Br. J. Pharmac. (1975), 53, 485-488

E. coli ENDOTOXIN SHOCK IN THE CAT; TREATMENT WITH INDOMETHACIN

J.R. PARRATT & R.M. STURGESS

Department of Physiology and Pharmacology, Royal College, University of Strathclyde, Glasgow G1 1XW

1 An earlier study had demonstrated that indomethacin, administered before *E. coli* endotoxin, abolished the initial pulmonary vasoconstriction and delayed the onset of the secondary shock phase that results from the intravenous injection of this agent in cats. The object of the present study was to determine whether indomethacin modified the shock phase when administered after endotoxin.

2 All the cats (whether or not they received indomethacin, 10 mg/kg) exhibited the characteristic features of the delayed shock phase that result from the administration of endotoxin (2 mg/kg). These included systemic hypotension, hypoglycaemia, reductions in arterial pH, cardiac output and systolic ejection time and an increase in arterial lactate. Five out of the ten animals given indomethacin survived 4 h compared with four out of twelve in the control (endotoxin alone) group.

3 These results do not support the suggestion that antipyretic-analgesic drugs like indomethacin may be of benefit when given during bacteraemic or septic shock. They do support the suggestion that the acute pulmonary changes (hypertension and decreased compliance) that occur in this species within a few minutes of endotoxin administration ultimately contribute to the severity of the shock phase.

Introduction

The results of a previous study (Parratt & Sturgess, 1974) demonstrated that pretreatment with indomethacin had two effects on the response of cats to *E. coli* endotoxin. Firstly, it prevented the acute pulmonary hypertension and oedema that occur within 1-2 min of endotoxin administration. Secondly, it delayed the systemic hypotension (a characteristic of the shock phase) and significantly increased survival time (e.g. 6 out of 15 cats were alive at 6 h compared with only 1 out of 25 in the control endotoxin series). More relevant to any possible clinical application of these experimental results is the question whether antipyretic-analgesic drugs modify the course of endotoxin-induced shock once it has started. The purpose of the present study was to attempt to answer this question.

Methods

Twenty-two cats of either sex were anaesthetized with sodium pentobarbitone (30 mg/kg by intraperitoneal injection) and ventilated with room air using a Palmer positive-pressure pump. The stroke volume of the pump was adjusted after thoracotomy to give an adequate degree of oxygenation (mean arterial Po_2 80 ± 4 mmHg); to achieve this a degree of hyperventilation was necessary (Parratt & Sturgess, 1974). No attempt was made to alter stroke volume during the course of the experiments. Rectal and mid-oesophageal temperatures were recorded with direct recording thermocouples (Ellab, Copenhagen). Systemic arterial pressure, pulmonary artery pressure and the electrocardiogram were measured as previously described (Parratt, 1973) and recorded on an ink-jet writing recorder (Elema-Schönander Mingograph 81). Mean pulmonary artery pressure was obtained by electronic integration, systolic ejection time was measured from the beginning of the upstroke of the central aortic pressure pulse to the trough of the incisural notch and cardiac output was measured by thermodilution. Arterial blood samples were analysed for O_2 and CO_2 tensions and pH with appropriately calibrated electrode systems (Radiometer, Copenhagen). Arterial lactate was measured by the Hohorst enzymatic method and plasma glucose by means of a Beckman analyser.

All the cats received, by slow intravenous injection, 2 mg/kg of the same batch of *E. coli* endotoxin (Difco, 055:B5) suspended in 0.9% w/v sodium chloride solution. Thirty minutes later ten of the cats received indomethacin (10 mg/kg, dissolved in a phosphate buffer pH 8) injected

slowly into a femoral vein. The various cardiovascular and metabolic parameters were measured each hour after the administration of endotoxin.

Results

The administration of endotoxin produced a marked elevation of pulmonary arterial pressure, with the maximum effect 3-4 min after the end of the injection. The degree of pulmonary hypertension was similar in the two groups i.e. those that were to serve as controls (no further treatment) and those that were to receive indomethacin. The values were (in the control group) 19 ± 1 mmHg (systolic); 10 ± 1 mmHg (diastolic) and 13 ± 1 mmHg (mean) immediately before endotoxin and 40 ± 5 mmHg; 23 ± 3 mmHg and 30 ± 4 mmHg respectively 3 min after endotoxin administration. In the group that were later to receive indomethacin the corresponding values were 23 ± 1 mmHg (systolic); 11 ± 1 mmHg (diastolic) and 15 ± 1 mmHg (mean) before endotoxin and 43 ± 4, 28 ± 3 and 32 ± 3 mmHg respectively 3 min after endotoxin. All these changes were significant ($P < 0.001$). There were also similar effects in the two groups with regards to systemic hypotension, bradycardia and the occurrence of ventricular arrhythmias; these initial effects of endotoxin have been described in detail in earlier studies (Parratt, 1973; Parratt & Sturgess, 1974).

When injected slowly 30 min after the endotoxin, indomethacin had no significant effect on systemic blood pressure (97 ± 8 mmHg systolic, and 58 ± 5 mmHg diastolic, immediately before, and 101 ± 12 mmHg and 57 ± 9 mmHg 5 min after, the administration of indomethacin), or on heart rate (216 ± 10 and 208 ± 11 beats/min) or mean pulmonary pressure (17 ± 2 mmHg and 16 ± 2 mmHg). There was no evidence of the hypertensive response which has been described following indomethacin administration in the dog subjected to haemorrhagic or endotoxin shock (Collier, Herman & Vane, 1973).

The cardiovascular effects of *E. coli* endotoxin are summarized in Table 1. There was a developing systemic hypotension, a reduction in systolic ejection time, in cardiac output and in stroke volume (1.6 ± 0.2 ml/beat before endotoxin and 1.2 ± 0.4 ml/beat at 3 hours). There was also a substantial metabolic acidosis, with a three to four-fold increase in arterial lactate (Table 3) and a reduction in arterial Po_2, probably as a result of the pulmonary oedema and increased shunting that occurs after endotoxin administration in this species (Parratt, 1973). Although there was a considerable individual variation, the plasma glucose tended to fall during the shock phase (Table 3). This hypoglycaemic response has also been observed in the dog and in man (Berk, Hagen, Beyer & Gerber, 1970; Griffiths, Groves & Leung, 1973). Of the 12 cats administered endotoxin alone four were alive at 4 h and one of these survived to 6 h; these results are similar to those obtained in the earlier series (Parratt & Sturgess, 1974).

The results of administering indomethacin after the endotoxin shock phase had started are summarized in Tables 2 and 3. There was probably

Table 1 Haemodynamic effects of *E. coli* endotoxin (2 mg/kg i.v.) in anaesthetized cats

	Control (pre-endotoxin)	*Post-endotoxin*				
		1 h	*2 h*	*3 h*	*4 h*	*5 h*
Carotid artery pressure (systolic; mmHg)	105 ± 6	104 ± 10	87 ± 12	90 ± 14	89 ± 23	102
(diastolic; mmHg)	76 ± 6	61 ± 10	48 ± 8*	54 ± 11	51 ± 14	40
Pulmonary artery pressure (mean; mmHg)	13 ± 1	16 ± 2	15 ± 2	16 ± 1	16 ± 1	16
Heart rate (beats/min)	191 ± 12	239 ± 15	218 ± 14	204 ± 15	200 ± 25	180
Systolic ejection time (ms)	130 ± 9	100 ± 10	101 ± 7*	94 ± 10*	107 ± 14	125
Cardiac output (ml min^{-1} kg^{-1})	132 ± 18	126 ± 13	89 ± 25	103 ± 37	–	120
Peripheral vascular resistance (arbitrary units)	2.7 ± 0.3	2.6 ± 0.5	3.4 ± 0.9	3.4 ± 2.0	–	2.7
Number of survivors	12	10	10	7	4	1†

† This animal survived 6 hours
* $P < 0.02$.

Table 2 Haemodynamic effects of *E. coli* endotoxin (2 mg/kg i.v.) and indomethacin (10 mg/kg) when administered 30 min after the endotoxin

	Control (pre-endotoxin)	*Post-endotoxin 1 h*	*2 h*	*3 h*	*4 h*	*5 h*
Carotid artery pressure (systolic; mmHg)	115 ± 8	104 ± 10	103 ± 13	107 ± 12	100 ± 12	83
(diastolic; mmHg)	86 ± 8	61 ± 7*	66 ± 11	73 ± 10	66 ± 13	56
Pulmonary artery pressure (mean; mmHg)	15 ± 1	16 ± 1	16 ± 1	15 ± 1	16 ± 1	14
Heart rate (beats/min)	215 ± 13	221 ± 11	217 ± 14	235 ± 15	240 ± 14	223
Systolic ejection time (ms)	118 ± 8	102 ± 7	95 ± 7*	86 ± 9*	88 ± 11*	–
Cardiac output (ml min^{-1} kg^{-1})	101 ± 14	92 ± 13	93 ± 16	79 ± 14	81 ± 12	77
Peripheral vascular resistance (arbitrary units)	3.7 ± 0.5	3.9 ± 0.5	4.2 ± 0.9	4.6 ± 0.8	3.8 ± 0.7	2.1
Number of survivors	10	9	8	6	5	3†

† One animal survived 6 hours.
* $P < 0.05$.

Table 3 Comparison of arterial oxygen tension, pH, arterial lactate and plasma glucose in cats administered *E. coli* endotoxin. Those in Group B also received indomethacin (10 mg/kg i.v.) 30 min after the endotoxin

	Control		*Post-endotoxin 1 h*		*2 h*		*3 h*	
Group	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
P_{O_2} (mmHg)	83 ±6	77 ±3	69 ±5*	60 ±5*	84 ±8	66 ±7	89 ±11	72 ±9
P_{CO_2} (mmHg)	22 ±2	20 ±4	27 ±4	22 ±2	24 ±6	22 ±3	22 ±4	19 ±3
pH (units)	7.500 ±0.059	7.487 ±0.043	7.346 ±0.067	7.257 ±0.062*	7.293 ±0.032*	7.167 ±0.078*	7.232 ±0.082*	7.285 ±0.031**
Lactate (mg/100 ml)	7.6 ±1.1	9.4 ±1.9	23.5 ±3.0**	20.6 ±2.1*	37.6 ±4.0**	32.3 ±4.8**	41.4 ±6.7**	30.8 ±4.0**
Glucose (mg/100 ml)	115 ±11	103 ±11	119 ±29	119 ±17	97 ±20	108 ±33	59 ±21*	95 ±14

Values are mean ± s.e.mean. Group A $n = 12$, group B $n = 10$.
The corresponding values for spontaneously breathing cats are: P_{O_2} 94 ± 3 mmHg; P_{CO_2} 27 ± 2 mmHg; pH 7.335 – 0.020 units; lactate 6.7 ± 0.9 mg/100 ml and glucose 106 ± 6 mg/100 ml.
* $P < 0.01$; ** $P < 0.001$.

a tendency for the systemic blood pressure to be maintained for longer than in the control group (compare the diastolic blood pressure values at 2, 3 and 4 h in Tables 1 and 2) but this was certainly not as marked as when indomethacin was given prophylactically (Parratt & Sturgess, 1974). Cardiac output fell to the same extent as in the cats given endotoxin alone and there was no modification of the endotoxin-induced metabolic acidosis (Table 3) or reduction in arterial P_{O_2}. Five of the ten animals survived 4 h compared with four out of 12 in the control group. Only one animal survived 6 hours.

Discussion

This study was undertaken because of the observation that pretreatment with indomethacin increased survival following *E. coli* endotoxin in

the cat (Parratt & Sturgess, 1974), a result similar to that obtained with this, and with other antipyretic-analgesic drugs (sodium salicylate, acetylsalicylic acid, flufenamic acid and sodium α-4-*sec*-butylphenoxypropionate) in the dog (Northover & Subramanian, 1962; Erdös, 1968; Culp, Erdös, Hinshaw & Holmes, 1971). Clearly such studies are not relevant to the question whether such drugs might be used in the treatment of developed septic or bacteraemic shock in man although, because it prevents endotoxin-induced pulmonary changes in cats, the use of aspirin has been suggested as a therapeutic measure to help prevent further deterioration of established pulmonary damage in patients with gram-negative septicaemia (Murthy & Greenway, 1972). We would suggest that because therapeutic success in septic shock is far from satisfactory (the mortality in patients with gram-negative bacteraemia is about 40%; Barnett & Sanford, 1969) any new approach to the problem should be actively investigated. In this respect the present results were disappointing. All the cats exhibited the characteristic features of the shock phase i.e. a reduced arterial oxygen tension, a marked metabolic acidosis and reductions in systolic ejection volume and time and in cardiac output. There was no clear evidence of increased survival. This is in contrast to the effect of pretreatment with indomethacin (Parratt & Sturgess, 1974) and suggests that the acute pulmonary changes (hypertension and decreased compliance) that occur in this species within a few minutes of endotoxin administration ultimately contribute to the severity of the shock phase. If these changes are prevented by the prior administration of indomethacin, survival time is increased despite the severity of the secondary shock phase. These results might also suggest that the beneficial effects of indomethacin pretreatment result from inhibition of prostaglandin biosynthesis or of platelet aggregation rather than to stabilization of lysosomal membranes, since the latter would presumably also occur when indomethacin is administered during the shock phase.

This work was supported by the Medical Research Council. We wish to thank Miss Joyce Hendrie and Mrs Elizabeth Petrie for technical assistance. R.M.S. is the holder of a University of Strathclyde open scholarship.

References

BARNETT, J.A. & SANFORD, J.P. (1969). Bacterial shock. *J. Am. med. Ass.*, **209**, 1514-1517.

BERK, J.L., HAGEN, J.F., BEYER, W.H. & GERBER, M.J. (1970). Hypoglycemia of shock. *Ann. Surg.*, **171**, 400-408.

COLLIER, J.G., HERMAN, A.G. & VANE, J.R. (1973). Appearance of prostaglandins in the renal venous blood of dogs in response to acute systemic hypotension produced by bleeding or endotoxin. *J. Physiol., Lond.*, **230**, 19-20P.

CULP, J.R., ERDÖS, E.G., HINSHAW, L.B. & HOLMES, D.D. (1971). Effects of anti-inflammatory drugs in shock caused by injection of living *E. coli* cells. *Proc. Soc. exp. Biol., N.Y.*, **137**, 219-223.

ERDÖS, E.G. (1968). Effect of nonsteroidal anti-inflammatory drugs in endotoxin shock. *Biochem. Pharmac.*, **17**, Suppl., 283-291.

GRIFFITHS, J., GROVES, A.C. & LEUNG, F.Y. (1973). Hypertriglyceridemia and hypoglycemia in gram-negative sepsis in the dog. *Surgery, Gynec. Obstet.*, **136**, 897-903.

MURTHY, V.S. & GREENWAY, C.V. (1972). Aspirin and pulmonary lesions in endotoxin shock. *Am. Heart J.*, **84**, 581-582.

NORTHOVER, B.J. & SUBRAMANIAN, G. (1962). Analgesic-antipyretic drugs as antagonists of endotoxin shock in dogs. *J. Path. Bact.*, **83**, 463-468.

PARRATT, J.R. (1973). Myocardial and circulatory effects of *E. coli* endotoxin; modification of responses to catecholamines. *Br. J. Pharmac.*, **47**, 12-25.

PARRATT, J.R. & STURGESS, R.M. (1974). The effect of indomethacin on the cardiovascular and metabolic responses to *E. coli* endotoxin in the cat. *Br. J. Pharmac.*, **50**, 177-183.

(Received December 2, 1974.)