Effect of intraluminal oxygen on endotoxin absorption in experimental occlusion of the superior mesenteric artery

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SUMMARY Absorption of bacterial endotoxin has been shown to occur after release of the occluded superior mesenteric artery in the rat. A significant reduction in endotoxin absorption was observed as a result of the infusion of gaseous oxygen into the bowel lumen during the period of ischaemia (p < 0.001). The methods of endotoxin identification and assay are described, and the possible reasons for this effect of intraluminal oxygen are discussed.

The role of bacterial endotoxins in the genesis of the circulatory collapse associated with acute ischaemia of the intestine is still the subject of debate. Some authors (Zweifach et al., 1958; Williams et al., 1968) deny the importance of endotoxaemia in this condition, but the majority consider that significant amounts of endotoxin reach the circulation, because of increased absorption through the damaged mucosa (Nelson and Noyes, 1952; Kobold and Thal, 1963; Milliken et al., 1965).

The reduction in mortality resulting from the introduction of oxygen into the bowel lumen of rats subjected to superior mesenteric artery occlusion has been described previously (Shute, 1975). In that study two identical groups of rats were subjected to occlusion of the superior mesenteric artery (SMA) under general anaesthetic. Thirty minutes after starting arterial occlusion 20 ml of gaseous oxygen was introduced into the small bowel lumen of each of one group of rats, while the other group acted as controls. The SMA clamp was released in all animals after a total of 120 minutes occlusion and the mortality was observed 48 hours later. The mortality of the control group at this time was 89%, while that of the oxygen treated group was 39%. This difference was highly significant p < 0.005 (chi square test).

The present study was designed to evaluate the effect of the administration of intraluminal oxygen on the absorption of endotoxin after experimental occlusion of the superior mesenteric artery in the rat. Endotoxin absorption was assessed by the method of Pieroni et al. (1970), who showed that submicrogram quantities of endotoxin could be detected by assessing the mortality after simultaneous intraperitoneal injection of the endotoxin and 12.5 µg actinomycin D into mice, because the latter drug enhances the lethal effect of endotoxin by up to 225 000 times.

Methods

ENDOTOXIN ASSAY (Table 1)
To confirm the efficacy of the Pieroni endotoxin assay, the following experiment was carried out.

Twenty female Swiss A2G mice weighing 20-25 g were injected with 1 ml normal saline and 12.5 µg actinomycin D in 0.25 ml saline. Forty similar mice were injected with 0.005 µg Escherichia coli 055:B5 lipopolysaccharide/endotoxin (Difco, England) in 0.5 ml saline, and a further 50 mice received the same amounts of actinomycin D and endotoxin simultaneously. All injections were given intraperitoneally, and the ampoules of actinomycin D were pooled to avoid the problem of variation in potency between

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D 12.5 µg</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ml normal saline</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endotoxin 0.005 µg</td>
<td>50</td>
<td>39</td>
<td>78</td>
</tr>
<tr>
<td>Actinomycin D 12.5 µg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endotoxin 0.005 µg</td>
<td></td>
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</tbody>
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Received for publication 30 November 1976
batches. The mortality in each group was noted at 48 hours.

No mice died among those given actinomycin D or endotoxin alone, but 39 (78%) of those given both substances together were dead 48 hours later, confirming that these agents act synergistically to produce an increased lethal effect in mice.

**Operative preparation**

Adult male Wistar rats were anaesthetised with intramuscular droperidol 2 mg and fentanyl 0·05 mg. A midline abdominal incision was made and the superior mesenteric artery (SMA) and vein (SMV) were isolated so that the SMA could be clamped, and blood samples could be taken from the SMV.

**Control study**

In fifteen rats, 2 ml blood were taken from the SMV immediately after this vessel was isolated. These control samples were pooled and spun down, and 0·5 ml aliquots of supernatant plasma were assayed for endotoxin by injection intraperitoneally into 20 mice together with 12·5 µg actinomycin D.

**Superior mesenteric artery occlusion**

After exposing the superior mesenteric vessels the SMA of each of 100 rats was occluded with a bulldog clamp for 120 minutes, then released. Two millilitre samples of blood were then taken immediately from each SMV, pooled and centrifuged. The supernatant plasma was then used as follows.

To assess the inherent toxicity of this plasma, 0·5 aliquots were injected alone intraperitoneally into 30 mice.

Plasma, 0·5 ml, and 12·5 µg actinomycin D were injected into a further 40 mice.

Clearly, deaths caused in this group by the combination of rat plasma and actinomycin D could be attributable to synergism between the drug and many constituents of plasma, including endotoxin. The remaining plasma was therefore used in the following experiments to identify the toxic component.

Plasma, 10 ml, was heated to 70°C for 30 minutes to denature the protein, then injected into 20 mice in 0·5 ml aliquots together with 12·5 µg actinomycin D.

Plasma, 10 ml, was autoclaved in a sealed container for 20 minutes at 130°C and 25 lb/sq. in. pressure. The resulting mixture was reconstituted to its original volume with normal saline, and 0·5 ml aliquots were injected into 20 mice together with 12·5 µg actinomycin D.

Fifty mice were made tolerant to endotoxin by receiving intraperitoneal injections of increasing doses (up to 100 µg) of *E. coli* 055:B5 lipopolysaccharide daily over a four day period. Tolerance to endotoxin was assessed by injecting 20 of these mice intraperitoneally with 12·5 µg actinomycin D and 0·5 µg of the lipopolysaccharide—that is, 100 x the original test dose. The remaining 30 'tolerant' mice received 0·5 ml of the plasma and 12·5 µg actinomycin D. The mortality of each group was observed at 48 hours.

**Effect of intraluminal oxygen**

The SMA of each of 30 anaesthetised rats was occluded with a bulldog clamp. Thirty minutes later, 10 ml gaseous oxygen was injected into the small bowel lumen through a 25 g needle which was pushed through the bowel wall. After a further 90 minutes the SMA clamp was released, and 2 ml blood was immediately taken from each SMV, pooled, and centrifuged; 0·5 ml samples of the supernatant plasma were assayed for endotoxin as before in 40 mice, the mortality being noted at 48 hours.

**Statistical analysis**

The significance of the different mortalities was determined using the chi square test.

**Results—mortality of mice at 48 hours (Tables 2 and 3)**

**Table 2  Mortality of mice at 48 hours**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rat plasma</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>+ actinomycin D</td>
<td>30</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Test rat plasma alone</td>
<td>40</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Test rat plasma + actinomycin D</td>
<td>40</td>
<td>9</td>
<td>23</td>
</tr>
</tbody>
</table>

**Table 3  Identification of toxic agent**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test rat plasma + actinomycin D</td>
<td>40</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Test rat plasma heated to 70°C + actinomycin D</td>
<td>20</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>Test rat plasma autoclaved + actinomycin D</td>
<td>20</td>
<td>14</td>
<td>70</td>
</tr>
<tr>
<td>Test rat plasma + actinomycin D</td>
<td>30*</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Endotoxin tolerant mice.
SUPERIOR MESENTERIC ARTERY OCCLUSION

1. Two (7%) of the mice injected with rat plasma taken after 120 minutes SMA occlusion died.
2. Twenty-six (65%) of those mice given this plasma and actinomycin D were dead at 48 hours. The mortality in this group was significantly higher than the mortality of those mice given similar plasma alone (p < 0.001), or of mice given control plasma and actinomycin D (p < 0.001).
3. Eleven (55%) of the mice given actinomycin D and plasma heated to 70°C died.
4. Fourteen (70%) of those given autoclaved plasma and actinomycin D died. The mortalities in these two groups are not significantly different from the mortality of mice in group 2 which were given unheated post-SMA occlusion plasma and actinomycin D.
5. None of the endotoxin ‘tolerant’ mice, which were given actinomycin D and 100 × the previous test dose of endotoxin, died, confirming that tolerance to endotoxin had indeed been induced. Only one (3%) of these endotoxin tolerant mice given post-SMA occlusion plasma and actinomycin D died. This mortality is significantly lower than in group 2 (non-tolerant) mice given the same treatment (p < 0.001).

INTRALUMINAL OXYGEN

Nine (23%) of the mice given actinomycin D and post-SMA occlusion plasma from oxygen treated rats died. This is significantly lower than the 65% mortality of mice in group 2 which were given actinomycin D and plasma from untreated rats (p < 0.001).

Discussion

Many different tests have been described for the detection of circulating endotoxins. Among these are pyrogenic effect in rabbits (Beeson, 1947), Thomas’ skin test (Thomas, 1956), chick embryo injection (Smith and Thomas, 1956), and the limulus lysate test (Rojas-Corona et al., 1969). Various agents have been shown to enhance the lethal effect of endotoxin in experimental animals, including Thorotrast (Beeson, 1947), lead acetate (Selye et al., 1966), pertussis vaccine (Pieroni and Levine, 1967), insulin (Pieroni and Levine, 1969), and pretreatment with BCG (Shands et al., 1969). Berry (1964) first demonstrated enhancement of the lethal effect of endotoxin in mice by actinomycin D. Pieroni et al. (1970) showed that submicrogram quantities of E. coli 026:B6 endotoxin could be detected by enhancement of its lethal effect in mice by giving them 12.5 μg actinomycin D.

The present experiments using purified endotoxin confirm the findings of Pieroni et al., for, while no mice died when given actinomycin D or endotoxin alone, 80% of those injected with both agents died before 48 hours after injection.

The negligible mortality of mice treated with pre-occlusion rat plasma and actinomycin D suggests that no significant amount of endotoxin was present in this plasma. Whereas plasma taken after SMA occlusion caused no deaths when given alone, the addition of 12.5 μg actinomycin D resulted in 65% mortality. This indicates that some toxic agent, possibly endotoxin, acting synergistically with actinomycin D, had appeared in the plasma during the SMA occlusion. The tests performed to identify this toxic substance showed that it is heat stable, and that it is ineffective when given to mice which are known to be tolerant to endotoxin. These results strongly suggest that the toxic factor is, indeed, endotoxin.

There was a significantly lower mortality in mice injected with actinomycin D and plasma taken from rats which had received intraluminal oxygen during the period of SMA occlusion. This indicates that the oxygen had somehow decreased the amount of endotoxin in the SMV blood. This may have occurred because of a decrease in the number of anaerobic endotoxin-producing organisms in the bowel lumen, or because of diminished absorption of available endotoxin. The former explanation is unlikely in view of the relatively short period of exposure of the anaerobes to oxygen, and because death of these organisms would, in the short term, lead to the release of large quantities of endotoxin. A much more likely explanation is that the decreased absorption of endotoxin from the bowel lumen is due to previously described maintenance of the mucosal ‘barrier’ by intraluminal oxygen (Shute 1975).

It has been shown that significant absorption of endotoxin occurs into the circulation after revascularisation of ischaemic intestine, and that this is reduced by treatment with intraluminal oxygen. It is suggested that this is a factor in the decrease in mortality which has been shown to occur (Shute, 1975) when oxygen is injected into the bowel lumen during experimental occlusion of the superior mesenteric artery.

I am grateful to Professor N. L. Browse for his help in the preparation of this paper and to Miss Diana Hill for her secretarial assistance. I also gratefully acknowledge the financial aid received from the Wellcome Trust which made this work possible.

References


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*Gut* 1977 18: 567-570
doi: 10.1136/gut.18.7.567

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