JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 220-223

Vol. 100, No. 1
Printed in U.S.A.

Effect of Bile Acids on the Intestinal Absorption of Endotoxin in Rats

L. T. KOCSÁR, L. BERTÓK, AND V. VÁRTERÉSZ

"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

Received for publication 11 March 1969

The absorption of tritium-labeled *Escherichia coli* O89 Westphal-type endotoxin from the peritoneal cavity of rats was diminished by bile by 23% and by sodium deoxycholate by 47%, respectively. Practically, there is no endotoxin absorption from the intestinal tract of normal rats. The bile duct of rats was chronically cannulated for experimental purposes. A significant amount of perorally administered endotoxin absorbed from the intestinal canal into the blood in the rats treated thus. Absorption was demonstrated by the lethal effect of endotoxin on rats previously hypersensitized by lead acetate, and by the radioactivities found in the blood samples. The intestinal absorption of endotoxin in rats, rendered bile-deficient, may be prevented by sodium deoxycholate. Supported by their experimental findings, we emphasize the important role of bile acids in the defense mechanism of the macroorganism against bacterial endotoxins.

It is well known that gram-negative bacteria, *Escherichia coli* strains, or their endotoxins, may induce so-called enterotoxemia in new-born children, calves, and piglets (27). However, under experimental conditions, bacterial endotoxins produce a fatal shock only if administered parentally (4). Thus, the main problem in the elucidation of the pathogenesis of these enterotoxemias is the absorption of endotoxin from the intestines. Earlier studies on endotoxin absorption gave contradictory results (2, 7, 9, 10, 13, 14, 17, 21, 22, 24, 25, 30, 31).

In our previous experiments, neither tolerance, nor any toxic effect could be induced by a large dose of perorally administered endotoxin in rats. No toxic effect was experienced even in rats hypersensitized to endotoxin by lead acetate and treated with a histamine liberator compound, 48/80, or X-ray irradiated, to damage the intestinal mucosa (3). In other earlier experiments, we could not demonstrate any change in toxicity and serological reactivity of perorally administered endotoxin in the gastrointestinal tract of rats, when endotoxin was reisolated, by the warm phenol-water method, from the intestinal content (1). However, in their physicochemical studies on bacterial endotoxins Rudbach and co-workers demonstrated a reversible inactivation of endotoxin in vitro by sodium deoxycholate treatment (23). Since the intestinal tract always contains various bile acids, which may have an effect similar to that of sodium deoxycholate, in a preliminary experiment we tried to remove the intestinal content from a ligated intestinal loop of rats by washing out with physiological saline prior to the intraintestinal injection of endotoxin. We found that the intestinal contents, or the surface-active agents within, have an important role in the defense mechanism against endotoxin absorption under physiological conditions in vivo. This has induced us to launch this series of experiments.

MATERIALS AND METHODS

Female rats of Wistar origin (120 to 200 g each) were used. The following compounds were used: *E. coli* O89 lipopolysaccharide Westphal-type endotoxin, prepared in this laboratory by the warm phenol-water method (28) and tritiated by Wilzbach's method (29); lead acetate (Fisher Scientific Co., Fair Lawn, N.J.); sodium deoxycholate (Reanal, Budapest, Hungary); and bile, collected by sterile syringe from the gall bladders of starved rabbits immediately after killing. The specific activity of the tritium-labeled endotoxin was 572 μc/mg; the LD_{50} of both labeled and nonlabeled endotoxin preparations was 4 mg per rat.

The following surgical method was used. Because rats have no gall bladder, their common bile ducts were cannulated (8) under pentobarbital sodium anesthesia. After median laparatomy, a polyethylene tube was introduced into the common bile duct, fixed to the abdominal wall, and ducted out on the surface of the skin just below the processus xiphoideus. Every operation was done under aseptic conditions. After surgery, the animals were treated with penicillin and kept in individual cages. The animals were fed with commercial rat pellets. Only those animals were used which

were controlled for the undisturbed cannulating of bile a week after the operation. A polyethylene tube was used for the gavage (peroral treatment) of the rats.

Endotoxin-hypersensitivity in rats was produced by intravenous (iv) dose of lead acetate (5 mg of lead acetate per 100 g of body weight) by the method of Selye and co-workers (5, 6, 26).

Blood samples were collected from the tail vein of the rats. The radioactivity in blood sera was measured in Kinard's (12) solution.

RESULTS

In the first part of our experiments we studied the effect of bile and of a certain bile acid, sodium deoxycholate, on the absorption of endotoxin from the peritoneal cavity. The first thing was to establish, by way of intraperitoneal injection, the absolutely nontoxic dose of freshly pooled rabbit bile and sodium deoxycholate for rats. The absolutely nontoxic dose proved to be 0.2 ml of bile and 5 mg of sodium deoxycholate per 100 g of body weight, respectively. Before administering the endotoxin it was incubated in vitro, either with bile or sodium deoxycholate, for 1 hr, at 37 C. The endotoxin so treated was tested on 60 rats (Fig. 1).

As it appears from Fig. 1, bile reduces the absorption of tritiated endotoxin by 23%, and sodium deoxycholate by 47%, respectively, 1 hr after administration.

Next, one had to elucidate whether radioactivity measured in the blood samples is due to the original labeled endotoxin itself or originates from the disintegration products of the preparation, or whether there is any parallelism between the radioactivity and biological activity of endotoxin. To elucidate this problem, rats were rendered hypersensitive to endotoxin by lead acetate (5, 6, 26). These rats were used for testing the biological activity (endotoxin content) of sera from rats treated with ^{3}H-endotoxin intraperitoneally (ip) (Table 1).

As shown in Table 1, normal rat serum failed to provoke any reaction in the endotoxin-hypersensitive animals. On the other hand, sera from rats treated with ^{3}H-labeled endotoxin induced 100% mortality in hypersensitized animals. This finding suggests that radioactivity measured in the blood of rats treated with ^{3}H-labeled endotoxin (ip) corresponds to a certain amount of endotoxin, 3 μg or more. Namely, as demonstrated earlier, 3 μg of endotoxin is necessary to provoke a fatal shock in the lead acetate-treated rats (5, 6).

In the second part of our experiments, we studied the intestinal absorption of ^{3}H-labeled endotoxin in rats with chronically cannulated bile ducts. It is well known that no measurable quantity of endotoxin absorption from the intestinal tract can be detected in normal rats (3). However, when endotoxin was administered perorally into bile-deficient rats (induced by bile fistula), a significant amount of endotoxin absorption could be demonstrated. The arrangement and results of one of our experiments are in Table 2.

As shown in Table 2, the groups 1 and 2 served as controls. Practically none of the control animals died. On the contrary, when endotoxin was given perorally, and, after an interval of 3 hr, lead acetate iv, all of the animals died of typical endotoxin shock within 24 hr. At autopsy,

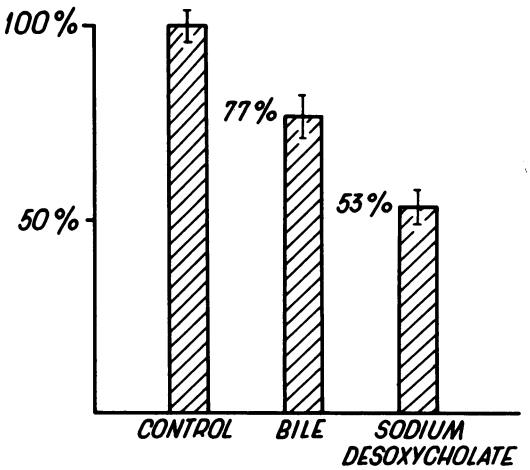

FIG. 1. *Effect of bile or sodium deoxycholate on the absorption of tritium-labeled endotoxin (1 mg) from the peritoneal cavity into the blood of rats 1 hr after administration.*

TABLE 1. *Toxic effect (endotoxin content) of sera from rats, treated with ^{3}H-labeled endotoxin (ip), in rats rendered endotoxin-hypersensitive by lead acetate*

Group[a]	Treatment	Death ratio (dead/total)
1	—	Killed
2	^{3}H-endotoxin (5 mg ip)	Killed (at 3 hr after treatment)
3	Lead acetate (5 mg iv) + normal rat serum (from group 1; 1 ml iv)	0/5
4	Lead acetate + ^{3}H-endotoxin-treated rat serum (from group 2; 1 ml iv)	5/5

[a] Blood from donor groups, 1 and 2, was collected by sterile puncture from the aorta abdominalis. Recipient groups, 3 and 4.

one could demonstrate the pathological changes characteristic of endotoxin shock.

The absorption of tritiated endotoxin was controlled by the determination of radioactivity in blood samples 90 min after the treatment. Table 3 shows the mean values of blood radioactivities in normal (without surgery) and in bile cannulated rats, respectively.

As it appears from Table 3, only a minimal amount of radioactivity absorbs from the intestines in normal rats. On the other hand, about five to six times higher activities can be detected in rats rendered bile deficient experimentally 90 min after the peroral administration of ^{3}H-endotoxin. We assume the majority of radioactivity measured in the blood of normal animals originated from the basic impurity and disintegration products of the endotoxin preparation.

Finally, in a preliminary experiment, we attempted to prevent by deoxycholate the intestinal absorption of endotoxin in rats with experimental bile fistula. The arrangement and result of this experiment are shown in Table 4.

As shown in Table 4, five out of six of the bile-deficient rats treated with endotoxin perorally and hypersensitized by lead acetate died. However, the nontoxic dose of sodium deoxycholate protects the majority of animals against the lethal effect of endotoxin.

TABLE 2. *Effect of chronic bile fistula on the absorption of perorally administered ^{3}H-endotoxin in rats*

Group	Treatment	Death ratio (dead/total)
1	^{3}H-endotoxin (5 mg per os after 0 hr) + lead acetate (5 mg iv after 3 hr)	0/5
2	Bile fistula + lead acetate (5 mg iv)	1[a]/5
3	Bile fistula + ^{3}H-endotoxin (5 mg per os after 0 hr) + lead acetate (5 mg iv after 3 hr)	5/5

[a] Died of intercurrent bile peritonitis.

TABLE 3. *Radioactivities in blood samples 90 min after per os administration of ^{3}H-endotoxin (5 mg)*

Group	Operation	Activities[a]
1	None	23,100 ± 1,650
2	Bile fistula	120,500 ± 6,760

[a] Expressed in counts per min.

TABLE 4. *Protective effect of sodium deoxycholate on the intestinal endotoxin absorption in rats with bile fistula, rendered hypersensitive to endotoxin by lead acetate*

Group	Treatment[a]	Death ratio (dead/total)
1	Bile fistula + lead acetate iv	1[b]/6
2	Bile fistula + endotoxin at 0 hr + lead acetate after 3 hr	5/6
3	Bile fistula + sodium deoxycholate + endotoxin at 0 hr + lead acetate after 3 hr	2/6

[a] Lead acetate, 5 mg iv. Endotoxin, 10 mg per os. Sodium deoxycholate, 40 mg, + endotoxin, 10 mg, per os after 1 hr of incubation in vitro of both at 37 C.

[b] Died of intercurrent bile peritonitis.

DISCUSSION

The absorption of endotoxins from the intestinal tract of mammals, both under physiological and pathological conditions, is a long-standing, but still current, problem. The majority of researchers could not demonstrate endotoxin absorption from the intestinal canal (2, 3, 7, 10, 13, 17, 24, 31). However, as found by Rauss and co-workers (18–20), Kétyi (11), and Ocklitz and co-workers (15, 16), the perorally administered endotoxin, as an antigen, is able to produce a specific immunity. The experiments of Rudbach and co-workers (23) permit an explanation of this discrepancy. We could suppose that in the gastrointestinal tract, bile acids, as surface-active agents, disintegrate (inactivate) the endotoxin macromolecule into smaller, atoxic fragments, still having antigenic properties. This disintegration is reversible. Namely, Rudbach and co-workers (23) proved that the surface-active agents, e.g. sodium deoxycholate, can disintegrate endotoxin in vitro and thus reversibly abolish some of its biological effects. In our present in vivo experiments we have found that the physiological surfactants, the bile acids, play an important role in the gastrointestinal tract in relation to the toxic properties of bacterial endotoxins. Provided the rats were rendered bile-deficient by the chronic cannulation of their common bile duct, the absorption of perorally administered endotoxin from the intestinal tract into the blood could be provoked in a quantity that was sufficient to elicit a typical endotoxin shock, experimental enterotoxemia. On the other hand, in a preliminary experiment, we could demonstrate some protective effect of sodium deoxycholate against the experimental enterotoxemia, as described above.

Our experimental findings suggest that bile acids play an important role in the defense mechanism of the macroorganism against bacterial endotoxins.

However, the mechanism of endotoxin absorption from the gastrointestinal tract of bile-deficient rats requires further elucidation.

ACKNOWLEDGMENTS

We are indebted to B. Jankó, E. Molnár, and Z. Urbán for their excellent technical assistance.

LITERATURE CITED

1. Berczi, I. 1968. Stability of *Escherichia coli* endotoxin in the rat gastro-intestinal tract. J. Pathol. Bacteriol. **96**:487–491.
2. Berczi, I., K. Baitner, and T. Antal. 1966. Comparative assay of endotoxins by oral and parenteral administration. Zentralbl. Veterinäermed. Reihe B. **13**:570–575.
3. Berczi, I., L. Bertók, K. Baitner, and B. Veress. 1968. Failure of oral Escherichia coli endotoxin to induce either specific tolerance or toxic symptoms in rats. J. Pathol. Bacteriol. **96**:481–486.
4. Berczi, I., L. Bertók, and T. Bereznay. 1966. Comparative studies on toxicity of Escherichia coli lipopolysaccharide endotoxin in various animal species. Can. J. Microbiol. **12**:1070–1071.
5. Bertók, L. 1968. Effect of sulfhydryl compound on the lead acetate-induced endotoxin hypersensitivity of rats. J. Bacteriol. **95**:1974–1975.
6. Bertók, L. 1968. Effect of endotoxin tolerance on the lead acetate-induced endotoxin hypersensitivity of rats. J. Bacteriol. **96**:569.
7. Culbertson, W. R., W. Elstun, W. Cole, and W. A. Altemeier. 1959. Bacterial studies in irreversible hemorrhagic shock. A.M.A. Arch. Surg. **79**:185–189.
8. Ericksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. Proc. Soc. Exp. Biol. Med. **94**:578–582.
9. Greene, R., T. Wiznitzer, S. Rutenburg, E. Frank, and J. Fine. 1961. Hepatic clearence of endotoxin absorbed from the intestine. Proc. Soc. Exp. Biol. Med. **108**:261–263.
10. Hardy, E. G., G. C. Morris, E. M. Yow, B. W. Haynes, and M. E. DeBakey. 1954. Studies on the role of bacteria in irreversible hemorrhagic shock in dogs. Ann. Surg. **139**:282–286.
11. Kétyi, I., 1967. Theoretical and practical relations of optimal adjuvation and functional detoxification of antigens. Ann. Immunol. Hung. **10**:115–120.
12. Kinard, F. E. 1957. Liquid scintillator for the analysis of tritium in water. Rev. Sci. Instrum. **28**:293–294.
13. McCluskey, E. T., B. W. Zweifach, W. Antropol, B. Benacerraf, and A. L. Nagler. 1960. Pathogenesis of experimental shock. I. Absence of morphologic evidence for bacterial endotoxemia. Amer. J. Pathol. **37**:245–278.
14. Miler, I., J. Kostka, L. Simek, and A. Lanc. 1964. Fate of endotoxin in the intestine of newborn bacteria-free piglets monocontaminated with Escherichia coli. Folia Microbiol. (Prague) **9**:277–283.
15. Ocklitz, H. W., H. Mochmann, E. F. Schmidt, and L. Hering. 1967. Oral immunization of mice with a soluble protective antigen obtained from enteropathogenic serotypes of Escherichia coli. Nature (London) **214**:1053–1054.
16. Ocklitz, H. W., H. Mochmann, E. F. Schmidt, L. Hering, and I. Maruse. 1967. Laboratoriumsversuche über die Möglichkeiten einer oralen Immunisierung mit einem Natriumdesoxycholat-Extrakt aus Dyspepsiecolie-Stämmen. I. Mitteilung: Immunologische Eigenschaften. Die Antikörperbindung des Extraktes in Latextest. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Orig. **203**:300–306.
17. Parant, F., Parant, H. Charlier, E. Sacquet, and L. Chedid. 1966. Etude de la tolérance aux endotoxines chez la souris "sans germe" au moyen d'un antigéne 0 radioactif. Ann. Inst. Pasteur (Paris), Suppl. no. 3. **110**:198–207.
18. Rauss, K., and I. Kétyi. 1966. Immunological studies on shigellosis by the mouse model technique. 1. Anti-infective immunity of actively immunized mice. Acta Microbiol. Hung. **12**:377–386.
19. Rauss, K., and I. Kétyi. 1966. Immunological studies on shigellosis by the mouse model technique. 2. Anti-infective immunity of passively immunized mice. Acta Microbiol. Hung. **12**:387–393.
20. Rauss, K., I. Kétyi, and T. Angyal. 1966. Experimental shigellosis in mice. Pathol. Microbiol. **29**:95–110.
21. Ravin, H. A., and J. Fine. 1962. Biological implications of intestinal endotoxins. Fed. Proc. **21**:65–68.
22. Ravin, H. A., D. Rowley, C. Jenkins, and J. Fine. 1960. On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animals. J. Exp. Med. **112**:783–792.
23. Rudbach, J. A., R. L. Anacker, W. T. Haskins, A. G. Johnson, K. C. Milner, and E. Ribi. 1966. Physical aspects of reversible inactivation of endotoxin. Ann. N.Y. Acad. Sci. **133**:629–643.
24. Sanford, J. P., and H. E. Noyes. 1958. Studies on the absorption of Escherichia coli endotoxin from the gastrointestinal tract of dogs in the pathogenesis of "irreversible" hemorrhagic shock. J. Clin. Invest. **37**:1425–1435.
25. Schweinburg, F. B., P. B. Shapiro, E. D. Frank, and J. Fine. 1957. Host resistance in hemorrhagic shock. IX. Demonstration of circulating lethal toxin in hemorrahagic shock. Proc. Soc. Exp. Biol. Med. **95**:646–650.
26. Selye, H., B. Tuchweber, and L. Bertók. 1966. Effect of lead acetate on the susceptibility of rats to bacterial endotoxins. J. Bacteriol. **91**:884–890.
27. Sojka, W. J. 1965. Escherichia coli in animals. Eastern Press Ltd., London.
28. Westphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. Z. Naturforsch. **7b**:148–155.
29. Wilzbach, K. E. 1963. The gas exposure technique for tritium labeling. *In* S. Rotschild (ed.), Advances in Tracer Methodology, vol. 1., pp. 4–11. Plenum Press, New York.
30. Wiznitzer, T., F. B. Schweinburg, N. Atkins, and J. Fine. 1960. On the relation of the size of the intestinal pool of endotoxin to the development of irreversibility in hemorrhagic shock. J. Exp. Med. **112**:1167–1171.
31. Zweifach, B. W. 1961. Hemorrhagic shock in germfree rats. Ann. N.Y. Acad. Sci. **78**:313–320.