level of cAMP in the brain remains to be established. Although the direct administration of the monoamines noradrenaline and histamine into rat cerebral cortex causes prolonged activation of adenyl cyclase (Chou *et al.*, 1971) the E.C.T.-induced increase in brain cAMP content cannot be completely explained by the action of these amines, since electrical stimulation of guinea-pig cerebral cortex produces an accumulation of cAMP over and above that produced by either noradrenaline or histamine (Rall and Sattin, 1970). Currently the most plausible suggestion is that depolarization results in increased release of adenine-ribose compounds, which then stimulate cAMP formation (Rall and Sattin, 1970).

This study has shown that E.C.T. produced an increase in the urinary excretion of cAMP, which may be due to increased tissue levels of cAMP or to an increased rate of elimination. The direct electrical stimulation of slices of guinea-pig cerebral cortex, however, rapidly produces a greatly increased level of cortical cAMP (Kakiuchi *et al.*, 1969; Shimizu *et al.*, 1970), and, furthermore, evidence has been obtained to support the suggestion that cAMP mediates the physiological actions of neural transmitter substances in the central nervous system (Florendo *et al.*, 1971; Greengard and Kuo, 1970). Possibly, therefore, the antidepressant action of E.C.T. is mediated through an increased production of cAMP in the brain, which is reflected in the increase observed in the urine of E.C.T.-treated patients.

We thank Mr. Z. Carrasco, Sister E. Carlson, and other members of the staff of Tooting Bec Hospital for their help. We are grateful to the patients involved in this study for their co-operation.

This work was supported by a grant from the South-West Metropolitan Regional Hospital Board.

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Cephalexin Levels in Human Bile in Presence of Biliary Tract Disease

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British Medical Journal, 1972, 3, 441–443

Summary

Cephalexin was given to 24 patients before and after operation on the bile ducts and gall bladder. Two patients had obstructive jaundice. Samples of the bile were taken either directly from the gall bladder at operation or via the T-tube. Cephalexin was excreted in the bile, peak levels being obtained after two to three hours. These levels could be raised if probenecid was given concurrently. Higher levels were found in patients with functioning gall-bladders. A trial of cephalexin seems justified for the treatment of typhoid carriers.

Introduction

Cephalexin, a semisynthetic analogue of cephalosporin C (Wick, 1967), is a broad-spectrum antibiotic whose oral absorption is rapid and virtually complete (Perkins *et al.*, 1968). Rat liver homogenates rapidly decompose some cephalosporin analogues to less active metabolites, but cephalexin is not affected and is excreted unchanged in the bile of rats. The object of the present study was to determine the concentration of cephalexin in the bile of patients undergoing surgery for biliary tract disease, to ascertain what factors affect these levels, and to assess its potential therapeutic value.

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Patients

Group 1.—Nine patients with T-tubes in the common bile duct who had recovered from cholecystectomy and exploration of the common bile duct and were receiving a normal diet were given 1 g of cephalexin on an empty stomach or after a little light breakfast. Serum and bile samples were collected at hourly intervals for six hours. Two days later five of the patients were given 1 g of cephalexin together with 0·5 g of probenecid and samples were again collected over six hours. All these patients had normal liver function tests.

Group 2.—Thirteen patients undergoing cholecystectomy were given 500 mg of cephalexin at six-hourly intervals over the 24 hours before operation and 500 mg with the premedication (total dose 2·5 g). At operation bile was obtained from the gall bladder by needle aspiration and when possible from the common bile duct through a fine polyethylene catheter inserted

through the cystic duct. A sample of venous blood was obtained at the same time. A piece of the wall was taken from the excised gall bladder. These patients were divided into two groups according to the preoperative radiological findings—(*a*) six patients with no evidence from oral or intravenous cholangiography of cystic or common bile duct obstruction, and (*b*) seven patients shown by oral or intravenous cholangiography to have non-functioning gall bladders but normal common bile ducts. The patients in both these groups had normal liver function tests.

Group 3 consisted of two patients with obstructive jaundice from whom samples of bile and blood were similarly obtained at operation. One was shown by preoperative transhepatic cholangiography to have stones in the common bile duct and the other to have a carcinoma of the lower end of the common bile duct.

Methods

SAMPLES

All specimens of serum and bile were frozen on collection and kept at −20°C until assayed.

MICROBIOLOGICAL ASSAY

Cephalexin was assayed microbiologically by a cup-plate method with the use of *Bacillus subtilis* ATCC 6633, enabling concentrations of about 1·5 μg/ml to be detected. The organism was grown on agar slopes at 37°C for seven days and the spores were washed off and stored at −20°C. At three-monthly intervals the suspensions were heated to 60°C to kill off vegetative cells. The medium (Oxoid peptone 0·5%, Oxoid lab-lemco 0·3%, sodium citrate 1·0%, Oxoid agar No. 3 1·2%; pH 7) was melted and inoculated at 48°C with a dilution of spore suspension in distilled water previously shown to give just confluent growth after overnight incubation. Glass plates 23 cm by 23 cm, with a 1-cm square section aluminium frame, were sterilized by swabbing with acetone and flaming; 150 ml of seeded molten medium was then poured into each plate on a levelled surface. When the medium had cooled six rows of six holes were made in the seeded agar with a stainless-steel puncher 6 mm in diameter.

A fresh stock solution of cephalexin (2 mg/ml) was made every day. Standard solutions of cephalexin from 1-20 μg/ml were made up in horse serum and in pH 6·0 phosphate buffer.

Bile was diluted with pH 6·0 phosphate buffer and patients' serum with horse serum. Duplicate volumes of three dilutions of standard and two dilutions of each specimen were used to fill the 36 holes in a predetermined random distribution. Specimens of gall-bladder wall were weighed in sterile containers and ground with known volumes of sterile pH 6·0 phosphate buffer.

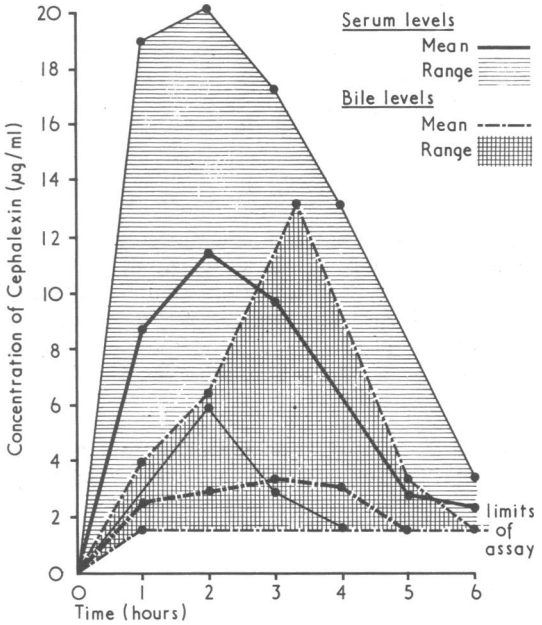
It had previously been found (Sales *et al.*, 1969) that the cephalexin levels in one jaundiced patient were extremely high. To exclude the possibility that the presence of bile or the use of horse serum rather than human serum might influence the assay result, several comparisons were made of the zones of inhibition produced by known concentrations of cephalexin added to normal or jaundiced human serum and to horse serum.

Results

No difference was shown in the zone diameters produced by standard concentrations of cephalexin in normal and jaundiced human serum or horse serum. A typical result is shown in Table I. The concentrations of cephalexin found in the samples from patients in group 1 are shown in the Chart. There was wide variation in both the bile and serum levels over the six-hour period studied. Probenecid given to block the renal excretion of

TABLE I—*Zone Diameters in mm (in triplicate) Obtained from Standard Additions of Cephalexin to Horse Serum and Jaundiced Human Serum*

Concentration of Cephalexin (μg/ml)	Horse Serum			Jaundiced Serum		
1	0	0	0	0	0	0
3	18	18	17·5	17·0	18·5	18·0
5	21·5	22·0	21·5	23·0	21·5	22·0
10	28·5	27·5	28·0	26·5	27·0	26·0
20	31·5	32·0	32·5	32·0	30·5	31·5



Concentrations of cephalexin in samples from patients in Group 1.

cephalexin in five patients produced a statistically significant (analysis of variance P <0·025) increase in cephalexin levels in the bile (Table II).

In group 2 patients after multiple doses high levels of cephalexin (14·4-92 μg/ml) were achieved in the bile unless radiology indicated that gall-bladder function was impaired, when levels of <1·5-27·2 μg/ml were obtained (Table III). At the time of operation the serum cephalexin levels were 3 μg or less per ml except in one patient with a non-functioning gall bladder (Table III, Case 13) in whom the concentration in the gall-bladder bile was 12·0 μg/ml when that in the serum was 18·4 μg/ml. This patient was also unusual in having 19·5 μg of cephalexin per ml of common duct bile and 7·4 μg/g of gall-bladder wall. The concentration in common duct bile was measured in three other patients. One who had no cephalexin measurable at other sites (Case 9) had no measurable cephalexin in the common duct bile. The other two both had high gall-bladder bile concentrations; the one with a functioning gall

TABLE II—*Bile Levels of Cephalexin (in μg/ml) with (P) and without (C) Probenecid in Five of the Nine Patients in Group 1*

		Time in Hours					
		1	2	3	4	5	6
Case 1..	C	0·0	0·0	5·8	7·0	1·75	3·2
	P	0·0	7·0	7·2	21·4	10·0	7·2
Case 2..	C	0·0	18·8	14·0	8·7	4·8	1·65
	P	18·4	34·0	24·0	9·0	6·3	5·1
Case 3..	C	3·45	2·3	3·0	0·0	0·0	1·7
	P	3·3	4·8	2·7	3·7	3·0	1·5
Case 4..	C	0·0	2·5	1·5	2·8	8·0	8·9
	P	9·2	6·1	3·5	3·0	1·5	0·0
Case 5..	C	2·9	6·5	7·2	3·35	1·9	0·0
	P	7·2	32·2	12·0	2·2	1·5	0·0

Difference significant by analysis of variance (P <0·025).

bladder (Case 3) had 40 μg/ml of common duct bile, and the other, with a non-functioning gall-bladder (Case 8), had 9·6 μg/g. In the two patients with complete common bile duct obstruction no antibiotic was excreted in the bile.

TABLE III—*Cephalexin Concentration in Gall-bladder Bile and Wall of Group 2 Patients in whom Gall Bladder was Radiologically Functioning or Non-functioning*

Case No.	Cephalexin Concentration	
	Gall Bladder Bile (μg/ml)	Gall Bladder Wall (μg/g)
	Functioning	
1	40	2·6
2	40	2·9
3	92	13·6
4	14·4	<1·5
5	29·0	2·4
6	50·0	5·0
	Non-functioning	
7	7	<1·5
8	27·2	2·3
9	<1·5	<1·5
10	8·6	<1·5
11	7·8	<1·5
12	1·7	2·0
13	12·0	7·4

Discussion

The results confirm that cephalexin is rapidly absorbed from the alimentary tract and show that it is excreted in the bile, peak levels being obtained after two to three hours. This contrasts with the findings of Acocella *et al.* (1968), who showed that orally-administered ampicillin, erythromycin, novobiocin, and tetracycline appeared irregularly in the bile, and peak levels were usually delayed for 6-12 hours. Probenecid administered shortly before or simultaneously with the cephalexin in five of the patients in group 1 significantly increased the biliary levels of the antibiotic in all five over a six-hour period.

In the patients given multiple doses of cephalexin before cholecystectomy (Group 2) high antibiotic levels were found in the gall-bladder bile of those with radiologically functioning gall bladders (Table III). Most of the patients with normal liver function but non-functioning gall bladders had much lower levels, but measurable antibiotic was present in all but one case.

In patients with common bile duct obstruction penicillin (Zaslow *et al.*, 1950), oxytetracycline, erythromycin, and spiramycin (Leurat *et al.*, 1957) are excreted, though not always in therapeutic concentrations. Rifampicin, which generally reaches high levels in the bile, is not excreted where there is complete obstruction (Khan and Scott, 1967), nor is ampicillin (Mortimer *et al.*, 1969) or, judging from the present two cases (group 3), cephalexin. With one exception, levels in the gall-bladder wall were generally less than 3 μg/g unless the levels in the bile with which the wall was in contact were unusually high (Table III). The levels of cephalexin found in the radiologically functioning gall bladders after multiple doses exceeded the minimum inhibitory concentration (Waterworth, 1971) for sensitive strains of *Escherichia coli* and *Salmonella typhi*, but not those for *Streptococcus faecalis* or *Pseudomonas aeruginosa*. When the gall bladder was radiologically non-functioning, levels above the mean inhibitory concentrations for sensitive enterobacteria were achieved in most cases.

The resistance of *Str. faecalis* and a number of enterobacteria (Waterworth, 1971) makes cephalexin unlikely to have any particular advantage over other antibiotics used in the treatment of infections associated with obstructive biliary tract disease. In addition, its oral route of administration may preclude its use in seriously ill patients. If it is to be used, probenecid may be given to increase its biliary levels.

On the other hand, the extreme sensitivity of typhoid bacilli (Waterworth, 1971) and the levels of cephalexin obtained in the bile suggest that a trial of its use in salmonella carriers may be worth while. It was suggested by Tynes and Utz (1962) and by Bullock (1963) that the treatment of salmonella carriers with ampicillin was less successful in the presence of gall-bladder disease because of failure of the antibiotic to penetrate into the lumen of the gall bladder (Mortimer *et al.*, 1969). In the present trial cephalexin appeared in concentrations greater than the mean inhibitory concentration for *Salm. typhi* in the gall-bladder bile of all but one patient with radiological evidence of non-function of the gall bladder.

We would like to thank Mr. R. A. Payne, of the North Middlesex Hospital, London, for allowing us to study patients under his care, and Dr. J. Grabinar for his technical help.

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