Role of endotoxin in glycerol-induced renal failure in the rat

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Summary

1. A relationship between bacterial endotoxin absorbed from the gut and acute renal failure has been postulated. Experiments employing either the endotoxin-tolerant state or the enhancement of endotoxin injury were undertaken to test this relationship in rats.

2. Endotoxin tolerance was induced by the administration of increasing doses of *Escherichia coli* 026 lipopolysaccharide. The severity of renal injury was assessed at various times after glycerol administration in endotoxin-tolerant and control animals. At 48 h, endotoxin-tolerant rats had higher urine volume and creatinine clearance than the non-tolerant control animals. In rats studied 72 h after glycerol, functional and anatomical assessment showed the endotoxin-tolerant rats to have lower serum urea concentrations and also less renal histological injury than the non-tolerant, control animals.

3. Lead acetate, which potentiates endotoxin injury, or diluent alone was administered to rats after glycerol. At 2, 3 and 10 days later there was a twofold increase in mortality in the lead acetate-treated animals.

4. A small dose of endotoxin (0.1 mg) was shown to be innocuous in control rats. Also, all rats given glycerol alone were alive 24 h later. In contrast, administration of the same dose of endotoxin simultaneously with glycerol resulted in an 80% mortality at 24 h.

5. These studies demonstrate enhancement of glycerol-induced renal injury by endotoxin and support a possible role for endotoxin in this model of acute renal failure.

Key words: endotoxin, glycerol, lead acetate, renal failure.

Introduction

The cause of acute renal failure resulting from sepsis, crush injury or prolonged shock remains obscure, although severe renal vasoconstriction is probably a key pathophysiological event in this process (Oken, 1973). The administration of intramuscular glycerol to dehydrated rats (Oken, Arce & Wilson, 1966; Thiel, Wilson, Arce & Oken, 1967) is widely used as a model of this disease. More recently, a role for bacterial endotoxins in the renal failure accompanying liver injury has been postulated (Wardle & Wright, 1970; Bailey, 1976; Wilkinson, Moodie, Stamatakis, Kakkar & Williams, 1976) and endotoxaemia has been demonstrated by the Limulus assay in patients with acute renal failure (Wilkinson *et al*., 1976; Wilkinson, Arroyo, Gazzard, Moodie & Williams, 1974; Wardle, 1974).

Clinical conditions associated with acute renal failure, such as hypovolaemic shock, may decrease the ability of the liver to detoxify endotoxin, or may promote increased intestinal endotoxin absorption (Cuevas & Fine, 1972a,b). We thus sought to determine if endotoxin played a role in the pathogenesis of acute glycerol-induced haemoglobinuric renal failure, by observing the effect of endotoxin
tolerance on the development of renal injury, and testing the effect of lead acetate in enhancing endotoxin toxicity in this model (Selye, Techweber & Bertok, 1966), and also by studying the effects of dehydration and glycerol on endotoxin sensitivity in the rat.

**Materials and methods**

Female Holtzman albino rats (Holtzman Co., Madison, Wis., U.S.A.), weighing 140–160 g, were used in all experiments. Each experimental group was from a single shipment, and the animals were allowed to acclimatize for 7–10 days before use. The animals were individually caged and Tekland 4% Fat Rat Diet (Teklad Mills, Winfield, Iowa, U.S.A.) and tap water were given *ad libitum* until the time of dehydration, when both were removed.

Freeze-dried lipopolysaccharide prepared from *Escherichia coli* 026, B6 (Boivin), obtained from Difco Laboratories (Detroit, Mich., U.S.A.), was freshly reconstituted in pyrogen-free sodium chloride solution (150 mmol/l; saline) before intraperitoneal injection. The lead acetate was purchased from Baker Chemical Co. (Phillipsburg, N.J., U.S.A.) as the trihydrate salt and was prepared daily in sterile pyrogen-free water.

Serum alanine aminotransferase (EC 2.6.1.2; glutamic–pyruvic transaminase) activities were determined in Sigma–Frankel units with the standard 505 substrates from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Serum urea concentrations were determined on a Beckman Analyzer by a modification of the urease method of Gentzkow (1942). Urine and serum creatinine concentrations were measured with a modification of the alkaline picrate method of Brod & Sirota (1948).

**Tolerance studies**

To analyse the possible modification of glycerol-induced renal injury by the state of endotoxin tolerance. Rats were paired by weight into tolerant and non-tolerant groups. Saline-suspended endotoxin was injected as follows: day 0, 0.01 mg; day 1, 0.025 mg; day 2, rest; day 3, 0.2 mg; day 4, 0.6 mg; day 5, rest; day 6, 1.0 mg. The injection volume was 0.5 ml in each case. Control rats were given 0.5 ml of saline intraperitoneally at the same times.

Forty-eight hours after either the last endotoxin or saline injection, food and water were removed, and 24 h later, 1.0 ml/100 g rat weight of 0.59 g of glycerol/ml (6400 mmol/l) in sterile water was injected into the femoral muscle under light ether anaesthesia. The animals were bled by aortic puncture and killed 24 h, 48 h or 72 h after the glycerol. Two groups of animals were studied in individual metabolic cages to collect urine.

Liver and kidney sections were obtained at the time of killing, fixed in 10% formalin, sectioned and stained with haematoxylin and eosin. The renal sections were interpreted without knowledge of the group of origin. The renal lesions were graded from the injury to the tubular and interstitial portions of the kidney, as there was no appreciable glomerular damage in either control or experimental animals by light microscopy. Tubular necrosis was expressed as a percentage by counting the number of necrotic and intact tubules in at least 10 high-powered fields in each section.

To establish if lead acetate would also heighten glycerol-induced renal injury, lead acetate, 0.5 ml of 10 mg/ml (26 mmol/l) solution, was given to rats into the femoral vein, which was exposed by sharp dissection under light ether anaesthesia. The lead acetate solution or the water diluent was given to two groups of weight-matched rats, followed by intraperitoneal injection of various amounts of endotoxin. Deaths were recorded each 24 h after the lead administration. To assess the nephrotoxic and hepatotoxic potential of lead acetate, or its water diluent, three groups of ten rats were dehydrated for 24 h. The first group received 0.5 ml of the lead acetate solution through the exposed femoral vein; the second group received 0.5 ml of sterile water via the same route; the final group was operated only. The animals were again anaesthetized 48 h later and aortic blood was drawn for urea and creatinine concentrations and determinations of alanine aminotransferase activity. To determine if administration of the water diluent would contribute to the renal pigment load, seven rats were injected intravenously with 0.5 ml of sterile water, and aortic blood, haemoglobin, packed cell volume and plasma haemoglobin measurements made 2 h later.

Three additional groups of 20 rats were studied. Ten rats in each group were given lead acetate solution and the other ten an equivalent volume of sterile water. Under continued light ether anaesthesia all rats received glycerol. One group was followed for 2 days, another for 3 days and the third group for 10 days after injection. Deaths were recorded in each group and surviving rats were bled at the time of killing.
Endotoxin and acute renal failure

Endotoxin enhancement of glycerol injury

To determine if rats treated with glycerol have an increased sensitivity to endotoxin, groups of ten rats were given glycerol intramuscularly, after 24 h without water, followed by an intraperitoneal injection of 0-001 mg, 0-05 mg or 0·1 mg of *E. coli* 026 lipopolysaccharide. An additional experiment was performed in a group of ten rats that were not dehydrated. Deaths were recorded at 24 h, and serum was obtained from survivors.

In the tolerance studies, any rat whose weight change was greater than 1 SD from the statistical comparison was deleted. Values are given as mean ± SEM, and where applicable, differences were compared by unpaired t-test.

**Results**

**Tolerance studies**

Animals made tolerant to increasing doses of endotoxin over 7 days gained weight at the same rate as control rats. The non-tolerant animals had higher serum alanine aminotransferase activities at 24 h (*P* < 0·01) and 48 h (*P* < 0·025) than the tolerant animals, but at 72 h values were similar in both (Table 1). Histologically, the livers of both tolerant and non-tolerant rats were similar, exhibiting minimal changes with some slight sinusoidal congestion, mild pericentral necrosis, and scattered, discrete areas of intralobular necrosis and inflammation.

In contrast to the changes in serum alanine aminotransferase, serum urea concentrations in the tolerant rats were lower in comparison to non-tolerant control animals as time increased after injury. This difference was significant at 72 h (*P* < 0·025).

For the first 24 h after glycerol, endotoxin-tolerant rats produced more urine than non-tolerant control rats (1·8 ± 0·6 ml compared with 0·3 ± 0·3 ml; difference not significant). Most of the non-tolerant animals were virtually anuric. From 24 to 48 h after glycerol administration, endotoxin-tolerant rats were polyuric, producing more than twice as much urine as the saline-treated control rats (18·6 ± 2·4 ml vs 8·8 ± 3·0 ml; *P* < 0·025). The serum creatinine concentrations at 48 h after glycerol were greater in the endotoxin-tolerant rats [0·18 ± 0·03 (tolerant) and 0·08 ± 0·03 (non-tolerant) ml/min × 100 per 100 g rat weight; *P* < 0·05]. In the 72 h histology sections, 17·1 ± 3·7% of tubules were necrotic in rats from the tolerant group, which was significantly less (*P* < 0·001) than the 40·4 ± 5·7% of such necrotic tubules in those control animals who were not made tolerant to endotoxin.

**Enhancement of toxicity with lead acetate**

Two groups of control experiments were performed to assess the potential toxic effects of lead acetate or the water diluent in rats. Three groups of ten dehydrated rats given lead acetate in water intravenously, the water diluent alone, or sham-operated control rats were killed 48 h after treatment. The serum urea and serum creatinine concentrations and alanine aminotransferase activities from blood obtained by aortic puncture were virtually identical in all three groups, suggesting that neither the lead acetate nor its diluent was nephrotoxic or hepatotoxic in the dose administered.

An additional seven rats received 0·5 ml of water intravenously. These animals, bled by aortic puncture 2 h later, had normal haemoglobin concentrations, 13·5 ± 0·3 g/dl, and packed cell volumes of 40·2 ± 1·1%; plasma haemoglobin was undetectable, indicating that the water diluent did not contribute significantly to the pigment load handled by kidneys when lead acetate in water was administered with the glycerol.

In a final control study, four groups of five rats each were killed 24 h after administration of *E. coli* 026 lipopolysaccharide in doses from 0·001 mg to 48 h and the serum creatinine concentrations 48 h after glycerol were greater in the endotoxin-tolerant rats [0·18 ± 0·03 (tolerant) and 0·08 ± 0·03 (non-tolerant) ml/min × 100 per 100 g rat weight; *P* < 0·05]. In the 72 h histology sections, 17·1 ± 3·7% of tubules were necrotic in rats from the tolerant group, which was significantly less (*P* < 0·001) than the 40·4 ± 5·7% of such necrotic tubules in those control animals who were not made tolerant to endotoxin.

### Table 1. Serum alanine aminotransferase activities and serum urea concentrations of tolerant and non-tolerant rats killed at 24 h intervals after glycerol administration

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Alanine aminotransferase activity</th>
<th>Concen. of urea (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerant</td>
<td>Non-tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td>24</td>
<td>55 ± 5·5</td>
<td>78 ± 5·4*</td>
</tr>
<tr>
<td>48</td>
<td>21 ± 1·7</td>
<td>37 ± 5·2**</td>
</tr>
<tr>
<td>72</td>
<td>18 ± 0·9</td>
<td>19 ± 1·1</td>
</tr>
</tbody>
</table>

*P* < 0·01, tolerant vs non-tolerant; **P* < 0·025, tolerant vs non-tolerant.
0·1 mg. Even the highest dose of endotoxin did not cause elevation of serum urea concentrations or alanine aminotransferase activities in these animals.

In contrast to the control studies, simultaneous administration of glycerol and lead acetate proved to be very toxic. The mortality rate in each group of rats, followed for 2, 3 or 10 days after administration of glycerol and either lead acetate or the water diluent, was higher in the lead acetate-treated animals at each time (Fig. 1). Furthermore, in animals surviving 10 days, a dramatic difference in morbidity was still apparent. Two of four remaining lead acetate-treated rats were severely azotaemic, whereas the serum urea concentrations in the surviving diluent-treated animals were all near normal.

Endotoxin activity was confirmed to be potentiated by lead acetate. Rats given doses of endotoxin (0·1 mg or less) that were innocuous to control animals uniformly succumbed when lead acetate was administered concurrently (Table 2).

**Enhancement of endotoxin toxicity after glycerol administration**

Although normal rats failed to show significant renal or hepatic damage at endotoxin doses as high as 0·1 mg, rats given glycerol after dehydration were exquisitely sensitive to endotoxin. In 24 h, 18% of rats receiving 0·01 mg, 60% of rats receiving 0·05 mg and 80% of rats receiving 0·1 mg of lipopolysaccharide died. Even without prior dehydration, glycerol administration caused 40% mortality in rats given 0·05 mg of endotoxin.

**Table 2. Enhancement of lethality of endotoxin by lead acetate in rats**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Dose of endotoxin (mg)</th>
<th>Dose of lead acetate (mg)</th>
<th>Deaths/no. of rats in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-1</td>
<td>5-0</td>
<td>10/10</td>
</tr>
<tr>
<td>2</td>
<td>0-01</td>
<td>5-0</td>
<td>10/10</td>
</tr>
<tr>
<td>3</td>
<td>0-00 (saline)</td>
<td>5-0</td>
<td>0/10</td>
</tr>
<tr>
<td>4</td>
<td>0-00 (water)</td>
<td>5-0</td>
<td>0/10</td>
</tr>
</tbody>
</table>

**Discussion**

Acute renal failure usually occurs in the clinical setting of hypotension associated with sepsis and trauma. The factors promoting acute renal failure might lead to both an increase in circulating endotoxin and a decrease in a host's ability to detoxify it. The effects of endotoxin on the renal circulation resemble the clinical and experimental features of acute renal failure. At low doses of endotoxin renal blood flow may temporarily increase and a diuresis result (Lucas, Rector, Werner & Rosenberg, 1973), but larger doses result in hypotension and intense renal vasoconstriction (Gillenwater, Dooley & Frohlich, 1963). Hypotension diminishes the phagocytic function of the reticuloendothelial system in the liver and allows enterically absorbed endotoxin to reach the systemic circulation (Hershey & Altura, 1969; Olcay, Kitahama, Miller, Drapanas, Trejo & Diluzio, 1974). Cuevas & Fine (1972a, b) have found evidence of increased intestinal endotoxin absorption in both septic and non-septic shock in rabbits. Woodruff, O'Carroll, Koisumi & Fine (1973) suggested a major clinical role for endotoxin of enteric origin in shock associated with major trauma, but without a focus of infection. In liver injury due to a dietary deficiency of choline, endotoxin causes renal haemorrhage and necrosis at doses that are innocuous in control rats (Nolan & Ali, 1968). Circulating endotoxin has also been shown to correlate with the development of oliguric renal failure in patients with massive liver necrosis (Wilkinson et al., 1974) and obstructive jaundice (Bailey, 1976; Wilkinson et al., 1976).

The acute myohaemoglobinuric renal failure seen in the glycerol model was once attributed to tubular blockade, with backflow from tubular casts. Oken et al. (1966) have shown by micropuncture that the casts were secondary and not causative. They suggested that the features of acute renal failure were due to intense renal vaso-
constriction after the glycerol insult. These haemodynamic alterations after glycerol have been verified and found to include significant reduction in cardiac output as well as renal blood flow (Hsu, Kurtz & Waldinger, 1977). Saline infusions or plasma volume expansion prevented the glycerol-induced renal lesion. We used a total of 3 ml of saline in each rat over 10 days to suspend the endotoxin used to induce tolerance in our animals. Non-tolerant rats were given the same saline load in order to serve as controls. No animal received any saline for 72 h before glycerol administration. The results of previous studies suggest that small quantities of saline have little effect on intrarenal renin and do not impede the development of glycerol-induced renal failure in rats (Flamenbaum, McNeil, Kotchen, Lowenthal & Nagle, 1973).

In a recent study, small pieces of kidney transplanted to the ear chambers of rabbits were observed after glycerol administration (Hobbs, Chuslip, Kincaid-Smith & McIver, 1976). Within minutes the intratubular and glomerular capillaries blanched, with changes in the arterioles and arteries occurring later. In view of these early vasoconstrictive changes, it is of interest that endotoxin causes similar vasoconstriction of the renal vasculature within 5 s after injection into an isolated perfused kidney (Hinshaw, Bradley & Carlson, 1959), and within 15–30 s after the intravenous injection of a lethal dose in an intact dog (Abernathy, 1957).

Rats are readily made tolerant to increasing doses of bacterial endotoxins (Abrams, 1967), and this tolerance protects against heterologous as well as homologous strains (Abernathy, 1957). A series of doses of endotoxin similar to that in the present study has been shown to protect against carbon tetrachloride-induced hepatic necrosis (Nolan & Ali, 1973), and the induction of tolerance has also been shown to protect mice against the renal damage associated with endotoxin administration (Bates & Margolin, 1968). The rise in serum alanine aminotransferase activity at 24 h after glycerol administration to the non-tolerant rats, which returns to normal over the next 2 days, showed that liver damage occurred. The induction of tolerance significantly reduced the extent of this rise in enzyme activity. Since there was also a concomitant difference in the serum urea concentration reached between the tolerant and the non-tolerant groups, it is possible that this protection of the liver in the tolerant animals reduced the entry of endotoxin into the systemic circulation.

In the first 24 h, rats in both tolerant and non-tolerant groups were oliguric, although the endotoxin-tolerant animals did produce more urine than the control rats; as the time from the injury increased a difference became apparent between the groups, the tolerant rats progressing more rapidly to a polyuric recovery phase with improved clearances within 24–48 h. In addition to the functional differences, the histological differences were more evident as time from the insult increased. At 72 h, although severe injury was still manifest in the non-tolerant group most tolerant rats had only minimal evidence of damage.

The synergistic effect of the concurrent administration of lead acetate and endotoxin was originally reported by Selye et al. (1966), and has been widely confirmed (Wilkinson et al., 1976; Bertok, 1968; Filkins, 1970; Trejo & DiLuzio, 1971). Lead salts enhance the lethal effect of endotoxin (Filkins & Buchanan, 1973) and we have confirmed this in our studies. A dose of 0·1 mg of endotoxin caused no rise in either the serum alanine aminotransferase activity or the serum urea concentration of normal animals, but all rats receiving even 0·01 mg died when this was given concurrently with lead acetate. Similarly, the simultaneous administration of lead acetate and glycerol increased both the mortality and the degree of renal injury in survivors when compared with animals given glycerol and diluent alone, suggesting that the lead potentiated the effect of circulating endotoxin after the glycerol insult.

Since it is postulated that small quantities of endotoxin reaching the systemic circulation may contribute to the renal ischaemia after glycerol, we have tested the sensitivity of glycerol-treated rats to endotoxin. At endotoxin doses ranging from 0·01 mg to 0·1 mg, 18–80% of glycerol-injured rats died in 24 h. These doses of endotoxin failed to elevate the serum alanine aminotransferase activity or serum urea concentrations in non-glycerol-treated control animals and no rats expired 24 h after glycerol alone. Furthermore, even without prior dehydration, glycerol given with 0·05 mg of bacterial lipopolysaccharide caused a mortality rate of 40%.

These observations demonstrate that endotoxin enhanced glycerol-induced renal injury and that the state of endotoxin tolerance partially protected animals from this injury, suggesting that endotoxin of enteric origin may contribute to the pathogenesis of this model of acute renal failure. Studies of the haemodynamic alterations, and specific identification of endotoxaemia after glycerol, are needed to evaluate this hypothesis further.
Acknowledgments

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References


