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Influence of Thorotrast Blockade and Acute Renal Artery Ligation on Disappearance of Staphylococcal Enterotoxin B from Blood

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The protein toxin staphylococcal enterotoxin B (SEB) produces a clinical syndrome somewhat similar to that of gram-negative endotoxins. Our studies indicate a further similarity to endotoxin in that SEB is rapidly cleared from the plasma of monkeys. However, data reported herein show that an effective Thorotrast blockade of the reticuloendothelial system (RES) does not produce a significant change in the rapid disappearance kinetics of SEB. This observation contrasts with what is known regarding endotoxin clearance. Furthermore, other data reported herein indicate that acute bilateral renal artery ligation produces a profound delay in SEB clearance from plasma. A possible mechanism for renal degradation of SEB is theorized. The RES may play a less important role in the clearance of SEB. This finding contrasts with the demonstrated importance of the RES in clearance and detoxification of gram-negative endotoxin.

Similarities in the toxic manifestations of bacterial endotoxin and the protein toxin staphylococcal enterotoxin B (SEB) have been noted with increasing frequency (9, 12). Both materials are lethal in microgram quantities after parenteral injection of experimental animals. Fever, leukopenia followed by leukocytosis, adrenal cortical hyperfunction, shock, and blood clotting abnormalities have been observed with both materials. Furthermore, studies utilizing isotopically labeled SEB and endotoxin have indicated that both materials are rapidly cleared from the plasma of experimental animals (1, 2, 9).

Carey and co-workers (2) and Beeson et al. (1) postulated that the reticuloendothelial system (RES) is the major site of localization of endotoxin. In addition, these workers reported that the immune state seemed to increase the removal rate of endotoxin from the circulation. The mechanism for such an increased clearance rate is not well defined; however, Perreault and co-workers (8) indicated that the more efficient clearance of endotoxin was mediated by the RES.

On the basis of several diverse observations, Sugiyama (12) concluded that enterotoxin may involve the RES in a fashion similar to endotoxin. He showed that Thorotrast potentiates the emetic stimulus of intragastrically administered enterotoxin, and, in addition, he noted that parenterally administered enterotoxin alters the phagocytic capacity of the RES in a fashion comparable to endotoxin (12, 13).

Recent reports (5, 9) have shown that SEB has a rapid clearance rate from the plasma of experimental animals similar to that of endotoxin; in direct contrast to endotoxin, the immune state is associated with a profound delay in removal of SEB. This somewhat conflicting observation implies that the role of the RES in defense against SEB may be different from that postulated for bacterial endotoxin. This conflict was recognized by Israel and co-workers (7), who used a crude staphylococcal culture filtrate in rabbits. They observed that the RES played no part in host defense against staphylococcal toxin, but, in contrast, the kidneys were found to exert a protective influence.

Crawley et al. (4) indicated that relatively high concentrations of radioisotopically labeled SEB appeared within the liver, lungs, and kidneys after intravenous challenge. Localization within the liver and lungs could be explained on the basis of RES affinity, but important renal localization on this basis would be doubtful. Our studies indicate

that the kidney may be an important site of at least partial metabolic degradation of SEB and that the RES is not vital for SEB localization.

MATERIALS AND METHODS

Healthy *Macaca mulatta* monkeys weighing 2.5 to 3.5 kg were studied in a manner previously described (9) in which bilaterial saphenous vein catheters were used for injection of test materials and for rapid serial sampling of venous blood.

Acute bilateral renal artery ligations by the transabdominal approach under Pentothal anesthesia were performed in certain animals which were then studied immediately after regaining consciousness.

RES "blockade" was accomplished in other monkeys by the intravenous (iv) administration of 3 ml of Thorotrast (Testagar, Inc., Detroit, Mich.) per kg 3 hr prior to the administration of isotopically labeled test materials.

Highly purified SEB, as described by Schantz and his co-workers (10, 11, 14), was labeled with ^{131}I by a method described in an earlier report (9). Radioiodinated aggregated albumin (RIAA) was kindly supplied by Squibb Laboratories, New Brunswick, N.J. RIAA in a concentration of 10 mg/ml with a specific activity of approximately 1.31 μc/mg was used.

Challenge with SEB-^{131}I was by the iv route with a dose of 1 mg/kg of body weight, an amount which is usually lethal. In other experiments, RIAA was given iv in a dose of 3 mg/kg of body weight. Each animal was used for a single experiment only, and none had known previous immunological experience with either SEB or RIAA.

Heparinized blood samples (4 ml) were collected from monkeys given SEB-^{131}I at 1.5, 5.5, 15, 30, 60, 180, 300, and 420 min postchallenge. Because of more rapid disappearance kinetics, blood from monkeys given RIAA was obtained at 1.5, 3.5, 5.5, 15, 30, 60, 120, and 180 min postchallenge. The blood was centrifuged at 1,600 $\times$ g in a PR-International Centrifuge for 30 min. Plasma protein-bound ^{131}I and free ^{131}I were determined in a manner described earlier (9).

Data were plotted on semilogarithmic paper as the percentage of initial plasma radioactivity, with the counts per minute of the 1.5-min sample assigned a value of 100. In terms of injected radioactivity, approximately 80 to 90% could be recovered in the 1.5-min bleeding based on a 7% blood value (4).

RESULTS

Figure 1 shows the clearance of RIAA in the various experimental situations. Both total plasma and trichloroacetic acid-precipitable protein-bound radioactivity are shown as the means $\pm$ 1 SE. In the control animals, it is apparent that disappearance from the plasma was exceedingly rapid. The mean half disappearance time ($t_{1/2}$) of the rapid initial portion (first 30 min) of the protein-bound ^{131}I curve was 5.5 min, with a range of 4 to 6 min. Deiodination of RIAA, as evidenced by the progressive separation of the two curves, occurred relatively rapidly. The clearance kinetics and deiodination of RIAA in the monkey thus appear to be virtually identical to those reported in the dog by Iio and Wagner (6). The administra-

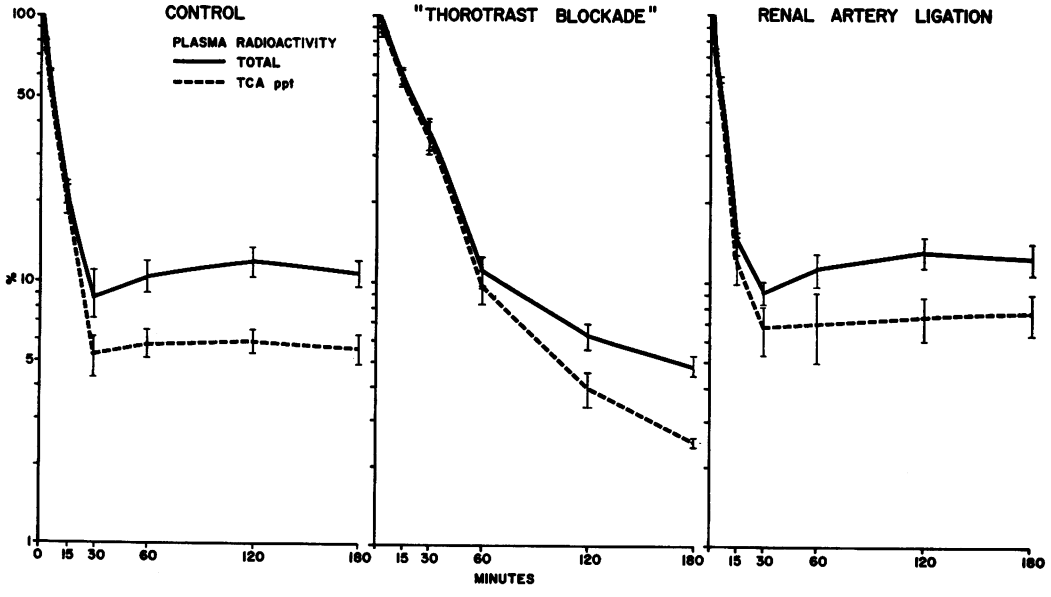

FIG. 1. *Disappearance of radioiodinated aggregated albumin from monkey plasma after Thorotrast blockade and renal artery ligation (means of six monkeys each).*

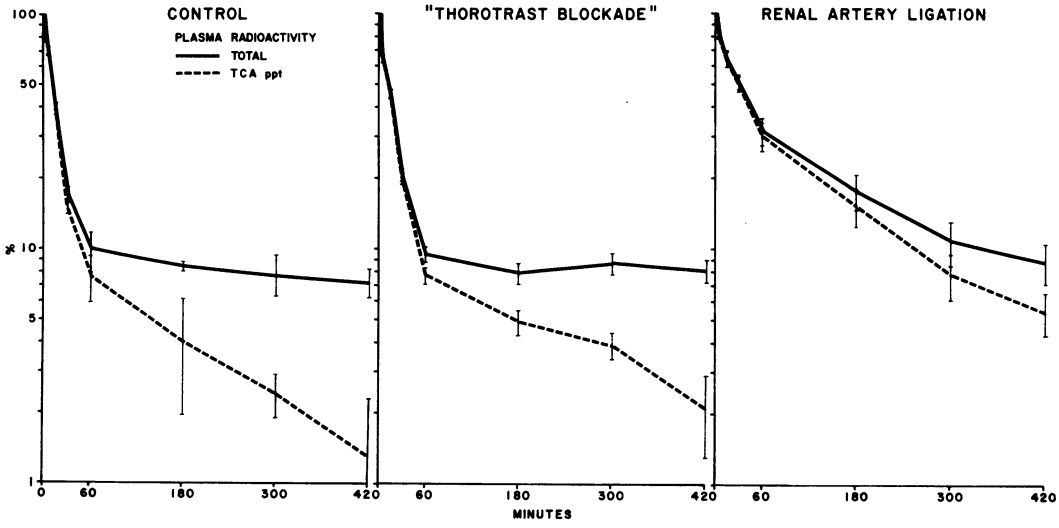

FIG. 2. *Disappearance of radioiodinated SEB from monkey plasma after Thorotrast blockade and renal artery ligation (means of six monkeys each).*

tion of Thorotrast 3 hr prior to RIAA produced a marked change in clearance and deiodination patterns. The mean $t_{1/2}$ of protein-bound ^{131}I was 19 min, with a range of 18 to 22, which is significantly delayed in comparison with controls ($P < 0.001$). The rate of deiodination was also delayed. In contrast, bilateral renal artery ligation performed less than 3 hr prior to administration of the aggregated albumin appeared to have no effect on its clearance or deiodination. The mean $t_{1/2}$ of RIAA of animals with bilateral renal artery ligations was 6 min, with range of 5 to 7.

The disappearance of SEB-^{131}I from the plasma of control monkeys is shown in Fig. 2. As with RIAA, the clearance and deiodination of this material were also rapid, although the phenomena were observed over a 7-hr period. The mean $t_{1/2}$ for SEB-^{131}I, with the use of the rapid initial (first 60 min) portion of the curve, was 8 min, with a range of 7 to 9. The influence of Thorotrast given 3 hr prior to SEB-^{131}I is also shown in Fig. 2. There was no apparent alteration in the clearance or deiodination of the toxin as a result of "Thorotrast blockade." The mean $t_{1/2}$ of SEB-^{131}I was 7.5 min, with a range of 7 to 8.5. Therefore, a dose of Thorotrast previously shown to be effective in RES blockade did not significantly affect clearance or deiodination kinetics of SEB-^{131}I. The influence of acute renal artery ligation is shown in the third part of Fig. 2; it is obvious by inspection that both clearance and deiodination of the labeled toxin were slower than in the control animals. The mean $t_{1/2}$ was 28 min, with a range of 19 to 32 ($P < 0.01$). This change in clearance and deiodination kinetics which followed bilateral renal artery ligation suggests that the kidney has an important function in the early metabolic alteration of SEB-^{131}I.

DISCUSSION

Although the mechanism by which Thorotrast interferes with normal RES function is uncertain, it is apparent from our studies that Thorotrast administration to monkeys can delay the removal of RIAA from the plasma. This observation is not unexpected, in that Iio and Wagner showed that this material was localized within the RES (6). These same authors indicated that metabolic degradation and probable deiodination of RIAA occur within the RES. Pretreatment of monkeys with the Thorotrast, given in a dose shown to be effective in delaying clearance of RIAA, appeared to have no effect whatever on the clearance and metabolic alteration of SEB-^{131}I. This conclusion is based on unaltered clearance rates and deiodination patterns of Thorotrast-treated monkeys in comparison with controls. Both groups of animals, control and Thorotrast-treated, cleared SEB-^{131}I at a rapid rate with no significant difference between them.

This observation supports earlier studies which indicated that SEB was bound to various tissues and that subsequent administration of SEB antiserum was able to reverse this binding relationship (9). It is unlikely that SEB would localize within cells of the RES and remain immunologically unaltered so that subsequent antiserum administration could effect removal of toxin from

the RES. Finally, if the RES were the major site of SEB localization in the unimmunized animal, then type-specific antiserum against SEB would be expected to speed rather than delay its clearance from the circulation on the basis of analogous studies performed with bacterial endotoxin (8).

Reports of relatively high concentrations of SEB-^{131}I localized within the kidney parenchyma, coupled with the knowledge that the kidneys possess abundant deiodinase activity, suggested to us that SEB-^{131}I might be at least partially metabolized at this site. Although acute bilateral renal artery ligation had no significant effect on plasma clearance and deiodination of RIAA, marked inhibition in clearance and deiodination of SEB-^{131}I was observed after this manipulation.

The overall importance of the kidneys in the detoxification or degradation of SEB is not readily apparent from our studies. However, Israel and co-workers (7), using a crude staphylococcal culture filtrate, found that nephrectomy potentiated lethality of the toxin. These workers concluded that the kidneys removed the toxin from the circulation initially but subsequently released it or one of its metabolites to exert a lethal effect elsewhere.

Still to be determined is the actual site or sites of localization of SEB within the kidneys and the manner by which the toxin arrives at these sites. The very rich vascular network and dual capillary system within the kidneys might suggest that the toxin is simply bound to the vascular endothelium in a fashion similar to that of numerous drugs. The fact that the kidney seems at least to deiodinate a large proportion of SEB-^{131}I would indicate that an intracellular process is involved. With the recognition that our data do not permit any further definition of the exact renal site of degradation of the toxin, numerous possibilities are likely. The information to date indicates that molecular size may be a major factor in determining the metabolic fate of SEB. It may be postulated that SEB, being a relatively small protein [molecular weight, 35,000 (14)], is able to diffuse freely from the renal vascular compartment into renal parenchyma. Equally probable is the postulation that SEB is filtered at the glomerulus and is subsequently reabsorbed by the tubular epithelial cells in a fashion similar to that observed with albumin. The renal tubular epithelium is rich in deiodinase activity and may be the site at which deiodination and perhaps further metabolic degradation occur.

RIAA, with a molecular weight of about 600,000, is probably not filtered in the glomerulus or diffusible from the renal vasculature, and, therefore, deiodination appears not to occur within the kidney. In a previous report, we established that the immune state is associated with an inhibition in clearance and dehalogenation of SEB-^{131}I (9). It is conceivable that a toxin-antitoxin complex which is formed in an immunized animal is analogous to a macromolecule which is not filtrable at the renal glomeruli or diffusible from the renal vasculature.

Our studies do not completely exclude the RES as a possible site for some metabolic alterations of SEB; however, it is clear that the kidneys are a site of at least partial metabolic degradation of the labeled toxin. The fact that Thorotrast blockade was not associated with any change in clearance or deiodination of SEB-^{131}I suggests that the RES is less vital in these functions.

Our findings would appear to support earlier work by Israel and co-workers (7), who showed that the RES has no important function in defense against a crude staphylococcal culture filtrate; however, the kidneys were found to exert a protective influence. The similarities between our findings and those of Israel et al. (7) suggest that the action of the crude culture filtrate may have represented activity of enterotoxin or enterotoxin-like materials contained within the filtrate.

Finally, the mechanism by which SEB is cleared from the circulation appears markedly different from that observed with bacterial endotoxin. Although it is clear that host responses to the toxins are similar, the postulated similarity of their relationships to the RES is not clear. Further studies establishing the importance of the kidneys in the pathogenesis of enterotoxemia are required.

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