Effect of Colistin on Reduction of Biliary Flow Induced by Endotoxin of *E. coli*

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In order to evaluate whether an antiendotoxin agent can inhibit the action of endotoxin of Escherichia coli *on biliary flow, we used 18 models of isolated perfused pig liver divided into four groups: (A) five perfused livers (control), (B) five perfused livers with 2 mg/100 ml of endotoxin added, (C) five perfused livers with 2 mg/100 ml of endotoxin added and treated with 1,500,000 IU of colistin, and (D) three perfused livers with 1,500,000 IU of colistin with no endotoxin. The livers were isolated and perfused according to a technique previously described by our laboratory. The viability of the perfusions was controlled by means of the mitochondrial respiration test. Transaminase and LDH levels were measured in the perfusion circuit. A significant reduction of the biliary flow was found in the group with endotoxin of* Escherichia coli *($P < 0.002$). There was no reduction of biliary flow after addition of 1,500,000 IU of colistin ($P < 0.001$). No significant changes were observed in the other parameters measured, and no increase of the bile flow was observed in the colistin group. From our results we conclude that colistin is an inhibitor* in vitro *of the diminished biliary flow induced by endotoxin of* Escherichia coli.

It seems to be well demonstrated that endotoxin absorbed from the intestinal lumen plays an important role in the hepatic and extrahepatic manifestations of liver disease (1–4). It has been suggested that the possible failure of the endotoxin detoxification process might initiate or perpetuate hepatic damage (5). Among the multiple effects that have been attributed to endotoxin, its ability to decrease bile flow has been demonstrated experimentally (6, 9).

The present study examines the effects of colistin, a therpeutic agent presumed to have antiendotoxin activity, in inhibiting the cholestatic action induced by endotoxin.

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MATERIALS AND METHODS

Experimental Animals. Iberian strain swine, 6–8 months old and weighing 20–22 kg, were used.

Endotoxin. *Escherichia coli* endotoxin commercially obtained from Difco Laboratories (*E. coli* 0127 and 138) was used. The endotoxin was solubilized in pyrogen-free saline immediately before use.

Analytical Determinations. Transaminase, lactic dehydrogenase, and alkaline phosphatase were reagent grade and purchased from commercial sources.

Colistin. This agent was commercially obtained from IFL (Spain) in vials containing 25 mg colistin metasulfonate (equivalent to 1,500,000 IU of colistin base).

Perfusion Procedure. The swine were kept fasting for 24 hr preceding surgery, although they were permitted free water intake. One hour before surgery, and while still in the animalarium, they were premedicated with Thalamonal, 2.5 mg intravenously. Anesthetic induction was carried out with thiopental sodium, 12 mg/kg body weight. Tracheostomy was performed immediately after induction, and a Rusch No. 14 latex cannula with inflatable cuff was inserted and connected to a Harvard-type respirator. Anesthesia was maintained with a mixture of halothane and oxygen (proportion 2:3), with the respirator set at a rate of 14 ventilations per minute. During the induction phase, ketamine hydrochloride was adminis-

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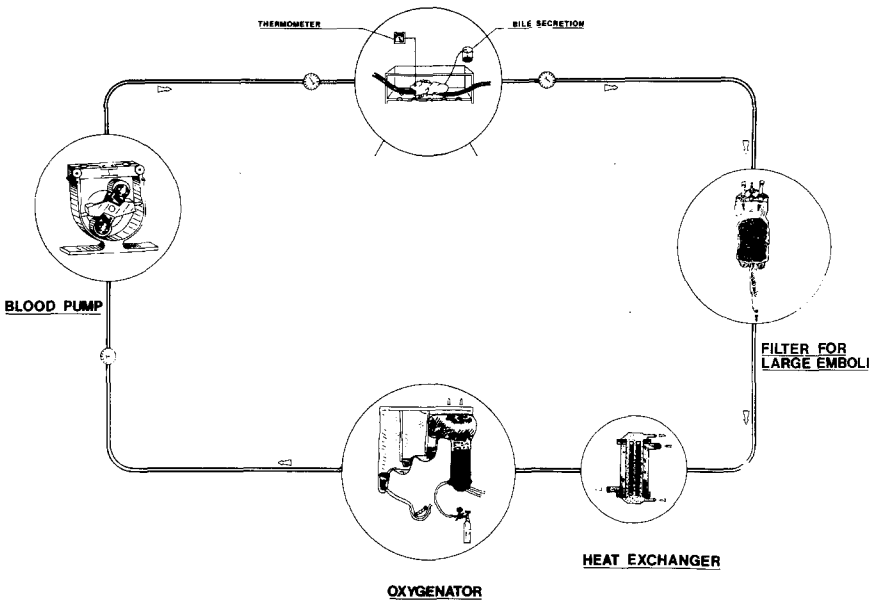

Fig 1. Liver perfusion circuit.

tered at a dosage of 20 mg/kg body weight, intramuscularly. Whenever needed, a muscular relaxant agent (Pavulon) was administered as an adjuvant to anesthesia. Control of anesthesia was effected over the corneal and palpebral reflexes.

Once anesthesia was achieved, and intravenous drip was maintained with 500 ml isotonic saline to which had been added 10 mEq sodium bicarbonate and 3 mg/kg heparin, while blood samples were collected for baseline analytical controls.

Median laparotomy was carried out and, with minimum liver manipulation, the portal vein, hepatic artery, supra- and infrahepatic inferior vena cava and common bile duct were dissected.

After ligature of the cystic duct for exclusion of the gallbladder, the common bile duct was cannulated with a polyvinyl catheter. The liver was then removed and weighed, and the portal and infrahepatic vena cava were cannulated. Immediately after removal, the liver was washed with Ringer–lactate solution with 50 mg heparin and 20 mEq/liter sodium bicarbonate at a temperature of 4° C, with the help of a pump (Sarns roller pump) and at an approximate portal flow of 400–600 ml/min, variable according to the liver weight. The temperature of the liver after washing ranged between 12 and 14° C, and the total ischemia time was 12 ± 2 min (X ± SD).

After washing, the liver was placed in a closed chamber, resting with its diaphragmatic aspect on a multiperforate silicone membrane, and was connected to a closed perfusion circuit which had been previously primed with autologous blood diluted 1:1 with Ringer–lactate solution (Figure 1).

Perfusion over the portal vein was carried out with a Travenol roller type blood pump (model 5M 0053) after oxygenation with a Rigg-Kyvsgaard pediatric-type oxygenator and heating in a Travenol heat exchanger, keeping the liver at a temperature of 36–38° C throughout the perfusion time.

After passing through the liver and exiting via the inferior vena cava, the blood was passed through a silicone filter, isolated from the atmosphere, and thereafter led to the oxygenator. The oxygenator's capacity was 1.200 ml, and the capacity of the full circuit was 1.500 ml. Perfusate lost in the circuit was compensated by adding autologous blood so as to maintain the initial priming volume and priming hematocrit. The temperature of the liver and of the perfusion circuit was continuously monitored by means of a Sarns telethermometer, and the blood flow and pressures both at the entrance to the liver and at the entrance to the oxygenator were monitored by means of Mycotrol blood flow meters and Telco M5 pressure manometers.

The bile flow was collected via the previously inserted catheter in the common bile duct and measured every 60 min during the full perfusion time.

The following analytical measurements were carried out at 30-min intervals: blood gases, pH, Po_2, Pco_2 (Eschweiler Combi-Analysator); blood biochemistry (Technicon SMA 12/60 Swords), and transaminase following the Reitman-Frankel method (DADE Laboratories, Spain).

The viability of the preparations was assessed by the mitochondrial respiration test (7, 8).

Experimental Preparations. Four different groups of perfused livers were studied: (A) five perfused livers with no modification to the above procedure, which were used as controls; (B) five perfused livers to which 2 mg/dl endotoxin were added in the reservoir of the perfusion circuit; (C) five perfused livers to which 2 mg/dl endotoxin and 1,500,000 IU colistin were added in the reservoir of

TABLE 1. BILIARY FLOW DURING PERFUSION TIME (ML/HR)

Time (hrs)	*Group A*	*Group B*	*Group C*	*Group D*
1	1.8 ± 0.4	0.36 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
2	1.7 ± 0.3	0.29 ± 0.3	1.5 ± 0.2	1.8 ± 0.4
3	2.0 ± 0.2	0.46 ± 0.2	1.7 ± 0.3	1.5 ± 0.5
4	1.6 ± 0.4	0.20 ± 0.3	1.8 ± 0.3	1.6 ± 0.4
5	1.5 ± 0.5	0.50 ± 0.3	1.8 ± 0.4	1.8 ± 0.4
6	1.8 ± 0.4	0.36 ± 0.4	2.1 ± 0.4	2.9 ± 0.2

the perfusion circuit; and (D) three perfused livers to which 1,500,000 IU colistin were added in the reservoir of the circuit.

Statistical Analysis. All results are expressed as mean ± standard deviation; the plus–minus test has been used to compare the means, and the 5% level was taken to be significant.

RESULTS

Bile Flow. Comparing the three treatment groups with the control group, we have observed a significant reduction of the bile flow ($P < 0.002$) in group B, which had received endotoxin only, as had been previously demonstrated (6, 9) (Table 1). On the contrary, there was no reduction of the bile flow in group C, which had received 1,500,000 IU colistin, in spite of its having received the same dose of endotoxin as group B. There was no modification of the bile flow, as compared with the control group, in the three preparations treated only with colistin (Figure 2).

In none of the groups did we observe significant change in the perfusate pressure, although this parameter had been most carefully monitored during the perfusion time, especially in the case of the two endotoxin groups.

Hepatic Enzyme Release. We have studied the effect of endotoxin on the release of enzymes (GOT, GPT, LDH, and alkaline phosphatase), but we have detected no significant modifications in any of the groups.

Optic Microscopy. A liver wedge was collected from all specimens for histologic study. None of the specimens showed significant alterations.

DISCUSSION

The present study seems to demonstrate that the cholestatic action of the *Escherichia coli* endotoxin may be inhibited by the effects of an antiendotoxin agent such as colistin and that this effect is not due to any choleretic action of colistin.

Cholestasis in patients with infection by gram-negative bacteria is a phenomenon quite familiar to pediatricians, as it is more frequent in the neonatal period (1, 10). In the adult, this phenomenon is much less frequent and usually much more moderate (4), except in cases of several sepsis (11–13) or in pregnancy (14). In many of these patients alkaline phosphatase and cholesterol plasma levels are not elevated, which speaks against a mechanism of decreased bile flow. However, liver histology reveals intrahepatic cholestasis with Kupffer cell hyperplasia and no evidence of cellular necrosis. In the neonate, the most frequent etiologic agent is *Escherichia coli,* and the most frequent localization is the urinary system (1, 15). In the adult patient, however, diverse types of severe abdominal infection may be responsible for the syndrome (11–14, 16).

The pathogenesis of hepatic involvement during nonhepatic bacterial infections might be related to direct invasion of the parenchyma by the infectious agents or to structural or functional modifications caused by circulating toxins. The frequent appearance of cholestasis, often without bacteremia, and the similarity of the manifestations during infections caused by diverse gram-negative bacteria suggest that circulating endotoxin has a pathogenetic role in the hepatic alterations observed in these conditions.

Endotoxin is a lipopolysaccharide complex situated exteriorly on the cell membrane of the bacterial cell wall (23). The intrinsic toxicity of the toxin is

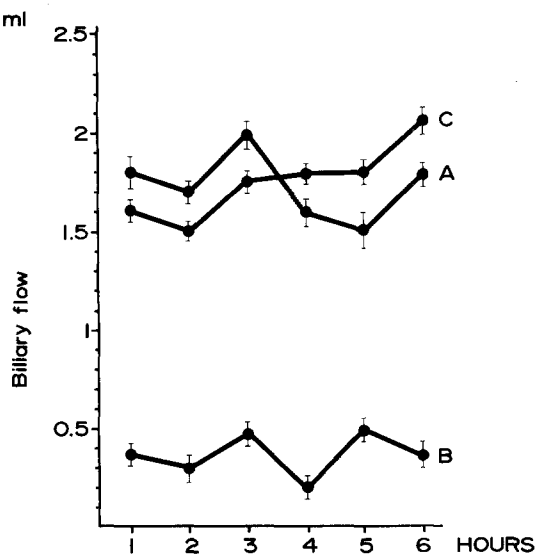

Fig 2. Effect of colistin on the bile flow rate diminished by *E. coli* endotoxin in the isolated pig liver. Group A, control group; group B, 2 mg/dl endotoxin was added in the reservoir of the perfusion circuit; group C, 2 mg/dl *E. coli* endotoxin plus 1,500,000 IU colistin was added in the perfusion circuit.

linked to the lipid moiety (lipid A), while the polysaccharide moiety (O-antigen) mediates the serological specificity. The lipid A portion has a similar structure in all Enterobacteriaceae, a circumstance that might explain the similarity of biological effects of endotoxins derived from different sources.

In order to verify the hypothesis that jaundice associated with gram-negative infection might be due to the cholestatic effects of the endotoxin, the effects of the endotoxins of *Escherichia coli* and *Salmonella enteritidis* on the bile flow have been studied on isolated livers (6, 17). These studies have demonstrated that both endotoxins cause a diminution of the bile flow, which is dependent on the administered dose and on the excretion of organic anions. Analysis of the action of the lipopolysaccharide complex on the bile flow in the same experimental preparation reveals a selective reduction of the non-bile-salt-dependent bile fraction.

It is generally accepted that the non-bile-salt-dependent bile fraction is regulated by the activity of the membrane Na^+, K^+, and ATPase which controls the active transport of sodium (18). Accordingly, the demonstration that both endotoxins, at identical concentrations, exert a selective inhibition on the activity of Na^+, K^+, and ATPase, which are dependent on the administered dose, would support the conclusion that the cholestatic effect of the lipopolysaccharide complex is exerted mainly on the non-bile-salt-dependent bile fraction (19).

Endotoxins show a great affinity for biological membranes. Shands (20) has suggested that the lipopolysaccharide complex might induce disorganization of such membranes and that this phenomenon might trigger some of its biologic effects. From the results of our observations and those of other authors using experimental models, both *in vitro*, we might derive a reasonable explanation of the cholestatic effect during infections caused by gram-negative bacteria. In this sense, the effects of the lipopolysaccharide complex could be increased, with a resulting cholestatic syndrome (22, 23).

If the endotoxin or the products derived therefrom initiate or aggravate a hepatic lesion and influence the peripheral manifestations of that lesion, such as functional renal failure and erosive gastritis (28), we would think that an antiendotoxin agent which is able to modify toxicity might be protective against such effects, and it is evident that all efforts in this direction would be conducive to beneficial therapeutic results. Rifking (24) used polymyxin B as a potent neutralizer of the effects of endotoxin; this neutralizing action seems to be independent of its antibacterial activity, since other antibiotics, such as gentamicin sulfate, do not have this action despite possessing a similar antibacterial spectrum. On the other hand, it has been demonstrated that the maximal neutralizing action of polymyxin B is exerted on lipopolysaccharides derived from a strain of *Pseudomonas* which is resistant to the antibacterial action of the drug (25).

Our study does not allow differentiation between an *in vitro* neutralization of endotoxin by colistin and a reversal of the endotoxin effects induced in the liver by this antibiotic. However it might be that colistin would act as polymyxin B.

The mode of action of polymyxin B seems to be that of formation of electrostatic and hydrophobic interactions with the endotoxin molecule and would be thus related to its cationic and detergent properties (26). Electromicroscopic studies have demonstrated the dissolution of the structure of the lipopolysaccharide after incubation with polymyxin B (27).

The present study shows that colistin, a drug pharmacologically similar to polymyxin B, has a neutralizing effect on the endotoxin, at least *in vitro*. Our results, show that colistin protects against the cholestatic action induced by the endotoxin, and it is possible that it also inhibits its systemic actions.

There is some evidence regarding the need for endotoxin in order that other hepatic toxins may exert their toxic effects. As polymyxin B is highly toxic, its clinical success is limited; nevertheless, it is possible that other less toxic agents which are able to break up the lipopolysaccharides might have success in cases of hepatic involvement.

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