused a greater weight increase.

Bile-to-liver (a) bile-to-plasma (b) Liver-to-plasma (c) concentration ratios of RBG were separately calculated and compared as shown in Figure 4. The change in RBG concentration ratios after cimetidine treatment decreased gradually and continued to decrease after 120min. The liver accumulated less of RBG after cimetidine treatment. Liver to plasma RBG concentration ratios decreased in animals treated with 80mg/kg and very rapidly after 30min for those treated with 10 mg/kg (Fig 4c). The control animals are observed to retain RBC specially the 80mg/kg dose gave a much greater retention than 10mg/kg. All the ratios were decreased at 120min.

DISCUSSION

Identifying the existence of potentially interfering substance that is clinically useful either as prophylaxis or for routine differential diagnosis of hepatobiliary diseases is obviously to reduce the toxic effect of the secretive substances. An important parameter in determining the rates of such secretion of a compound into the bile is the biliary flow¹⁴. The production of bile is also a function of hepatic cells¹⁵. Most compounds that will affect biliary flow will therefore affect the liver function. The effect of cimetidine on volume and rate of bile flow in Table 1 shows that cimetidine treatment increases bile flow, which may be due to the action of metabolizing enzyme inducers because phenobabitone have equally been observed to increase biliary flow by a number of microsomal enzyme inducers¹¹.

Figures 1a and b show that cimetidine increases both the plasma concentration of RBG excreted and bile level. The observed increase in biliary flow indicates that treatment with normal dose of cimetidine for 30 min alters the response and suggests that it is likely to affect the reabsorption of fluid along the bile duct.

Though, the concentration of RBG in bile of cimetidine treated rat was lower than that of the control 30 min after RBG administration, the RBG concentration continues to decrease for up to 120 min (Fig. 2a and b). The RBG concentration in the liver are however higher than the controls and are observed to remain high at 120 min after administration (Fig. 2a and b). These responses show that the administered drug has a negative effect on the function of the liver as regard the excretion of RBG by causing retention of RBG in liver cells.

Despite a single dose and the time depended nature of the study, significant correlation are observed when rats were treated with tetrachloride – dibenzo-p-dioxin (TCDD) on the biliary excretion of Indocyanine Green (ICG) ^{16,17}. Thus the use of RBG may be equally favoured in liver dysfunction and could be a good substitute for ICG. Since cimetidine enhances the RBG uptake but inhibits its excretion by the hepatic cells, it is possible that this observation perhaps may have led to the decrease in total RBG excreted and slight increases in the liver weight and RBG liver concentration (Fig. 3). By calculation the ratios of bile-to-liver, bile-to-plasma and liver-to-plasma were compared. For unknown reasons, the ratio decreases even with higher doses (Fig. 4).

In conclusion, the results suggest that cimetidine increases the RBG plasma concentration and simultaneously decreases biliary excretion of the same compound. The study does not address mechanism responsible for this change.

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