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Failure of drugs that selectively inhibit thromboxane synthesis to modify endotoxin shock in conscious rats

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1 The effects of two thromboxane synthetase inhibitors (dazoxiben and UK 38485) were investigated on the cardiovascular and metabolic effects of *Escherichia coli* endotoxin infusion in the conscious, unrestrained rat.

2 Infusion of *E. coli* endotoxin (41.7 ng kg^{-1} min^{-1}) for 4 h produced a fall in mean arterial pressure, an increase in heart rate, a transient hyperglycaemia (at 1 h) followed by hypoglycaemia (evident at 6 h), an elevation in plasma lactate and a profound thrombocytopenia.

3 The above changes were accompanied by a marked elevation in plasma thromboxane B_2 concentrations (e.g. endotoxin-treated 935 ± 150 pg ml^{-1} at 1 h compared with pre-endotoxin values of 125 ± 30 pg ml^{-1}).

4 The administration of either dazoxiben (30 mg kg^{-1} i.v., given 30 min before starting the endotoxin infusion) or UK 38485 (15 mg kg^{-1} given 30 min before, and again 4 h after, starting the endotoxin infusion) prevented the rise in plasma thromboxane B_2 concentrations.

5 Neither dazoxiben nor UK 38485 prevented the metabolic, cardiovascular or thrombocytopenic effects of endotoxin and did not modify mortality.

6 These results suggest that, although large amounts of thromboxane are generated in response to endotoxin, they do not play an important role in the major pathophysiological consequences of acute endotoxaemia.

Introduction

Plasma thromboxane concentrations (as detected by radioimmunoassay of thromboxane B_2) are elevated in various models of endotoxin shock (Cook *et al.*, 1980; Coker *et al.*, 1983). This observation, together with the potent vasoconstrictor and platelet aggregatory properties of thromboxane A_2 (Samuelsson *et al.*, 1978) make it a possible mediator of various responses to bacterial endotoxin, such as vasoconstriction in the pulmonary, renal and mesenteric vascular beds and thrombocytopenia. Moreover, thromboxane synthesis inhibitors have been shown to block pulmonary vascular responses to endotoxin in the anaesthetized cat (Ball *et al.*, 1983) and to reduce 24 h mortality in endotoxin-treated rats (Halushka *et al.*, 1983). We were interested to examine the possible role of thromboxane in a conscious rat model of endotoxin shock in which endotoxin is infused over several hours rather than administered as a bolus.

Methods

Male Sprague Dawley rats (200–230 g) were used throughout and allowed free access to food and water until 18 h before the experiment, when food but not water was withdrawn. The animals were prepared 48 h before the experiment by the implantation of catheters in the right external jugular vein and right common carotid artery. The catheters were loosely tied to the psoas muscle and exteriorised on the dorsal surface of the neck. During the experiment the rats were housed individually in metabolic cages with the catheters connected to a swivel unit allowing the animals free movement in all directions (Brown *et al.*,1982). The arterial catheter was used for blood sampling and the continuous recording of blood pressure using a Bell and Howell transducer Type 4-327-I, coupled to a Devices chart recorder. Heart rate was measured intermittently from the blood pressure record. Drugs and endotoxin (or saline) were infused or injected through the venous catheter. Endotoxin (*E.*

coli lipopolysaccharide, Difco Laboratories type 026-B6) was dissolved in 0.9% w/v sodium chloride solution (saline) and administered as a 10 mg kg^{-1} dose over a 4 h period (41.7 ng kg^{-1} min^{-1}; Harvard Slow Infusion Pump). Blood samples (0.2 ml) were removed before starting the infusion, at hourly intervals from the start of the infusion for 6 h and also at 10 h and at 24 h. The samples were analysed for platelets and for plasma concentrations of glucose and lactate. Platelets were measured by the method of Brecher & Cronkite (1950), plasma glucose was determined with the Beckman Glucose Analyser (Beckman R11C) and lactate was measured using a Boehringer reagent kit. In some experiments venous thromboxane B_2 (TxB_2) concentrations were determined in 0.2 ml plasma using the radioimmunoassay described by Coker *et al.* (1982). Tritiated TxB_2 and TxB_2 antibody were obtained from the Institut Pasteur, Paris. For these determinations, 2 ml venous blood samples (inferior vena cava) were removed from rats anaesthetized with ether; separate groups of rats were used for each time interval studied. All values are expressed as mean ± s.e.mean. Statistical significance was usually assessed using the Wilcoxon signed rank or Mann Whitney U tests for paired or unpaired observations as appropriate. Fischer's exact probability test was used to assess the statistical significance of differences in mortality produced by the drugs.

Drugs used

Dazoxiben and UK 38485, (3 - (1H-imidazol - 1 - yl methyl) - 2 - methyl - 1H - indole - 1 - propanoic acid, Pfizer Laboratories) were dissolved in saline or alkaline saline. Dazoxiben was injected 30 min before starting the endotoxin infusion in a dose of 30 mg kg^{-1}. UK 38485 was injected in a dose of 15 mg kg^{-1} 30 min before and 4 h after starting the endotoxin infusion.

Results

Effects of endotoxin or saline

The infusion of saline did not modify blood pressure (Table 1), heart rate (Table 2) or the plasma concentrations of glucose (Table 3) and lactate (Table 4) either during the 4 h infusion period or over the following 20 h. Infusion of *E. coli* endotoxin produced a reduction in mean blood pressure detectable at 1 h and reaching a nadir of about 90 mmHg by 4 h (Table 1). In surviving rats, blood pressure had shown some recovery by 24 h. Heart rate increased by about 40 beats per min at 2 h and tended to remain elevated but with considerable fluctuations (Table 2). Plasma glucose concentrations were elevated at 1 h and declined thereafter, significant hypoglycaemia being present at 6 h (Table 3); in surviving rats, plasma glucose returned to control values by 24 h. Those animals that died had very low plasma glucose concentrations in the 1–2 h before death (mean 2.3 ± 0.5 mmol l^{-1}). Plasma concentrations of lactate increased by 1 h, remained elevated up to 10 h and had returned to control values by 24 h (Table 4).

Endotoxin administration also resulted in a profound thrombocytopenia, platelet concentrations decreasing to 6% of the pre-endotoxin value by 10 h and remaining depressed for up to 24 h (Figure 1). In control animals receiving a saline infusion the platelet count remained steady (337 ± 24, 352 ± 17, 326 ± 17, 345 ± 16, 318 ± 11 × 10^3 mm^{-3} at 0, 1, 3, 6 and 10 h respectively; $n = 4$ at each time point).

Table 1 The effects of a 4 h infusion of *E. coli* endotoxin (41.7 ng kg^{-1} min^{-1}) on mean arterial blood pressure in normal rats and in rats treated with dazoxiben (30 mg kg^{-1} i.v. given 30 min before starting the endotoxin infusion) or UK 38485 (15 mg kg^{-1} given both 30 min before, and 4 h after, starting the endotoxin infusion)

Treatment	*Mean arterial blood pressure* (mmHg) at time (h) after commencing endotoxin infusion				
	0	1	3	5	10
Saline infusion	116 ± 6 (4)	117 ± 10 (4)	110 ± 4 (4)	113 ± 4 (4)	113 ± 5 (4)
Endotoxin	114 ± 3 (12)	102 ± 3*** (12)	96 ± 5*** (12)	90 ± 5** (9)	88 ± 10 (6)
Endotoxin + dazoxiben	120 ± 2 (10)	93 ± 6*** (10)	95 ± 3*** (9)	96 ± 6*** (8)	79 ± 19 (3)
Endotoxin + UK 38485	122 ± 2 (9)	87 ± 5*** (9)	90 ± 11** (9)	96 ± 3*** (8)	101 ± 2* (6)

Each value is the mean ± s.e.mean. The number of observations is shown in parentheses.
Asterisks indicate statistically significant differences from pre-endotoxin values: *$P < 0.05$; **$P < 0.02$; ***$P < 0.001$

Table 2 The effects of a 4 h infusion of *E. coli* endotoxin (41.7 ng kg^{-1} min^{-1}) on heart rate in normal rats and in rats treated with dazoxiben (30 mg kg^{-1} given i.v. 30 min before commencing the endotoxin infusion) or UK 38485 (15 mg kg^{-1} given both 30 min before, and 4 h after, commencing the endotoxin infusion).

Treatment	*Heart rate* (beats min^{-1}) at time (h) after commencing endotoxin infusion				
	0	1	3	5	10
Saline infusion	476 ± 33 (4)	454 ± 18 (4)	461 ± 15 (4)	456 ± 26 (4)	443 ± 26 (4)
Endotoxin	420 ± 14 (12)	430 ± 16 (12)	457 ± 14 (12)	437 ± 19 (11)	418 ± 40 (6)
Endotoxin + dazoxiben	428 ± 15 (10)	477 ± 13 (10)	482 ± 20* (10)	426 ± 21 (8)	420 ± 79 (3)
Endotoxin + UK 38485	453 ± 13 (9)	450 ± 13 (9)	472 ± 18 (9)	489 ± 25 (8)	458 ± 44 (6)

Each value is the mean ± s.e.mean. The number of observations is shown in parentheses.
Asterisks indicate statistically significant differences from pre-endotoxin values: *$P < 0.05$;

Table 3 The effects of a 4 h infusion of *E. coli* endotoxin (41.7 ng kg^{-1} min^{-1}) on the plasma glucose concentration of normal rats and of rats treated with dazoxiben (30 mg kg^{-1} i.v. given 30 min before starting the endotoxin infusion) or UK 38485 (15 mg kg^{-1} given both 30 min before, and 4 h after starting the endotoxin infusion).

Treatment	*Plasma glucose* (mmol l^{-1}) at time (h) after starting endotoxin infusion				
	0	1	3	6	10
Saline infusion	5.2 ± 0.28 (4)	4.7 ± 0.22 (4)	5.3 ± 0.22 (4)	5.0 ± 0.13 (4)	5.5 ± 0.37 (4)
Endotoxin	4.9 ± 0.15 (12)	7.3 ± 0.43*** (12)	5.1 ± 0.52 (12)	3.3 ± 0.4*** (10)	4.2 ± 0.74 (6)
Endotoxin + dazoxiben	4.6 ± 0.17 (10)	6.9 ± 0.22*** (10)	4.6 ± 0.4 (8)	2.6 ± 0.71* (8)	4.5 ± 1.5 (3)
Endotoxin + UK 38485	5.2 ± 0.2 (9)	6.7 ± 0.22*** (9)	4.6 ± 0.5 (9)	3.7 ± 0.38* (7)	4.1 ± 0.78 (7)

Each value is the mean ± s.e.mean. The number of observations is shown in parentheses.
Asterisks indicate statistically significant differences pre-endotoxin values. *$P < 0.05$; ***$P < 0.01$.

Table 4 The effects of a 4 h infusion of *E. coli* endotoxin (41.7 ng kg^{-1} min^{-1}) on plasma lactate concentrations in normal rats and in rats treated with dazoxiben (30 mg kg^{-1} i.v. given 30 min before starting the endotoxin infusion) or UK 38485 (15 mg kg^{-1} given both 30 min before, and 4 h after, starting the endotoxin infusion).

Treatment	*Plasma lactate concentration* (mmol l^{-1}) at time (h) after commencing endotoxin infusion				
	0	1	3	6	10
Saline infusion	2.2 ± 0.25 (4)	2.5 ± 0.47 (4)	2.2 ± 0.19 (4)	2.1 ± 0.32 (4)	2.00 ± 0.25 (4)
Endotoxin	2.5 ± 0.13 (11)	3.0 ± 0.16*** (11)	3.8 ± 0.39*** (10)	4.8 ± 0.5** (8)	4.9 ± 0.6* (5)
Endotoxin + dazoxiben	2.3 ± 0.13 (10)	2.99 ± 0.13* (10)	3.8 ± 0.16*** (10)	4.9 ± 0.12*** (6)	5.0 ± 0.4 (3)
Endotoxin + UK 38485	2.3 ± 0.19 (9)	2.7 ± 0.12** (9)	3.5 ± 0.4*** (9)	5.3 ± 0.5*** (8)	5.0 ± 0.8* (6)

Each value is the mean ± s.e.mean. The number of observations is shown in parentheses.
Asterisks indicate statistically significant differences from pre-endotoxin values: *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$.

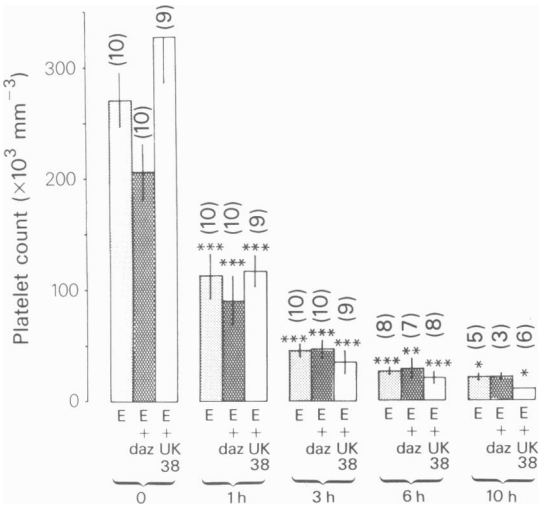

Figure 1 Blood platelet counts before, and at various times after, the start of endotoxin infusion. The rats received either endotoxin alone (E) or endotoxin together with either dazoxiben (E + daz) or UK 38485 (E + UK38). Each column represents the mean and a vertical line the s.e.mean. The numbers in parentheses refer to the number of observations. The platelet count did not change with time in animals receiving saline infusion in place of endotoxin (See text). Asterisks indicate statistically significant differences from appropriate pre-endotoxin values: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Plasma thromboxane (TxB_2) concentrations had increased seven fold at 1 h after starting the infusion and were still elevated, although less markedly so, at 4 h and 8 h (Figure 2). Animals infused with saline instead of endotoxin had very low TxB_2 concentrations at 1 and 4 h (84–164 pg ml^{-1} at 1 h; 76–108 pg ml^{-1} at 4 h). This endotoxin infusion produced a 75% mortality by 24 h (Table 5).

Effects of thromboxane synthetase inhibitors

Either dazoxiben or UK 38485 markedly suppressed the elevation in plasma TxB_2 concentrations normally induced by endotoxin (Figure 2). Dazoxiben augmented significantly the endotoxin-induced tachycardia at 1 h but not at other times (Table 2)

Table 5 Mortality, at 24 and 48 h, of rats receiving endotoxin infusion alone or in conjunction with dazoxiben or UK 38485

	Endotoxin only	*Endotoxin plus dazoxiben*	*Endotoxin plus UK 38485*
24 h	9/12 (75%)	5/9 (55%)	9/10 (90%)
48 h	9/12 (75%)	5/9 (55%)	9/10 (90%)

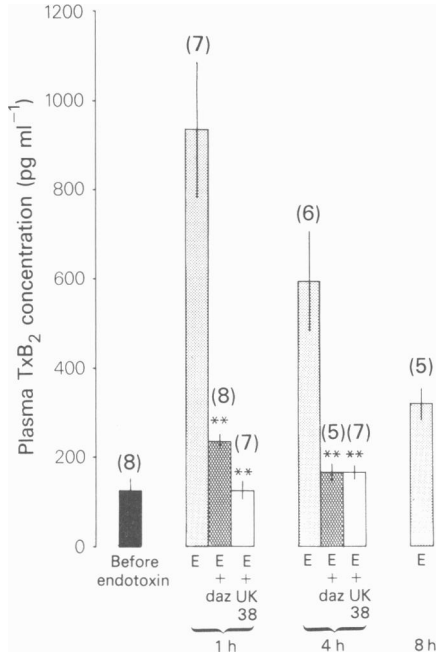

Figure 2 Plasma thromboxane (TxB_2) concentrations, as determined by radioimmunoassay, at various times before and after the start of endotoxin infusion. The rats received either endotoxin alone (E) or endotoxin together with either dazoxiben (E + daz) or UK 38485 (E + UK38). Each column represents the mean and a vertical line the s.e.mean. The numbers in parentheses refer to the number of observations. The values were obtained from rats killed at different times and thus were not sequential observations in the same groups of animals. Asterisks indicate statistically significant differences from control (i.e. endotoxin alone) values: $*P<0.05$; $**P<0.01$.

whilst UK 38485 increased the endotoxin-induced hypotension at 1 h (Table 1). Apart from these changes, neither drug modified any other response to endotoxin (Tables 3 and 4) and failed to increase the survival rate either at 24 or 48 h (Table 5).

Discussion

The metabolic effects of infusing endotoxin in the conscious rat are very similar to those observed after bolus injection (e.g. Adeleye *et al.*, 1981). On the other hand, endotoxin infusions did not result in the rapid and profound fall in systemic pressure seen after bolus injection, for example, in anaesthetized cats (Parratt, 1973) or in conscious rats (unpublished observations from this laboratory).

Differences between the effects of endotoxin when

given by continuous infusion and by bolus injection have been noted by others. Thus, Cohen *et al.* (1973) observed that infusing endotoxin in cats did not produce the severe initial pulmonary vasoconstriction and right ventricular failure seen following bolus injection (Greenway *et al.*, 1969). The major reason for using an infusion of endotoxin in the present study was the far greater reproducibility of the responses.

The observed rise in plasma TxB_2 concentrations following endotoxin administration is in agreement with other findings using different species, different protocols for endotoxin administration and different types of endotoxin (Cook *et al.*, 1980; Coker *et al.*, 1983). However, we were unable to demonstrate any protective effect of either of two thromboxane synthetase inhibitors against the initial hyperglycaemia, the later hypoglycaemia, the increases in plasma lactate concentrations, the tachycardia, hypotension or thrombocytopenia occurring in response to endotoxin infusion. Moreover, neither drug influenced survival. There is no clear explanation for the differences between the present results and those of Halushka's group (Wise *et al.*, 1980; Halushka *et al.*, 1983) who showed that thromboxane synthetase inhibitors reduced both the hypoglycaemia and the thrombocytopenia produced by endotoxin and markedly improved survival. These workers used a different endotoxin (*Salmonella enteritidis*) although they found a similar 24 h mortality rate (69%; Halushka *et al.*, 1983) to that seen in the present study (75%). The major difference between the results of the two studies may depend on the use of a single bolus injection of endotoxin compared to the continuous infusion used in our work. As discussed above, bolus injections of endotoxin have been reported to produce different effects from those produced by infusion. In the cat, the acute pulmonary vasoconstrictor response occurring following bolus injection but not infusion (Cohen *et al.*, 1973) appears to be due to thromboxane formation (Ball *et al.*, 1983). If the rat is similar then thromboxane synthesis inhibition may be expected to protect against the effects of bolus injection of endotoxin perhaps by preventing pulmonary vasoconstriction. Although it is obviously difficult to compare plasma TxB_2 concentrations between laboratories, especially when sampling times and radioimmunoassays are different, it may be noteworthy that peak TxB_2 concentrations in endotoxin-treated rats in the present study are only about half of those reported by Halushka's group (Cook *et al.*, 1980; Wise *et al.*, 1980; Halushka *et al.*, 1983). It is interesting that thromboxane synthesis inhibition using imidazole, whilst preventing increases in plasma TxB_2 concentrations, was ineffective in protecting rats against live *E. coli* organisms injected intraperitoneally (Fletcher *et al.*, 1983). It is likely that the time course of the endotoxaemia following such a protocol resembles more closely that found after a continuous infusion of endotoxin and that both models are more relevant to the clinical situation of septic shock with maintained circulating endotoxin levels.

The present work suggests that, although the concentrations of thromboxane are clearly increased by *E. coli* endotoxin, thromboxane is unlikely to be alone, or even primarily responsible for ultimate multi-organ failure in this form of shock.

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