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THE EFFECT OF BUCOLOME ON THE CANALICULAR BILE FORMATION AND SULFOBROMOPHTHALEIN TRANSPORT MAXIMUM IN THE DOG

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Abstract Bucolome (BC, 1-cyclohexyl-5-*n*-2,4,6-trioxoperhydropyrimidine), which was developed and used as a non-steroid, anti-inflammatory drug, has been reported to decrease the plasma bilirubin and sulfobromophthalein (BSP) levels in man and rats. Since this drug is also a choleretic, the effect of this drug on the BSP transport maximum (BSP Tm) and canalicular bile formation was studied in the dog. Intravenous administration of BC (10 mg/kg BW) increased the bile flow by 130 per cent in dogs without BSP infusion and 71 per cent in dogs with BSP infusion. Bile to plasma concentration ratio of [^{14}C]erythritol was decreased only 10 per cent by BC administration, and thus BC-induced choleresis was suggested to be primarily of canalicular origin. Neither BSP Tm nor bile salt excretion rate was significantly changed by BC administration. These results indicate that the increased clearance of BSP from the plasma by this drug can not be attributed to the enhancement of hepatic transport process. Furthermore, the results are compatible with the currently proposed view that the dependency of BSP Tm on the bile salt induced choleresis is not due to the increased canalicular bile flow, but due to the increased excretion of bile salts into the bile.

Bucolome (1-cylohexyl-5-*n*-butyl-2,4,6-trioxoperhydropyrimidine, Fig. 1), was originally developed as a non-steroid anti-inflammatory agent [1], but is also a potent choleretic [2]. Recently, its similarity in chemical structure to phenobarbital, a well known hepatic microsomal enzyme inducer, prompted investigators to examine its effect on hepatic dye clearance [3]. As was anticipated, it significantly enhanced the bilirubin [3] and BSP [4] plasma clearances. In contrast to the need for several doses of phenobarbital to enhance dye clearance, Bucolome (BC) was effective after a single administration. It has also been reported that after repeated administration of this drug, there is no induction of the microsomal enzymes [4]. Recently, it has been shown that the effect of BC on dye clearance is due to its ability to decrease binding of the dyes to plasma proteins [5].

There is little information available on its choleretic property. It is also possible that BC can facilitate the plasma clearance of dyes by enhancing the hepatic excretion function related to choleresis.

It was felt that further study of BC-induced choleresis, in terms of its effects on canalicular bile formation and sulfobromophthalein transport maximum (BSP Tm), might be worthwhile for several reasons. (1) If the hepatic BSP Tm is enhanced by a single injection of BC, the reported acceleration of BSP plasma clearance by this drug could be at least partly attributed to the increased excretion of the dye. (2) On the other hand, if BSP Tm is unchanged by this drug, and if it enhances the canalicular bile formation, it would contribute to the understanding of the increase in BSP Tm produced by bile salt-induced choleresis.

Since the report of O'Máille *et al.* [6] and Combes *et al.* [7] that the BSP Tm could be increased as the bile salt infusion rate increased, it has been widely accepted that this increase of BSP Tm is due to the increase of canalicular bile flow [8, 9]. However, several recent studies have challenged this hypothesis [10, 11, 12]. The contention of all these studies is that BSP Tm is increased not by the increase of canalicular bile flow, but by the increase in bile salts excreted into the bile canaliculus. The latter theory is based upon the findings that the BSP Tm can not be increased by a mere increase of canalicular bile flow unless it is accompanied by an increase in bile salt excretion. In these studies, an increased canalicular bile flow with unchanged BSP Tm was produced using several different drugs, such as theophylline [10, 12], methylumbelliferone [10] and SC 2644 [11]. In this report, we will also demonstrate that BC can

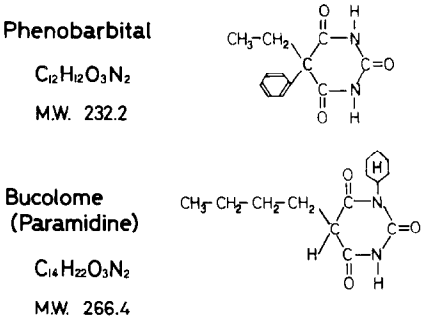

Fig. 1. Phenobarbital and Bucolome (1-cylohexyl-5-*n*-butyl-2,4,6-trioxoperhydropyrimidine).

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increase the canalicular bile flow in dogs, but not the BSP Tm.

MATERIALS AND METHODS

Experimental design. Thirteen mongrel and two beagle dogs of both sexes weighing 7–19 kg were used in the study. Dogs were anesthetized by an intravenous (i.v.) administration of sodium pentobarbital (35 mg/kg, Nembutal, Abbot, North Chicago) after fasting overnight. Additional doses were administered as needed.

Through an abdominal incision, the common bile duct was cannulated, and the cystic duct was ligated. A femoral vein was cannulated and sodium taurocholate (TC, Calbiochem, Los Angeles) was infused continuously at a constant rate of 7–9 μmole/min using a motor-driven syringe pump (Harvard Apparatus). Canalicular bile flow was measured by the erythritol clearance method [13]. About 20 μCi of [^{14}C]erythritol (Radiochemical center, Amersham) was dissolved in 20 ml of 5% glucose solution. Ten ml was injected i.v. and the rest was constantly infused over 4–5 hr. After an equilibrium period of 2 hr, bile was collected every 10 min in a graduated tube for three or more time intervals. Blood (1.5 ml) was drawn from an arterial catheter into a tube containing powdered heparin every 10 min at the midpoint of the 10 min bile collection period. BC* solution in saline (10 ml) was then infused i.v. over a period of 5 min (10 mg/kg). Bile collection was continued for another 60 min at 10-min intervals.

The BSP transport maximum (Tm) was determined in a series of separate experiments, however in three experiments, (Dogs 8, 9, 10), both BSP Tm and canalicular flow were studied simultaneously. The bile collection and the infusion of TC were performed as described in the erythritol study. Sulfobromophthalein sodium (BSP, Daiichi Kagaku, Tokyo) solution in 5% glucose was infused constantly at a rate of 0.4 mg/kg/min after the administration of a priming dose of 12 mg/kg. Bile was collected every 10 min. Ninety to 120 minutes after the beginning of the infusion, the bile flow became constant and BSP excretion rate also reached a plateau. Three to six bile samples were then taken to obtain a baseline BSP excretion rate (Tm). The effect of BC administration (10 mg/kg in 5 min) on bile flow and BSP excretion rate were evaluated for another 60–90 min. In three experiments (Dog 5, 6, 9), an additional continuous infusion (0.2 mg/kg/min) of BC was given to obtain a more constant choleresis. In Dog 9, a second dose (10 mg/kg followed by 0.2 mg/kg/min for 1 hr) was given 50 min after the first dose of BC, and samples collected for another hour. Plasma BSP concentrations were measured from the arterial blood samples taken at every 10 min during the bile collection period.

Arterial blood pressure and EKG were monitored continuously during the experiment. Rectal temperature was maintained at 37.5° ± 0.5° using a warmed table and a heating lamp.

* Sodium salt of BC in crystalline form was kindly supplied by Takeda Chemical Industries, Ltd. Osaka, Japan and was used without further purification.

Analytical procedures. The bile flow rate was estimated by weighing the bile sample, assuming the specific gravity of bile is 1.0.

In early experiments, ^{14}C activity in the plasma and bile was measured by the method reported by Wheeler *et al.* [13]. The color of bile pigment and BSP in the bile was bleached with the use of sodium hypochlorite. Later, the procedure for plasma sample preparation was changed to the use of Instagel (Packard, Downers Grove), which enabled us to omit several steps in the preparation of plasma samples. The correction for the quenching was performed using an internal standard. The activity was measured by a liquid scintillation counter (Aloka, Tokyo).

The concentration of BSP in bile and plasma was determined with the samples diluted with a suitable amount of alkaline saline (pH 9.4). The extinction was read at 580 nm in a Hitachi spectrophotometer. Standards were prepared in dog bile and plasma using BSP solutions of known concentrations. Bile salt concentration was determined enzymatically by the method of Talalay *et al.* [14] modified by Paumgartner *et al.* [15] using 3-hydroxysteroid dehydrogenase (Worthington Biochemical Corp, Freehold, N.J.). Methanolic bile solutions were prepared with 20 μl of bile diluted with 420 μl of methanol. The reaction mixture contained 2 ml of 1 M glycine buffer (pH 9.4) containing EDTA (5.6 m-mole/l), and hydrazine sulfate (0.4 mole/l), 0.2 ml of NAD solution (5.4 m-mole/ l), 0.2 ml of hydroxysteroid dehydrogenase solution (0.7 U/ml), and 0.04 ml of methanolic bile solution. The reaction mixture was incubated at 26 for 30 min and the extinction was read at 340 nm against appropriate blanks. For the standard bile salt solution, methanol solution of cholic acid (Calbiochem., Los Angeles) was used. It has been reported that, in the presence of BSP, this method gives abnormally low values for bile salt concentrations [16]. However, with the range of concentrations of bile salts (31.3–81.1 μmole/ml) and BSP (5.3–18.5 mg/ml) in the bile obtained in the present study, this cause of error was found to be less than 2 per cent, and thus correction was not performed.

Statistical analysis. Data were analysed using the Student *t*-test.

RESULTS

Figure 2 shows the results of a typical experiment for the erythritol clearance study without BSP infusion. The bile flow rate began to increase during the 5-min BC infusion period, and the maximal flow rate was observed in the first 30 min after BC adininstration. Thereafter, the bile flow rate showed a gradual decline, but the choleresis continued for more than 6 hr. The gradual decline of bile flow rate was prevented by an additional continuous infusion of BC (0.2 mg/kg/min) in three experiments (Dog 5, 6, 9), in which the bile flow rate was kept almost constant during the infusion period (Fig. 3).

The bile flow rates after BC administration, depicted in Tables 1 and 2, are the averages of the three highest flow rates. On the average, BC adminstration produced a 130 per cent increase in bile flow in seven dogs without BSP infusion (Table 1) and

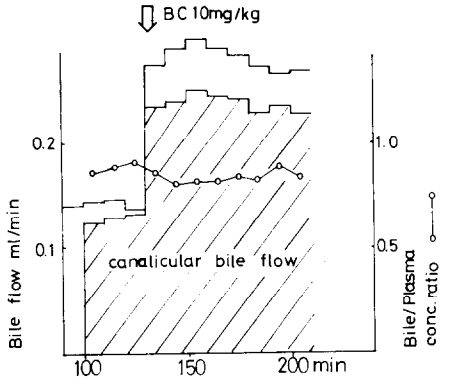

Fig. 2. The effect of Bucolome on the canalicular bile flow (Dog 2). I.v. administration of BC (10 mg/kg) increases the bile flow more than twice as the original bile flow, while bile plasma concentration ratio of erythritol (B/P) falls very slightly. The calculated cananicular bile flow also increases significantly, accounting for the most of the increased bile flow.

71 per cent in eight dogs with BSP infusion (Table 2). However, the B/P erythritol ratio decreased to a much smaller extent (approximately 10 per cent on average), so that the choleresis caused by BC seemed to be mostly of canalicular origin.

Figure 3 illustrates an example of the BSP Tm study and Table 2 summarizes all the results. BC significantly increased bile flow as in the erythritol study. However, the concentration of BSP decreased to an extent to compensate for the bile flow increase. Consequently, BSP excretion rate (Tm) was not changed significantly. As is shown in Fig. 3, the rise in BSP concentration in the plasma was not decreased after the administration of BC. In Table 2, bile salts excretion rates before and after BC administration are also shown. There was no significant change in bile salt excretion rates caused by BC administration.

DISCUSSION

BC was originally developed as a non-steroid anti-inflammatry drug [1]. Although the choleretic effect of this agent was noted during development, little attention has been given to this aspect until recently

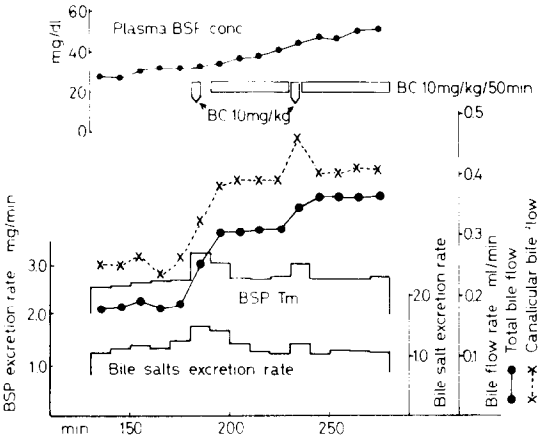

Fig. 3. The effect of Bucolome on the BSP Tm and bile salt excretion rate (Dog 9). BC administration (10 mg/kg followed by continuous infusion at a rate of 0.2 mg/kg/min) effected to increase the bile flow as well as canalicular bile flow. Second dose of BC further increased the bile flow. However, either BSP Tm or bile salts excretion rate was not changed during these choleretic periods.

Table 1. Effect of Bucolome on canalicular bile flow in the dog

Dog no.*	Body wt (kg)	Bile flow Before BC (μl/min)	Bile flow After BC† (μl/min)	Bile flow Increase (%)	B/P ratio Before BC	B/P ratio After BC†	Canalicular bile flow Before BC (μl/min)	Canalicular bile flow After BC† (μl/min)	Canalicular bile flow Increase (%)
1	13.0	60	122	103	1.45	1.10	83	126	52
2	15.0	139	298	114	0.92	0.83	127	227	79
3	12.0	100	232	132	0.92	0.74	92	159	73
4	12.6	150	388	159	0.79	0.66	113	234	107
5‡	7.8	55	174	216	1.19	1.08	66	188	185
6‡	8.0	169	289	71	0.98	1.01	166	295	78
7	11.5	101	214	112	0.59	0.77	60	165	175
Mean	11.4	111	245•	130	0.98	0.88	101	199•	107
S.D.	2.4	40.8	81.5	43.1	0.26	0.16	34.6	52.7	48.6
8	10.0	182	266	46	1.39	1.29	253	345	36
9‡	15.0	186	307 (357)§	65 (92)§	1.35	1.26 (1.12)§	251	390 (403)§	55 (61)§
10	19.0	200	412	106	1.43	1.16	288	476	65
Mean	14.7	189	328**	72	1.39	1.24**	264	404**	52
S.D.	3.7	7.7	61.5	25.0	0.03	0.06	17.0	54.3	12.0

* Dogs 1–7, without BSP infusion, Dogs 8–10, with BSP infusion.
† After i.v. administration of 10 mg/kg Bucolome.
‡ Initial dose (10 mg/kg) was followed by continuous administration of BC (0.2 mg/kg/min).
§ Values in parenthesis are those after second dose administration (see Fig. 3).
• Significantly different from values in control period ($P < 0.005$).
| N.S. ($P > 0.1$).
** Significantly different ($P < 0.01$).

Table 2. Effect of Bucolome on BSP Tm and bile salt excretion rate

Dog no.	Body wt (kg)	Bile flow rate			BSP Tm		Bile salts excretion	
		Before BC	After BC*	Increase	Before BC	After BC*	Before BC	After BC*
		(μl/min)		(%)	(mg/min)		(μmole/min)	
8	10.0	182	266	46	2.32	1.85	8.4	7.8
9†	15.0	186	307	65	2.61	2.64	11.7	10.9
			(357)‡	(92)‡		(2.61)‡		(10.2)‡
10	19.0	200	412	106	2.64	2.75	9.7	11.9
11	12.0	174	281	62	1.94	1.90	8.2	9.0
12	18.2	273	449	64	2.77	2.85	10.6	9.4
13	7.8	105	184	75	1.04	0.96	6.3	6.8
14	12.4	95	180	89	1.75	1.88	7.7	7.9
15	14.5	214	345	61	2.27	2.35	6.7	6.8
Mean	13.7	179	3038	71	2.17	2.15¶	8.7	8.8¶
S.D.	3.6	53.8	90.9	17.5	0.54	0.59	1.8	1.7

* After i.v. administration of 10 mg/kg Bucolome.
† Initial dose (10 mg/kg) was followed by continuous administration of BC (0.2 mg/kg/min).
‡ Values in parenthesis are obtained after second dose of BC (10 mg/kg + 0.2 mg/kg/min).
§ Significantly different from values in control period ($P < 0.001$).
¶ N.S. ($P > 0.5$).

and the mechanism of choleresis remains unknown [2]. On the other hand, Yamamoto *et al.* [3] reported a rapid decrease of the unconjugated billirubin levels in the plasma of patients with constitutional jaundice after the administration of this drug. They assumed this effect was due to enzyme induction in the liver microsomes, since the chemical structures of BC and phenobarbital are similar (Fig. 1).

Yatsuji *et al.* [4] further reported that BC enhanced the clearance of BSP in rats and decreased plasma bilirubin levels in jaundiced Gunn rats. They demonstrated, however, that unlike phenobarbital, BC did not enhance the enzyme activity of glucuronyl transferase in the liver homogenate nor the binding activity of ligandin (Y protein) in the liver. They further found altered *in vitro* binding of ICG with plasma proteins obtained from rats given BC. Yamamoto *et al.* [5] demonstrated that BC injected into Gunn rats decreased the binding of bilirubin to albumin and increased its distribution to various tissues including the nervous system. They concluded that the decrease in plasma bilirubin levels caused by BC is due to its effect of decreasing the binding of BSP to plasma proteins, possibly by competitive processes. Thus, the clinical use of BC for the treatment of neonatal jaundice lost a rationale, because of the increased concentration of bilirubin in the nervous system.

The magnitude and the duration of choleresis induced by BC observed in the present study are in agreement with a previous observation* in the dog with the same dose of BC (10 mg/kg). However, it is higher than was observed in rats or guinea pigs, in which the average increase rate was approximately 60 per cent.† Furthermore, the results of the present study have shown that BC increases the canalicular bile flow in the dog. However the canalicular choleresis caused by BC was not accompanied by an increase in bile salt excretion. Thus, BC produces an increase in the bile salt independent fraction of canalicular bile formation. It is also clear that BC-induced choleresis does not increase BSP Tm. This is a finding similar to that reported by Forker *et al.* [11] using a different choleretic, SC-2644.

According to a previous study [17], the plasma half life of BC in man and in other animals was long, ranging from 5 30 hr. The biliary excretion rate in the rabbit of i.v. administered BC and its metabolites is reported to be less than 2 per cent of the dose in the first 8 hr [17]. In the rat, only 5 per cent of the i.v. administered dose was recovered in the feces in 24 hr, while more than 50 per cent was recovered in the urine [18]. Assuming these values can be applied to the dog, 2 per cent of the presently used dose is only 7–10 μmoles of BC. Furthermore, the choleresis observed in the present study was for more than 6 hr, with a very gradual decline of bile flow. Even if we assume that the amount of BC (or its metabolites) excreted in dog bile is more than ten times that of rabbits, the degree and duration of choleresis are contrary to the explanation that BC-induced choleresis is due to the osmotic force of BC or its metabolites excreted in the bile.

As in the case of SC-2644, it is difficult to completely exclude the possibility that BC inhibits the BSP transport in the canalicular membrane by an unknown mechanism. However competitive inhibition of the transport process is very unlikely. It is possible that the canalicular choleresis produced by BC increased the BSP Tm and in some way also increased the reabsorption of BSP from the biliary tree. However, even if such a coincidence occurred with one drug, it would be an extraordinarily rare event to happen with several different drugs. The addition of a new member, BC, to the group of choleretics (SC-2644, theophylline, methylumbelliferone) seems to support the contention that BSP Tm is dependent on the bile salt excretion rate and not on the canalicular bile flow *per se*.

From the present results, it would appear that enhanced BSP clearance reported in a previous study [4] is not due to the enhanced transport of the dye

* H. Sato. Personal communication.
† K. Kitani. Unpublished observation.

in the liver. However in contrast with the previous work reporting the enhanced dye clearance in the rat [4], the rise in BSP concentration in the plasma in the present study was not significantly decreased by the BC administration. The cause of this discrepancy is not clear. Differences in the animal species, dose, the test procedure (clearance after single dye injection versus continuous infusion of the dye), and the condition of bile salts reserve and supply, all could be possibilities.

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