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The effects of the protease inhibitor, aprotinin, on the course of shock induced by endotoxin in cats

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1 The administration of endotoxin derived from *Escherichia coli* to anaesthetized cats resulted, within the first 5 min, in an initial increase in right atrial pressure and a reduction in systemic arterial blood pressure. Over the next 2 h shock was characterized by a reduced cardiac output, tachycardia, reduced arterial pH and an increased level of lactate. The survival rate at the end of the 8 h experimental period was only 10%.

2 The protease inhibitor aprotinin (Trasylol), given as a continuous intravenous infusion 1000 kallikrein inhibitor units (k.i.u.) $kg^{-1}h^{-1}$ together with a bolus of 40,000 k.i.u. kg^{-1}, significantly inhibited the severity and incidence of the initial endotoxin response (increase in right atrial pressure and systemic hypotension), perhaps suggesting the direct or indirect involvement of kinins.

3 Aprotinin did not reduce the delayed effects of endotoxin (sustained reduction in cardiac output, lacticacidosis), nor did it improve survival at 8 h. Indeed, there was some evidence that aprotinin exaggerated the delayed effects of endotoxin in this model.

Introduction

Plasma kinins have been implicated in the pathophysiology of circulatory shock as a consequence of their ability to lower systemic arterial pressure and increase capillary permeability (Hinshaw, 1971; Parratt, 1983a). The kallikrein-kinin system is activated early in experimental endotoxin shock in several species including primates (Nies *et al.*, 1968) and cats (Al-Kaisi *et al.*, 1977) as well as in clinical sepsis (Kimball *et al.*, 1972).

Previous experimental studies have shown that rapid kininogen depletion occurs in cats given endotoxin (Al-Kaisi *et al.*, 1977) and the main purpose of the present study was to investigate the effects of the protease inhibitor, aprotinin, on the development of the delayed shock phase in this model. It was given in a dose of 48,250 k.i.u. kg^{-1}. In this species, this dose has been shown to limit markedly the increase in plasma creatine phosphokinase resulting from coronary artery occlusion (Lefer & Spath, 1975); it also prevents the marked increase in the coronary sinus blood levels of bradykinin which result from coronary artery occlusion in anaesthetized dogs (Hashimoto *et al.*, 1975). This dose of aprotinin also reduced the increase in pulmonary extravascular fluid volume which resulted from myocardial infarction in cats, an effect attributable at least in part, to increased pulmonary capillary leakage (Massion *et al.*, 1975). Increased pulmonary capillary leakage is also a major consequence of endotoxin administration in this species (Parratt, 1983b).

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Methods

Twenty-one cats (mean weight 2.1 ± 0.1 kg) of either sex were fasted overnight and anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) administered intraperitoneally. Polythene cannulae were placed in the right atrium and the aortic arch for pressure measurements.

The trachea was cannulated and the animals allowed to breathe spontaneously. When necessary (e.g. immediately after endotoxin administration) the animals were ventilated with a Palmer positive-pressure respiration pump using room air at a rate of 20 strokes min^{-1} and a stroke volume of 25 ml kg^{-1}. A femoral vein was cannulated for drug and endotoxin administration.

All pressures were recorded as described previously (Parratt, 1973) with appropriate Elema-Schönander

or Statham transducers and recorded on a Mingograph 81 ink-jet writing recorder together with the electrocardiogram (normally lead II). Cardiac output was measured by a thermodilution technique using room temperature saline (0.9% w/v NaCl solution; Parratt, 1973). Body temperature was monitored from direct recording thermocouples in the oesophagus and rectum. Arterial blood samples were analysed for oxygen and carbon dioxide tensions and for pH with appropriate electrode systems (Instrumentation Laboratories) and arterial lactate was measured enzymatically by a Boehringer test combination. All cats received heparin (100 iu kg^{-1}) at the start of the operative procedures.

The cats were divided into three groups: (Group I) These cats ($n = 10$) were given intravenous injection of 2 mg kg^{-1} of *E. coli* endotoxin (0.55 B5 lipopolysaccharide B, Difco Laboratories, prepared by the Boivin method and suspended in saline). (Group II) These cats ($n = 8$) were given the Bayer kallikrein inhibitor aprotinin in a dose of 1000 kallikrein inhibitor (k.i.u.) $kg^{-1}h^{-1}$. The aprotinin was dissolved in saline and infused intravenously for 8.25 h beginning 15 min before the slow intravenous injection of *E. coli* endotoxin. Just prior to the endotoxin a bolus injection of aprotinin (40,000 k.i.u. kg^{-1}) was also administered. The total aprotinin dose was thus 48,250 k.i.u. kg^{-1}. (Group III) These cats ($n = 5$) received aprotinin alone, in the same dose as the Group II animals.

Statistical analysis

Student's *t* test for paired data was used in Group III and the Mann-Whitney U-test for non-parametric data was used for comparisons (within the groups) in Groups I and II. Student's *t* test for unpaired data was used for comparisons between groups.

Results

Haemodynamic effects of aprotinin

The administration of aprotinin (1000 k.i.u. $kg^{-1}h^{-1}$) over a 6 h period resulted in myocardial depression, as evidenced by a transient reduction in mean blood pressure (e.g. from 115 ± 13 mmHg to 92 ± 10 mmHg at 1 h and a return at 2 h to 112 ± 16 mmHg) and a more sustained reduction in cardiac output (from 346 ± 21 ml min^{-1} to 220 ± 39 ml min^{-1} at 1 h and 205 ± 29 ml min^{-1} at 6 h; $P < 0.05$). Heart rate was increased by aprotinin infusion (162 ± 15 beats min^{-1} to 208 ± 22 beats min^{-1} at 1 h and 218 ± 15 beats min^{-1} at 6 h). All five cats treated with this dose of aprotinin survived the 6 h experimental period.

Table 1 Initial responses of anaesthetized cats to the administration of endotoxin following the administration of either saline (controls) or aprotinin

	Control	Aprotinin-treated
Increase in mean right atrial pressure (mmHg)	+ 5.5 ± 1.0	+ 3.0 ± 0.7*
Maximum reduction in diastolic blood pressure (mmHg)	− 74 ± 15	− 16 ± 15*
Number of animals exhibiting a 'positive' response†	80%	25%

† Defined as an increase of more than 1.0 mmHg right atrial pressure and a reduction of more than 20 mmHg in diastolic blood pressure.
* $P < 0.05$ (Student's *t* test).

Effects of aprotinin on the initial responses to E. coli *endotoxin*

Within 1–3 min of endotoxin administration, the animals not pretreated with aprotinin (Group I) exhibited all the haemodynamic responses typical of this model (Parratt, 1973). There was a marked increase in right atrial pressure (of 5.5 ± 1.0 mmHg from a resting control value of 0.9 ± 0.4 mmHg) with reductions in systemic pressure, and in some cats, the occurrence of ventricular arrhythmias. In contrast, in the cats pretreated with aprotinin (Group II), endotoxin elicited a marked initial response in only two of the eight animals; in the six remaining animals there was only a slight increase in right atrial pressure (less than 1.0 mmHg) and insignificant decreases in arterial pressure (less than 20 mmHg). This is in contrast to the incidence and severity of the initial response in cats given only endotoxin (Table 1).

Effects of aprotinin on the delayed responses to E. coli *endotoxin*

The delayed cardiovascular effects of *E. coli* endotoxin in control animals are summarized in Table 2. After an initial hypotension during the first hour, blood pressure returned towards pre-endotoxin levels at 3 h, declining thereafter. There were significant reductions in cardiac output and in stroke volume (Table 2). The overall haemodynamic profile resulting from endotoxin administration in cats treated with aprotinin (Table 3) was not significantly different from that of the endotoxin alone group. Heart rate increased significantly during the late shock phase (e.g. 223 ± 15 beats min^{-1} pre-endotoxin to 260 ± 9 beats min^{-1} at 6 h, Table 3), and there was a gradual decline in

Table 2 Delayed haemodynamic effects of *E. coli* endotoxin in cats (Group 1)

			Time after endotoxin (h)							
	Control	*+0.5*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Mean arterial pressure (mmHg)	134(10) ±7	112*(9) ±8	78**(9) ±15	116(8) λo9	125(8) ±7	100*(8) ±15	76*(6) ±23	76*(6) ±23	94(1)	98(1)
Heart rate (beats min^{-1})	210 ±5	217* ±7	204 ±15	236* ±7	230* ±6	225* ±11	226 ±12	236 ±7	220	220
Right arterial pressure (mmHg)	0.9 ±0.4	0.0 ±0.5	−0.1 ±0.5	0.3 ±0.3	0.0 ±0.5	0.5 ±0.6	−0.1 ±0.5	−0.1 ±0.1	2.2	2.2
Cardiac output (ml min^{-1})	358 ±41		284* ±44	261* ±44	210* ±26	175* ±30	165* ±30	236 ±28	222	222
Stroke volume (ml $beat^{-1}$)	1.57 ±0.19		1.27** 0.16	1.16* ±0.21	0.89* ±0.11	0.85* ±0.23	0.81 ±0.16	1.16 ±0.23	1.0	1.0

* $P < 0.05$; ** $P <$ compared to control values.
Mann-Whitney U test for non-parametric data.
Values are means ± s.e.mean; number of observations (survivors) in parentheses.

cardiac output, stroke volume and right atrial filling pressure throughout the experimental period (Table 3).

The metabolic effects of *E. coli* endotoxin were not influenced by aprotinin treatment. There was a marked lacticacidosis developing at 1 h (7.1 ± 0.4 mg 100 ml^{-1} to 16.6 ± 1.4 mg 100 ml^{-1}; $P < 0.01$, Table 4) and later, a significant reduction in arterial Po_2. These effects of endotoxin are the same as those observed in cats not treated with aprotinin (Hughes *et al.*, 1981).

Overall survival was not improved by aprotinin pretreatment: 50% (4/8) of the animals had died by 4 h post-endotoxin in comparison with a 20% mortality in the endotoxin alone group at this time. All of the animals that survived the 8 h observation period exhibited, at this time, all the haemodynamic and metabolic signs of the delayed shock phase.

Discussion

In the present study pretreatment with aprotinin markedly reduced the initial cardiovascular responses to *E. coli* endotoxin, which in this species consist of a marked rise in pulmonary arterial pressure (and right

Table 3 Delayed haemodynamic effects following endotoxin administration in cats treated with aprotinin

			Time after endotoxin (h)							
	Control	0.5	1	2	3	4	5	6	7	8
Mean arterial pressure (mmHg)	88(8) ±4	104(8) ±7	100(8) ±8	115(6) ±6	95(4) ±15	104(4) ±21	114(3) ±18	102(3) ±15	63(2) ±8	47(2) ±5
Heart rate (beats min^{-1})	223 ±15	223 ±10	232* ±8	250* ±8	235 ±17	245 ±13	250 ±10	260* ±9	260* ±10	250* ±5
Right atrial pressure (mmHg)	0.8 ±0.5	0.5 ±0.4	−0.1* ±0.3	−1.2 ±0.9	0.0 ±1.5	−0.2 ±1.4	−0.3 ±1.0	−0.1 ±0.1	−1.2 ±0.2	−1.1 ±0.1
Cardiac output (ml min^{-1})	375 ±24		296 ±38	187** ±36	209* ±23	203* ±30	218* ±23	240* ±68	217* ±46	207* ±74
Stroke volume (ml $beat^{-1}$)	1.63 ±0.22		1.55** ±0.19	0.74** ±0.14	0.90** ±0.10	0.85** ±0.14	0.99** ±0.20	0.78** ±0.12	0.83* ±0.24	0.80* ±0.23

* $P < 0.05$; ** $P < 0.01$ compared to control values.
Mann-Whitney U test for non-parametric data.
Values are means ± s.e.mean; number of observations (survivors) in parentheses.

Table 4 The delayed metabolic effects of *E. coli* endotoxin in cats treated with aprotinin

	Time after endotoxin (h)								
	Control	+1	+2	+3	+4	+5	+6	+7	+8
Arterial lactate (mg 100 ml^{-1})	7.1(8) ±0.4	16.6**(8) ±1.4	17.0**(6) ±1.0	17.0**(4) ±1.1	19.00**(4) ±1.0	20.0**(3) ±1.4	20.0**(3) ±2.6	21.1(2) ±3.0	21.0(2) ±3.0
Arterial pH (units)	7.408 ±0.010	7.273* ±0.030	7.185* ±0.035	7.259* ±0.040	7.198* ±0.020	7.208* ±0.030	7.188* ±0.040	7.233 ±0.030	7.142 ±0.040
Arterial P_{O_2} (mmHg)	97.0 ±3	76* ±6	87 ±3	80 ±3	80 ±3	75* ±4	76* ±4	75 ±4	75 ±4

There was no significant change in arterial P_{CO_2} throughout the 8 h experimental period.
*$P < 0.05$; **$P < 0.01$ compared to control values.
Mann-Whitney U test for non-parametric data.
Values are means ± s.e.mean, with the number of observations in parentheses.

atrial pressure) and a reduction in systemic arterial pressure (Parratt, 1973). A majority of animals so treated failed to exhibit a significant initial response in that there was no marked change in right atrial pressure and in six of the eight cats given aprotinin the reduction in arterial pressure was less than 10 mmHg. This is in contrast to the marked reduction in arterial pressure observed when endotoxin is given to normal cats. It suggests that kinin generation may be involved in both endotoxin-induced systemic hypotension (partly a direct action) and the initial pulmonary changes. This latter effect must be indirectly mediated because there is powerful evidence that, in this species, endotoxin-induced pulmonary hypertension, increased airways resistance and reduced lung compliance are mediated by substances (thromboxane A_2, prostaglandin $F_{2\alpha}$) derived from arachidonic acid (Parratt & Sturgess, 1977; Coker *et al.*, 1983b). The most likely explanation for the observed effect of aprotinin is that it prevents bradykinin-induced prostanoid release or, less likely, that it may directly inhibit prostaglandin synthesis. There is good evidence for extensive interactions between the kallikrein-kinin and arachidonic acid systems (Ferreira & Vane, 1976) and bradykinin can stimulate the release of endoperoxides from lungs (Piper & vane, 1971). A previous study in this feline shock model has demonstrated rapid kininogen depletion resulting from endotoxin administration (Al-Kaisi *et al.*, 1977) and although it is not possible to draw definitive conclusions regarding the mechanism of actions of aprotinin in reducing the severity of the initial response, one likely explanation is that inhibition of kinin generation may play a primary role. The dose of aprotinin used is certainly sufficient to inhibit kinin production in other models (Hashimoto *et al.*, 1975).

The present study also demonstrates that aprotininin does not modify beneficially the ultimate course of endotoxin shock in the cat. Despite the reduction by aprotinin pretreatment of the incidence and severity of the initial response, this did not lead to an improved survival; indeed, the onset of the delayed phase appeared to be accelerated. This may have been due partly to the direct myocardial effects of aprotinin itself. Both Haberland (1978) and Schneider (1976) make the important point that very large doses have to be administered clinically for any benefit to be observed in shock and, further, that aprotinin needs to be given early for the best chance of success. This is not very surprising when we consider the early activation of the kallikrein-kinin system in shock (Hinshaw, 1971; Parratt, 1983). Both these condition were fulfilled in the present study. The drug was given both before and during shock in doses that have been used clinically (Kobald *et al.*, 1963) and that have been shown to inhibit kinin generation (Hashimoto *et al.*, 1975). Despite this there was no evidence of a beneficial effect of aprotinin under these conditions. However, the use of such a large dose seems to have resulted in non-specific cardiac depressant effects of aprotinin as distinct from its actions as a protease inhibitor. This effect on the myocardium may have obscured any protective action resulting from protease inhibition.

It is possible that differences in species and in the way shock was induced may account for this negative result. Certainly, there is evidence in other species (dogs, rats) that aprotinin reduces mortality in shock associated with trauma, burns, anaphylaxis and endotoxin (Back *et al.*, 1968; Sumida, 1979).

The development of a more specific and potent protease inhibitor would help in attempting to ascertain the contribution of the kallikrein-kinin system to the pathophysiology of shock. This may have particular relevance to the management of clinical shock since there is evidence for the activation of this system,

for example, during septicaemia (Aasen *et al.*, 1983). The elucidation of the role of the kallikrein-kinin system in shock is made more difficult by the considerable interaction between this system and renin-angiotensin and arachidonic acid cascade (Mullane & Moncada, 1980). Certainly a fuller understanding of the role of each of these systems in the development of circulatory shock would yield valuable information regarding possibilities for therapeutic intervention.

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References

AASEN, A.O., SMITH-ERICHSEN, N. & AMUNDSEN, E. (1983). Pathological plasma proteolysis in experimental endotoxemia and clinical septicaemia. The kallikrein-kinin system. In *Shock Research* ed. Lewis, D.H. & Haglund, I. pp. 243–255. Amsterdam: Elsevier.

AL-KAISI, N., PARATT, J.R. SIDDIQUI, H.H. & ZEITLIN, I.J. (1977). Feline endotoxin shock: effects of methylprednisolone on kininogen depletion on the pulmonary circulation and on survival. *Br. J. Pharmac.*, **60,** 471–476.

BACK, N., WILKENS, H. & STEGER, R. (1966). Proteinases and proteinase inhibitors in experimental shock states. *Am. N.Y. Acad. Sci.*, **146,** 491–507.

COKER, S.J., HUGHES, B., PARRATT, J.R. RODGER, I.W. & ZEITLIN, I. (1983). The release of prostanoids during the acute pulmonary response to *Escherichia coli* endotoxin in anaesthetized cats. *Br. J. Pharmac.*, **78,** 561–570.

FERREIRA, S.H. & VANE, J.R. (1976). Interaction between bradykinin and prostaglandins. In *Chemistry and Biology of the Kallikrein-kinin Systems in Health and Disease States.* ed. Pizano, J.F. & Austen, K.F. pp.;255–266. DHEW Publication No. 76 (National Institute of Health, Bethesda).

HABERLAND, G.L. (1978). The role of kininogenase, kinin formation and kininogenase inhibition in post-traumatic shock and related conditions. *Klin. Wschr.*, **56,** 325–331.

HASHIMOTO, K., HIROSE, M., FURUKAWA, S., HAYAKAWA, H. & KIMURA, E. (1975). Changes in kinin levels in coronary sinus blood and haemodynamics after the experimental occlusion of a coronary artery. In *New Aspects of Trasylol Therapy*, ed. Cantin, M., Haberland, G.L., Snells, G. & Selye, H. pp. 371–379. Stuttgart: Schattauer.

HINSHAW, L.B. (1971). Release of vasoactive agents and the vascular effects of endotoxin. In *Microbial Toxins*, Vol. V (Bacterial Endotoxins). ed. Kadis, S., Weinbaum, G. & Ajl, S.J. pp. 209–260. Academic Press: New York.

HUGHES, B., MADAN, B.R. & PARRATT, J.R. (1981). Polymyxzin B sulphate protects cats against the haemodynamic and metabolic effects of *E. coli* endotoxin. *Br. J. Pharmac.*, **74,** 701–707.

KIMBALL, H.R., MELMON, K.L. & WOLFF, S. (1972). Endotoxin induced kinin production in man. *Proc. Soc. exp. Biol. Med.*, **139,** 1078–1082.

KOBALD, E.E., LUCAS, R. & THAL, A.P. (1963). Chemical mediators in clinical septic shock. *Surgical Forum*, **14,** 16–19.

LEFER, A.M. & SPATH, J.A. (1975). Protective effect of protease inhibition in myocardial ischaemia. In *New Aspects of Trasylol Therapy*, ed. Cantin, M., Haberland, G.L., Schnells, G. & Selye, H. pp. 311–328. Stuttgart: Schattauer.

MASSION, W.H., BLÜMEL, G., ERHARDT, W. KRUEGER, P., PETROWICZ, O., WENDT, P. & HEINKELMANN, W. (1975). Effect of protease inhibitors in the early post-infarct phase. In *New Aspects of Trasylol Therapy*. ed. Cantin, M., Haberland, G.L., Schnells, G. & Selye, H. pp. 101–114. Stuttgart: Schattauer.

MULLANE, K.M. & MONCADA, S. (1980). Prostaglandin release and the modulation of some vasoactive hormones. *Prostaglandins*, **20,** 25–45.

NIES, A.S., FORSYTH, R.P., WILLIAMS, H.E. & MELMON, K.L. (1968). Contributions of kinins to endotoxin shock in the unanaesthetised rhesus monkey. *Circulation Res.*, **22,** 155–164.

PARRATT, J.R. (1973). Myocardial and circulatory effects of *E. coli* endotoxin: modifications of response to catecholamines. *Br. J. Pharmac.*, **47,** 12–25.

PARRATT, J.R. (1983a). Neurohumoral agents and their release in shock. In *Handbook of Shock and Trauma*, Vol. 1, Basic Science, ed. Altura, B.M., Lefer, A.M., Schumer, W. pp 311–336. New York: Raven Press.

PARRATT, J.R. (1983b). Pulmonary function in experimental endotoxin shock – an examination of the role of chemical mediators. In *Shock Research*, ed. Lewis, D.H. & Haglund, U. pp 129–145. Amsterdam: Elsevier.

PARRATT, J.R. & STURGESS, R.M. (1977). The possible roles of histamine, 5-hydroxytryptamine and $PGE_{2\alpha}$ as mediators of the acute pulmonary effects of endotoxin. *Br. J. Pharmac.*, **60,** 209–219.

PIPER, P.J. & VANE, J.R. (1971). The release of prostaglandins from lung and other tissues. *Ann. N.Y. Acad. Sci.*, **180,** 363–379.

SCHNEIDER, B. (1976). Ergebnisse einer Feldstudie über den therapeutischen Wert von Aprotinin beim traumatischen Shock. *Arzneim. Forsch.*, **26,** 3–19.

SUMIDA, S. (1979). Experimental studies on the effect of prophylactic proteinase inhibitor therapy on endotoxin shock. *Adv. exp. Med. Biol.*, **120,** 395–401.

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