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THE PLACE OF RIFAMYCIN-B-DIETHYLAMIDE IN THE TREATMENT OF CHOLANGITIS COMPLICATING BILIARY OBSTRUCTION

BY

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The rifamycins are a group of chemically related substances isolated from the fermentation broth of *Streptomyces mediterranei* (Sensi, Margalith & Timbal, 1959). Rifamycin B has been the most intensively studied of the natural rifamycins and chemical modification of this molecule has led to the production of two potent antibiotics. Rifamycin SV was the first to be synthesized and studied and is the subject of a recent extensive review (Bergamini & Fowst, 1965). Rifamycin-B-diethylamide is a more recently developed derivative which has comparable *in vitro* activity against a similar range of organisms (Pallanza, Fürész, Timbal & Carniti, 1965) but is significantly more active in experimental animal infections, probably because of greater tissue permeability (Fürész, Arioli & Scotti, 1965). Its structural formula is seen in Fig. 1. Its chief activity is against Gram positive organisms and the minimum inhibitory concentration of this drug for such organisms as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Diplococcus pneumoniae* lies between 0.01 and 0.1 μg/ml., which is the same order of potency as is found with penicillin G. The minimum inhibitory concentration for gram negative organisms is higher, and Pallanza and others (1965) report values of 20 μg/ml. for *Escherichia coli* and 50 μg/ml. for *Pseudomonas aeruginosa*. Dezulian, Serralunga &

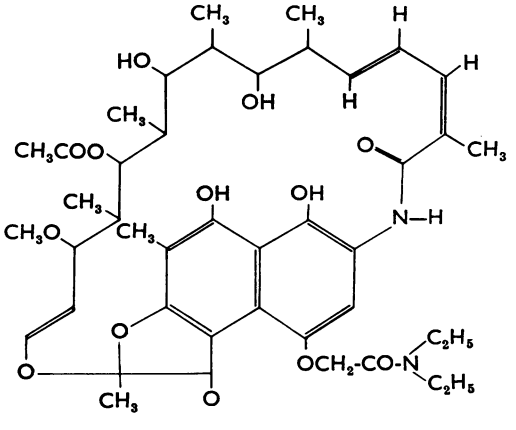

Fig. 1. Rifamycin-B-diethylamide.

Maffii (1966) have shown the compound to be without toxicity in dogs in doses of 100 mg/kg/day for 6 months, and, in patients given rifamycins to date, reports of toxicity are virtually confined to rare hypersensitivity phenomena (Bergamini & Fowst, 1965).

The rifamycins have been shown to be excreted in high concentration in the bile, where the antibiotic activity in animals and patients with an unobstructed biliary system reaches levels of 1,000–2,000 μg/ml. (Fürész *et al.*, 1965; Acocella, Lamarina, Tenconi & Nicolis, 1966). Hence, although the sensitivity of the usual organisms found to be responsible for bacterial cholangitis (the Gram negative commensals and group D. streptococci) is not comparable with that of the pyogenic cocci, the levels of the drug obtained in bile should theoretically be such as to inhibit even these less sensitive bacteria. The bile concentrations quoted, however, have been obtained in subjects with normal or barely compromised liver function and, in order to establish a place for this drug in the typical patient with cholangitis who has considerably impaired liver function, it was necessary to show whether effective bile levels could be achieved in such patients. In order to study this point we have measured the biliary concentration of the drug in a group of patients with varying degrees of biliary obstruction both with and without cholangitis to try to define the indications for its use.

METHODS

Twenty patients with a history of obstructive jaundice admitted to the Royal Free Hospital, London, have been studied. Of these five (Group A) had malignant tumours obstructing the large bile ducts and deep jaundice, eight (Group B) had choledocholithiasis and had not previously undergone biliary surgery, one patient (C) had recurrent cholangitis following a cholecystduodenostomy, one patient (D) had sclerosing cholangitis complicating ulcerative colitis, and five had had previous biliary surgery and had biliary strictures (Group E).

The specimens of bile for bacteriological study were taken preoperatively through the catheter used for obtaining a percutaneous cholangiogram by the method of Shaldon, Barber & Young (1962) or, in the instances where that technique failed, by direct puncture of the bile ducts during the course of laparotomy. Viable bacterial counts were carried out on the specimens (Miles & Misra, 1938). When percutaneous cholangiography was successful (10 patients) rifamycin-B-diethylamide was then given by an intramuscular injection of 300 mg in all except patient B7 who, on account of her low body weight, was given only 150 mg. Bile samples were then obtained at timed intervals through the cholangiography catheter, which was allowed to drain bile up to the moment of laparotomy from 1–3 hr later. These were the only bile samples it was possible to obtain from the patients in Group A, but in other patients, in whom a T tube was subsequently placed in the duct, samples were collected over a more extended period after a second injection of the drug on the second postoperative day. When percutaneous cholangiography was not successful (10 patients) the first injection of rifamycin-B-diethylamide was delayed until the first post-operative day and all the bile samples were taken from the T tube.

Blood was taken at 1 to 2 hr after the administration of the drug in patients A1–A4 and in the majority of the other patients three samples were taken at 1, 2 and 4 hr.

The concentration of rifamycin-B-diethylamide in bile was determined by the cup plate assay method using spore suspensions of *Bacillus subtilis* ATCC 6633 as the test organism. Pennassay base agar (Difco No. 2) was seeded with 0.8% of the test suspension and distributed in sterile petri dishes in 12 ml. volumes to give an agar depth of 4 mm. Plugs of agar were removed to give an internal cup diameter of 6 mm. These were filled with the sample under test alternating with the reference standard, thus compensating for variations in the thickness of the agar. The plates were incubated at 37° C for 18 hr. The zones of inhibition were then measured and the antibiotic concentration determined by reference to a standard curve. The same procedure was followed in determining antibiotic concentration in serum, except that Pennassay seed agar (Difco. No. 2) was used as the medium and *S. aureus* 209 P ATCC 6538 as the reference organism.

RESULTS

The concentration of rifamycin-B-diethylamide in bile

The findings are presented in Table 1 where the results of assays of the antibiotic in bile are combined with relevant clinical details and the result of bacteriological studies of bile. No distinction is made between T tube and percutaneous cholangiography samples since the two methods of collecting bile gave essentially similar results.

TABLE 1

RIFAMYCIN-B-DIETHYLAMIDE CONCENTRATION IN BILE COMPARED WITH THE DURATION AND DEPTH OF JAUNDICE AND THE BACTERIOLOGICAL FINDINGS

Subject	Rifamycin-B-diethylamide levels (hr after administration) (μg/ml.) 1	2	3	4	6	8	Serum bilirubin	Alkaline phosphatase (King-Armstrong units)	Duration of symptoms of obstruction (months)	Clinical cholangitis	Bacteria isolated	Minimum inhibitory concentration (μg/ml.)
Group A	Patients with malignant obstruction											
1	1·4	1·4	—	—	—	—	12·5	78	3	No	Sterile	
2	0	2·1	—	—	—	—	21·0	56	2	No	Sterile	
3	—	3·0	—	—	—	—	19·0	85	5	No	Sterile	
4	1·3	—	—	—	—	—	13·0	115	3	No	Sterile	
5	4·0	17·0	—	—	—	—	32·0	90	3	No	Sterile	
Group B	Patients with choledocholithiasis											
1	175	960	2200	1600	1350	—	0·7	20	3	No	Sterile	
2	350	1100	—	800	350	200	1·4	48	4	No	*E. coli*	60
3	43	600	690	—	65	40	1·6	24	6	No	Sterile	
4	5	370	510	—	450	275	1·0	40	3	No	*Str. faecalis*	15
5	85	275	500	420	310	—	2·0	34	14	No	*Str. faecalis*	15
6	15	130	340	410	500	290	3·0	80	12	Yes	*E. coli*	60
											Str. faecalis	15
											K. aerogenes	60
											Ps. pycocyanea	60
											P. mirabilis	15
7	100	150	295	390	500	380	3·1	132	4	Yes	*E. coli*	60
											Str. faecalis	15
8	80	240	300	280	270	155	8·0	38	11	Yes	*Ent. aerogenes*	500
Group C	Recurrent cholangitis without demonstrable obstruction											
1	150	550	720	520	475	230	1·6	28	7	Yes	*Str. faecalis*	15
											P. vulgaris	30
Group D	Sclerosing cholangitis complicating ulcerative colitis											
1	5	275	615	—	40	—	6·0	43	3	No	Sterile	
Group E	Patients with biliary strictures											
1	175	430	520	275	120	—	2·4	84	24	Yes	*E. coli*	60
2	—	70	230	400	350	300	4·5	95	48	Yes	*K. aerogenes*	60
3	40	—	125	200	70	30	3·0	108	33	Yes	*E. coli*	60
											Str. faecalis	15
4	0	15	65	105	85	5	6·5	111	24	Yes	*E. coli*	60
											Str. faecalis	15
5	0	25	100	45	30	—	3·0	54	58	Yes	*E. coli*	60
											K. aerogenes	60
											Str. faecalis	15

Among the patients with malignant obstruction of the major bile ducts, in all of whom obstruction was by clinical criteria complete, four produced antibiotic levels that were no greater in bile than in serum. In the fifth patient a modest degree of concentration in bile was achieved. The bile was sterile in all the members of this group.

The patients with choledocholithiasis without previous biliary surgery showed varying degrees of obstruction which had been present for varying periods of time. In the table the subjects are arranged in decreasing order of peak bile concentrations of antibiotic. It is clear that the peak bile concentration is inversely related to both the depth of jaundice and the duration of symptoms of obstruction. Bacteria were present in six of the eight patients and in only one patient was an insensitive organism found. In the other five patients the organisms were sensitive to the drug in a concentration at least eight times less than that in fact achieved.

The patient with recurrent cholangitis resulting from a cholecystduodenostomy but with no mechanical obstruction to bile flow produced an antibiotic level equal to 24 times the minimum inhibitory concentration of the least sensitive infecting organism.

The patients with biliary strictures had all had clinical jaundice constantly for over 2 years and all had had repeated attacks of cholangitis. In all the peak bile concentration exceeded the minimum inhibitory concentration of the infecting organism although by only a small margin in two.

Serum levels

The serum levels were inversely related to peak bile concentrations and this is illustrated in Fig. 2 where serum and bile levels for subjects B1 and E5 are contrasted.

Bacteriological findings in bile

Total viable bacterial counts in the patients with positive cultures ranged from 8×10^5 (B2) to 2×10^9 (E4).

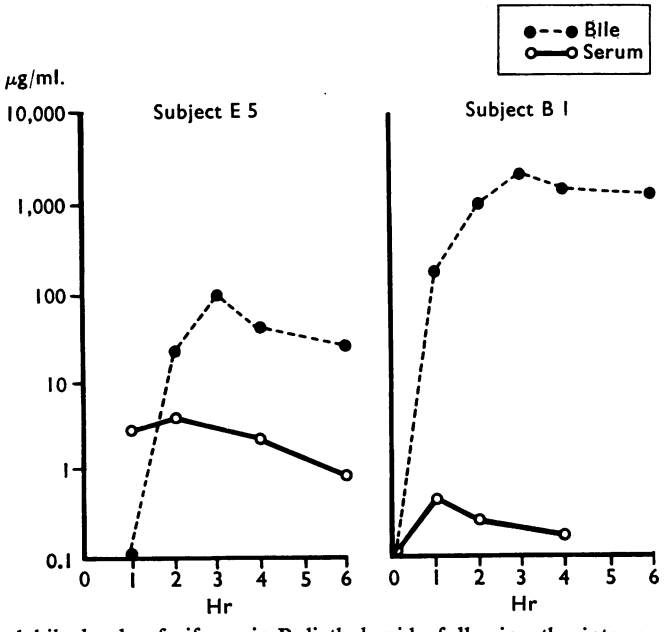

Fig. 2. Serum and bile levels of rifamycin-B-diethylamide following the intramuscular injection of 300 mg of the drug in a patient (E5) with poor liver function and a patient (B1) with good liver function.

DISCUSSION

Bacterial cholangitis is nearly always a complication of obstruction of the major bile ducts and the definitive treatment of the condition is clearly by surgery. The roles that can reasonably be allotted to antibiotics seem therefore to be two: to suppress clinical attacks when surgery cannot be undertaken, and as a prelude to surgery itself in an effort to render the infectivity of bile as low as possible. The latter seems justifiable in view of the demonstrated frequency of bacterial infection in choledocholithiasis as shown both in this study and others (Elkeles & Mirizzi, 1942; Edlund, Mollstedt & Ouchterlony, 1959).

Rifamycin-B-diethylamide could fill these roles. In 11 of the 12 infected patients the organisms isolated were inhibited by levels attained in bile. A much higher proportion of the organisms found in cholangitis would be expected to be inhibited by this drug than by other antibiotics that achieve effective bile levels—namely, novobiocin, erythromycin, the tetracyclines and among the penicillins especially ampicillin (Brette, Lambert & Truchot, 1965).

With rifamycin-B-diethylamide, as with any antibiotic, the results will be limited by the impossibility of permanently sterilizing an obstructed duct, and by the degree of impairment of liver excretory function. With the other antibiotics mentioned drug resistance on the part of the invading microbes is more likely to provide the limiting factor.

Impairment of the excretory function of the liver provides a very important limiting factor. If cholangitis had developed in the patients with malignant disease (perhaps as an early complication of surgery, which occurred in patient A5) it would not be possible to achieve therapeutic levels against typical Gram negative organisms. Nor can the levels achieved in patients E3, 4 and 5 be regarded as providing a large enough margin to be satisfactory. Deep jaundice and cholestasis of whatever degree prolonged beyond $1\frac{1}{2}$ yr are indications that the drug is unlikely to be effective.

Concentrations of rifamycin-B-diethylamide high enough to inhibit Gram negative bacteria are found in bile only when the drug is used in conventional doses. Such levels cannot be expected in gall bladder bile when the cystic duct is occluded and the drug is unlikely to be effective in acute cholecystitis. The role of antibiotics in this disease is in any case poorly defined and probably limited (Zazlow, 1953). Effective tissue levels would be expected to be equally as important as effective bile levels and the former cannot be obtained with the rifamycins.

The high levels of rifamycin SV reached in the bile are achieved at the expense of bilirubin excretion, which is impaired. Acocella, Nicolis & Tenconi (1965) have shown that after large doses of this drug elevation of serum bilirubin can be demonstrated. Similar mechanisms probably apply to rifamicin-B-diethylamide but we have not observed such an effect. Evidence provided by Maffii & Schiatti (1966) in animals and Acocella *et al.* (1966) in man suggests that the effect is less pronounced with the newer drug.

Impairment of bilirubin excretion is attributed to interference or competition after the point at which bilirubin is conjugated and also occurs with drugs such as sulphobromophthalein sodium and indocyanine green which have been used for many years. It can fairly safely be dismissed as a toxic hazard. The effect can be contrasted with that of novobiocin

which appears to interfere with bilirubin conjugation, causing an elevation of unconjugated bilirubin.

One may conclude that rifamycin-B-diethylamide is a safe drug, which used in patients with good or only moderately impaired hepatic excretory function, should effectively suppress cholangitis. The need for new effective drugs in this disease is a very real one in view of the increasing resistance of Gram negative organisms to those at present available. It is as pressing as the more widely recognized need for new drugs to treat resistant infections of the renal tract.

Our limited clinical experience shows that, although not an ideal drug for the reasons outlined, because its effectiveness will usually be confined to bile rather than the infected walls of the bile ducts and gall bladder, and because it requires parenteral administration, rifamycin-B-diethylamide will at times satisfy this need.

SUMMARY

1. The concentration of rifamycin-B-diethylamide has been studied in the bile of 20 patients with obstructive jaundice and the levels compared with the *in vitro* sensitivity of organisms isolated from the bile of 12 of these subjects who had cholangitis.

2. Adequate antibiotic activity in bile is shown to depend on the preservation of at least moderately good liver excretory function.

3. Despite this limitation it is suggested that the drug will have a place in the treatment of cholangitis in many patients.

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