Effect of ioglycamide (Biligram) on bile flow and biliary lipid secretion in man¹

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SUMMARY Twenty-one anicteric patients with a t-tube *in situ* were studied between the ninth and 11th postoperative days. Eleven patients were given an intravenous infusion of the biliary contrast agent ioglycamide (Biligram), while the other 10 acted as controls. Bile flow was recorded and the biliary concentrations of ioglycamide, bile salt, phospholipid, and cholesterol estimated in the two groups. The biliary excretion of ioglycamide was associated with a significant choleresis which was probably due to the obligatory coupling of the osmotically active contrast agent molecules with water. Biliary ioglycamide excretion did not significantly alter bile salt secretion rates. In contrast, the biliary secretion of both phospholipid and cholesterol was significantly lowered ($P < 0.001$). Unlike chenodeoxycholic acid, ioglycamide significantly reduced bile acid independent cholesterol secretion ($P < 0.01$), although secretion rate in terms of μmol of cholesterol per μmol of bile acid was essentially unchanged.

The biliary secretion of phospholipids and cholesterol is partially dependent on the simultaneous secretion of bile acid (Entenman *et al.*, 1969; Hardison and Francis, 1969; Nilsson and Schersten, 1970; Dowling *et al.*, 1971; Northfield and Hofmann, 1973), but the mechanism involved for this biliary lipid coupling is poorly understood. Small (1970) suggested that during their transit across the hepatocyte the bile acids might penetrate the canalicular membrane from the interior of the cell and specifically leech out lecithin (the main phospholipid found in bile) and cholesterol, leaving the membrane protein and other structural lipids intact. Several groups (Swell *et al.*, 1968; Hardison and Francis, 1969; Young, 1971), on the other hand, have suggested that a specific macromolecular complex of bile acid, lecithin, cholesterol, and water is assembled inside the hepatocyte before being excreted into bile.

It is now known that biliary cholesterol secretion is much less influenced by changes in bile acid secretion than is phospholipid excretion. This helps to

explain why human bile becomes saturated with cholesterol—that is, 'lithogenic'—at low bile acid secretion rates (Grundy *et al.*, 1972; Northfield and Hofmann, 1973). Oral chenodeoxycholic acid (CDCA) is an effective treatment for cholesterol gallstones (Danzinger *et al.*, 1972; Bell *et al.*, 1972b) because it 'uncouples' the normal bile acid/cholesterol relationship in bile with the result that the patient's bile is less than saturated with cholesterol even at low bile acid outputs (Northfield *et al.*, 1975). Any other compound like CDCA that is shown to affect bile acid/cholesterol coupling would be of interest, because (a) it might shed light on our understanding of the physiology of biliary lipid secretion, and (b) might have implications in the future development of other cholesterol gallstone dissolving drugs.

In the rhesus monkey it was found that the intravenous cholangiographic agent ioglycamide (Biligram, Schering Chemicals Ltd.) was not only a potent choleric (Whitney and Bell, 1976) but at the same time it significantly ($P < 0.01$) enhanced the ratio of bile salts + phospholipid/cholesterol in bile (Bell and Dowling, unpublished results). During studies of the biliary excretion of ioglycamide at different infusion rates (Bell *et al.*, 1978a) we decided also to examine, in man, the effect of this compound on bile flow and biliary lipid output. This paper reports our findings.

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Methods

PATIENTS

Twenty-one patients who had undergone cholecystectomy with exploration of the common bile duct for gallstones were studied. They all had common bile duct t-tubes through which cholangiograms were performed on the seventh or eighth post-operative day. The t-tubes were then clamped for two to three days to ensure that the enterohepatic circulation of bile salts had been re-established. The serum bilirubin levels of all patients were normal at the time of study. The experiments were started at 9.00 a.m. in the non-fasted state. At the beginning of the study the t-tube was unclamped and the bile allowed to drain by gravity. The first few millilitres of bile were discarded and then two 'baseline' 10-minute bile collections were made before the intravenous infusion of ioglycamide was started. Bile samples were then collected at 10-minute intervals for a further 120 minutes and their volumes recorded. Five patients were given a two-hour intravenous infusion of ioglycamide at a rate of 2 mg.kg body weight⁻¹ min⁻¹, while six patients received an infusion of ioglycamide at 4 mg.kg body weight⁻¹ min⁻¹ for one hour, followed by 1 mg.kg body weight⁻¹ min⁻¹ for a further 30 minutes. The intravenous infusions were controlled by a constant infusion pump (Ivac 531, Ivac Corporation, Harrow, Middlesex). The remaining 10 patients received no ioglycamide and acted as controls. All 21 patients were encouraged to drink liberal quantities of water throughout the period of study. Informed consent was obtained in every case.

BILE ANALYSIS

The bile samples were stored at -20°C. The specimens were thawed and thoroughly shaken before taking the various aliquots for analysis (Bell *et al.*, 1972a). Total bile acid concentration was measured enzymatically using Admirand and Small's (1968) modification of the method of Talalay (1960). The cholesterol content of the bile samples was assayed by an enzymatic method (Roda *et al.*, 1975) using the Boehringer Mannheim Kit (Boehringer Ltd., England). Biliary phospholipid was measured using the method of Bartlett (1959). Biliary ioglycamide levels were estimated using a spectrophotometric method (Bell *et al.*, 1978b).

STATISTICAL ANALYSIS

In each of the 21 patients, 14 consecutive 10-minute bile samples were taken for analysis. In each 10-minute period the bile flow (ml/min) and biliary secretion rate (μmol/min) of ioglycamide, bile salt, phospholipid, and cholesterol was estimated. In each

sample the following ratios were also calculated: (1) the bile salt plus phospholipid/cholesterol (BS + PL/C) and (2) phospholipid/cholesterol (PL/C). In addition, the secretion rates of bile salts, phospholipids, and cholesterol were also expressed as a percentage of their baseline values (baseline secretion being arbitrarily defined as the average of the first two 10-minute samples). Then, for each of the above variables and ratios, the mean values for the 10 control subjects were compared with those for the 11 patients given intravenous ioglycamide. The significance of difference found was assessed using an unpaired Student's *t* test.

In each individual patient the relationships between bile salt secretion rate (plotted on the ordinate or *y* axis) and (a) bile flow (*y* data) and (b) biliary cholesterol excretion (*y* data) were studied. The excretion rates of water and cholesterol and their respective correlation coefficient (*r*) were calculated from the regression equation $y = mx + b$, where *b* represents the *y* intercept, *m* is the slope of the regression line. The mean values for both *b* and *m* were calculated for the 10 controls and the 11 ioglycamide treated patients and the significance of any differences found assessed by an unpaired Student's *t* test. In addition, the relationship between biliary ioglycamide secretion (*x* data) and bile flow (*y* data) was calculated for each of the 11 patients given ioglycamide using the above regression equation. The mean slope and mean intercept relating ioglycamide output and bile flow was then calculated.

Results

BILIARY EXCRETION OF IOGLYCAMIDE

It can be seen from Fig. 1 that significant amounts of ioglycamide begin to appear in the bile 20 minutes after the start of the intravenous infusion. The rate of biliary excretion of the contrast agent was very similar in the two different intravenous infusion groups. For this reason we have combined the results of the two infusion groups and for the rest of this paper all 11 patients will be referred to as the 'ioglycamide treated group'.

EFFECT OF IOGLYCAMIDE ON BILE FLOW

The excretion of ioglycamide into bile was associated with a significant choleresis (Fig. 2). There was a statistically significant relationship between bile salt secretion and bile flow in each of the 10 control patients and nine of the 11 ioglycamide treated group. The mean ($\pm$ SEM) slope *m* of the 10 controls of 0.028 ± 0.009 (range 0.008-0.078) was not significantly different from that of the 11 ioglycamide treated patients (0.009 ± 0.001 —range 0.002-0.018). In contrast, the mean intercept *b*—that is, bile salt

Fig. 1 Biliary excretion of ioglycamide ($\mu\text{mol}/\text{min}$) following intravenous administration of the contrast agent at either 2 or 4 mg/kg body weight $^{-1}$ min $^{-1}$ —see text.

Fig. 2 Effect of ioglycamide on mean bile flow (ml/min).

independent bile flow—of the ioglycamide group 1.190 ± 0.103 (range 0.704–1.873) was significantly ($P < 0.001$) greater than that of the control group (0.310 ± 0.054 , range 0.001–0.608).

In 10 of the 11 patients given ioglycamide there was a highly significant r value relating ioglycamide

secretion to bile flow. The mean slope ($\pm$ SEM) was 0.023 ± 0.002 with a mean intercept on the bile flow axis of 0.934 ± 0.049 ml/min. As can be seen from Fig. 1 the ioglycamide secretion reached peak levels about one hour after the start of the intravenous infusion and was then maintained for the rest of the

experiment. The maximum secretion rate of ioglycamide achieved in the individual patients ranged from $11.4\text{--}38.1\text{ }\mu\text{mol/min}$. There was not a statistically significant relationship between baseline bile salt secretion rate and the maximum ioglycamide secretion rate achieved in the individual patient.

EFFECT OF IOGLYCAMIDE ON BILE SALT SECRETION

The baseline bile salt secretion rate was slightly higher in the ioglycamide treated group than in the controls, but this difference was not statistically significant. In both groups the bile salt secretion rates remained relatively constant for a further 60 minutes, and then gradually fell as the bile salt pool began to be 'washed out'. At no time were there significant differences between the two groups (Fig. 3). Similarly, when bile salt secretion was expressed in terms of percentage of baseline secretion there was once again no significant difference between the ioglycamide infused and control groups.

EFFECT OF IOGLYCAMIDE ON BILIARY PHOSPHOLIPID SECRETION

The mean ($\pm$ SEM) biliary phospholipid secretion rate in the first baseline samples from the control group of $9.25 \pm 1.49\text{ }\mu\text{mol/min}$ was slightly higher than that of the ioglycamide treated group ($7.68 \pm 0.69\text{ }\mu\text{mol/min}$). For purposes of comparison, changes in phospholipid secretion were therefore calculated in terms of percentage change in baseline secretion rates. It can be seen from Fig. 4 that within 30 minutes of the start of the of the ioglycamide infusion the phospholipid secretion rate in the treated group began to fall and by 40 minutes phospholipid secretion was significantly depressed. By the end of the experiment phospholipid secretion rate was only $17.7 \pm 5.1\%$ of baseline values in the ioglycamide group, compared with $72.1 \pm 12.2\%$ in the control group ($P < 0.01$).

EFFECT OF IOGLYCAMIDE ON BILIARY CHOLESTEROL SECRETION

The mean biliary cholesterol secretion rate in the first baseline samples from the control group of $2.80 \pm 0.40\text{ }\mu\text{mol/min}$ was similar to that of the ioglycamide treated group of $2.91 \pm 0.40\text{ }\mu\text{mol/min}$. Figure 5 shows that in the first 30 minutes after starting the ioglycamide infusion the patients secreted more cholesterol into their bile than the control group, but these differences were not significant. The biliary cholesterol output then fell progressively and by 70 minutes was significantly less ($P < 0.05$) in the ioglycamide treated group than the control subjects. By the end of the experiment cholesterol secretion was only $32.3 \pm 5.8\%$ of baseline values in the ioglycamide group, compared with $81.9 \pm 9.8\%$ in the control subjects ($P < 0.001$).

RELATIONSHIP BETWEEN BILE SALT SECRETION AND BILIARY CHOLESTEROL OUTPUT: EFFECT OF IOGLYCAMIDE

Since significant amounts of ioglycamide did not start to appear in bile (Fig. 1) until 20 minutes after the start of the intravenous infusion, only the last 10 samples were used to study the relationship between bile salt secretion and biliary cholesterol output. Table 1 shows the individual correlation coefficients, slopes, and intercepts on the biliary cholesterol axis of all 10 controls and 11 ioglycamide treated patients. The mean slope was very similar in the two groups, but the mean intercept on the biliary cholesterol axis—that is, the bile salt independent fraction of cholesterol flow—was significantly lowered by the ioglycamide infusion ($P < 0.01$).

EFFECT OF IOGLYCAMIDE ON THE

$$\frac{\text{BS} + \text{PL}}{\text{C}} \text{ RATIO}$$

The mean ratio in the two baseline samples was very

Fig. 3 Effect of ioglycamide on mean bile salt secretion ($\mu\text{mol/min}$).

Fig. 4 Effect of ioglycamide on mean biliary phospholipid secretion—phospholipid secretion expressed as percentage of baseline secretion.

Fig. 5 Effect of ioglycamide on mean biliary cholesterol secretion—cholesterol secretion expressed as percentage of baseline secretion.

similar in the two groups (Table 2). After 50 minutes of intravenous ioglycamide the mean $\frac{BS + PL}{C}$ ratio

was 24.6 ± 4.3 compared with 17.3 ± 2.4 in the control group. As can be seen from Table 2, at no stage were the differences in this ratio statistically significant. When, however, the mean ratio of the two baseline specimens was compared with the ratio of the 60 minute specimen using a paired t test the differences did become significant in the ioglycamide treated group ($p < 0.02$), but not in the controls.

EFFECT OF IOGLYCAMIDE ON BILIARY PL/C RATIOS

The mean PL/C ratio in the two baseline samples taken from the 10 controls was slightly higher than that of the ioglycamide group, but this difference was not significant (Table 3). The PL/C ratio remained fairly constant in the control group over the first 100 minutes of study, and then fell slightly over the last 40 minutes of the experiment. The PL/C ratio of the ioglycamide group fell significantly soon after the start of the infusion of biliary contrast material

and stayed depressed throughout the two hour period of study.

Discussion

Ioglycamide (Biligram) consists of two iodinated benzene rings joined by a polymethylene chain. It is

Table 1 Relationship between bile salt secretion and biliary cholesterol output: effect of ioglycamide

Patients	r	p	slope m	intercept b
Controls				
1	0.889	***	0.174	0.360
2	0.611	NS	0.027	1.892
3	0.014	NS	0.002	2.356
4	0.741	*	0.045	0.512
5	0.887	***	0.090	0.276
6	0.882	***	0.025	2.036
7	0.947	***	0.064	1.209
8	0.887	***	0.036	1.786
9	0.735	*	0.011	1.906
10	0.479	NS	0.015	1.814
Mean $\pm$ SEM	—	—	0.048 $\pm$ 0.016	1.414 $\pm$ 0.242†
Ioglycamide				
1	0.939	***	0.074	-0.023
2	0.527	NS	0.022	0.741
3	0.817	**	0.032	0.490
4	0.877	**	0.025	1.233
5	0.763	*	0.087	-0.524
6	0.940	***	0.048	0.376
7	0.985	***	0.087	0.298
8	0.897	***	0.065	0.118
9	0.742	*	0.047	0.532
10	0.576	NS	0.021	1.602
11	0.925	***	0.030	0.489
Mean $\pm$ SEM	—	—	0.054 $\pm$ 0.007	0.533 $\pm$ 0.170†

† $t = 3.031$, $P < 0.01$.

structurally very similar to iodipamide (Biligradin). The renal and hepatic handling of these two choangiographic agents is probably very similar (Sperber and Sperber, 1971). Both compounds are actively transported into bile and are subject to a transport maximum (Miller *et al.*, 1969; Burgener *et al.*, 1970; Whitney and Bell, 1976; Bell *et al.*, 1978b). Ioglycamide uses the same liver to bile transport system as many other organic anions including bilirubin and bromsulphthalein but not bile acids, the latter being secreted into bile by a separate mechanism (Sperber and Sperber, 1971).

We have shown that the biliary excretion of ioglycamide is associated with a significant choleresis which is independent of bile salt secretion. The choleresis is probably due to the obligatory coupling of the osmotically active contrast agent molecules with water as they are excreted into bile. The choleresis associated with the excretion of ioglycamide (23.0 ml/mol ioglycamide) is very similar to the values previously reported for iodipamide in the dog (Berk *et al.*, 1976).

Our results show that, in man, bile salt secretion is not significantly affected by the simultaneous excretion of ioglycamide, thus confirming our previous findings in the rhesus monkey using iodipamide (Whitney and Bell, 1972). This finding was not unexpected, since bile salts probably have a separate transport mechanism within the hepatocyte (see above), and it is now well known that the choleresis produced by the administration of bile acids actually enhances rather than depresses the biliary excretion

Table 2 Effect of ioglycamide on BS + PL/C ratio (mean $\pm$ SEM)

<i>Time in minutes</i>	<i>Baseline</i>		<i>10</i>	<i>20</i>	<i>30</i>	<i>40</i>	<i>50</i>	<i>60</i>	<i>70</i>	<i>80</i>	<i>90</i>	<i>100</i>	<i>110</i>	<i>120</i>
Controls	14.8	13.6	14.4	15.1	16.2	15.7	17.3	16.9	15.4	14.3	11.9	10.9	9.4	8.4
	$\pm$ 1.6	$\pm$ 1.7	$\pm$ 1.7	$\pm$ 2.0	$\pm$ 2.2	$\pm$ 2.1	$\pm$ 2.4	$\pm$ 2.3	$\pm$ 2.4	$\pm$ 2.6	$\pm$ 2.5	$\pm$ 2.5	$\pm$ 1.7	$\pm$ 1.7
Ioglycamide treated	16.6	15.3	15.4	16.7	16.6	18.5	24.8	22.9	20.9	18.3	14.1	14.5	12.5	10.2
	$\pm$ 1.9	$\pm$ 2.8	$\pm$ 1.9	$\pm$ 2.3	$\pm$ 1.5	$\pm$ 1.6	$\pm$ 4.3	$\pm$ 2.7	$\pm$ 2.7	$\pm$ 2.2	$\pm$ 1.9	$\pm$ 1.7	$\pm$ 1.7	$\pm$ 1.2
Statistical significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 3 Effect of ioglycamide on PL/C ratio (mean $\pm$ SEM)

<i>Time in minutes</i>	<i>Baseline</i>		<i>10</i>	<i>20</i>	<i>30</i>	<i>40</i>	<i>50</i>	<i>60</i>	<i>70</i>	<i>80</i>	<i>90</i>	<i>100</i>	<i>110</i>	<i>120</i>
Controls	3.4 ± 0.3	3.1 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	3.4 ± 0.3	3.3 ± 0.2	2.9 ± 0.2	2.9 ± 0.3	2.6 ± 0.2	2.6 ± 0.3
Ioglycamide treated	3.0 ± 0.3	2.8 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	2.7 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	2.3 ± 0.3	2.1 ± 0.3	2.2 ± 0.3	2.0 ± 0.3	1.5 ± 0.3
Statistical significance	NS	NS	*	**	***	**	NS	*	NS	*	NS	NS	NS	**

of a number of anionic dyes including bromsulphthalein (O'Maille *et al.*, 1966) and ioglycamide (Behan *et al.*, 1977).

Since ioglycamide did not affect bile salt secretion, we must conclude that the significant reduction in the output of biliary phospholipid and cholesterol observed during the ioglycamide experiments was independent of bile acids. Erlinger *et al.* (1975) have shown that lysine acetylsalicylate stimulates bile salt independent bile flow and depresses biliary phospholipid and cholesterol secretion in the dog. They postulated that other canalicular bile acid independent choleretics should similarly depress biliary cholesterol secretion simply as a result of their diluting canalicular bile and reducing micellar bile acid concentration (Erlinger *et al.*, 1975). Whether or not this is the mechanism involved in the present set of ioglycamide experiments is not known.

There was a significant correlation between bile salt and biliary cholesterol output in both the control group and the ioglycamide treated patients. The mean cholesterol excretory rate in the control group of 0.048 $\mu\text{mol}/\mu\text{mol}$ of bile acid was very similar to the previous reported value of 0.051 $\mu\text{mol}/\mu\text{mol}$ of bile acid (Schersten *et al.*, 1971). Similarly, the positive y intercept b on our control group of 1.414 μmol cholesterol per minute was very similar to the value of 1.49 μmol cholesterol per minute found by the same group (Schersten *et al.*, 1971). The slope of the regression line relating biliary cholesterol to bile acid output was unchanged by ioglycamide. When, however, the regression line was extrapolated back to zero bile salt secretion, the mean positive y intercept b of 0.533 μmol cholesterol per minute was significantly ($t = 3.03$, $P < 0.01$) lower than that of the control group.

Chenodeoxycholic acid exerts its cholesterol lowering effect by altering the slope of the regression line relating cholesterol and bile acid output but does not affect bile acid independent cholesterol secretion (Northfield *et al.*, 1975). Thus, the mechanism by which ioglycamide lowers biliary cholesterol excretion is quite different from chenodeoxycholic acid, as bile acid independent cholesterol secretion is significantly reduced but the cholesterol secretion rate in terms of μmol per μmol of bile acid was unaltered.

Ioglycamide, again unlike chenodeoxycholic acid, also significantly reduced biliary phospholipid output. The ratio of phospholipid/cholesterol fell significantly during ioglycamide infusion at a time when bile salt secretion was unaffected (Table 3). This is of interest, as Wheeler and King (1972) had previously found a fixed ratio of phospholipid/cholesterol in bile over a wide range of bile salt secretion rates, and concluded that the secretion of the two lipids in

response to bile acids was closely coupled. Other evidence (Smallwood and Hoffmann, 1976), like our own, suggests that biliary cholesterol and phospholipid secretion may be influenced independently.

The rise in the $\frac{\text{BS} + \text{PL}}{\text{C}}$ ratio—the so-called 'cholesterol solubilizing capacity' of bile (Isaksson, 1954)—noted during ioglycamide excretion is of particular interest, as one might, on physicochemical grounds, predict that such bile would be capable of dissolving cholesterol gallstones (Bell, 1974). Ioglycamide might therefore prove to be a prototype for the future development of an orally administered drug for dissolving cholesterol gallstones.

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