Onderstepoort J. Vet. Res., 56, 207–209 (1989)

RESEARCH COMMUNICATION

ADVERSE EFFECTS OF A PROPOSED EQUINE SUBLETHAL ENDOTOXIN MODEL

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ABSTRACT

STADLER, P. & VAN AMSTEL, S. R., 1989. Adverse effects of a proposed equine sublethal endotoxin model. *Onderstepoort Journal of Veterinary Research*, 56, 207–209 (1989).

Commercially available *Escherichia coli* 055: B5 lipopolysaccharide was administered intravenously experimentally at a dosage of 10 µg/kg to 2 horses. Various clinical and clinico-pathological parameters were monitored before and after the endotoxin administration. Because of a hopeless prognosis, and for humane reasons, euthanasia was applied on both horses 6 h after administration. Values recorded for the different parameters, including the blood lactate level, were consistent with a lethal condition. It would appear that an intravenous dose of 10 µg/kg of endotoxin is potentially lethal to horses.

INTRODUCTION

Endotoxaemia is a well-known and potentially lethal condition, quite ofter encountered in equine veterinary medicine. Possible treatment regimes for sublethal endotoxaemia in the horse were investigated in this project. The clinical and clinico-pathological changes caused by sublethal endotoxaemia under local experimental conditions were determined as the first step.

MATERIALS AND METHODS

Two Thoroughbred and Arab cross-bred horses were used. Horse A was a 25-year-old gelding with a body mass of 370 kg and the other a 15-year-old mare with a body mass of 425 kg (Horse B). Five weeks before the commencement of the experiment the left arteria carotis externa of each horse was translocated to a subcutaneous position, according to the method described by Tavernor (1969). They were fed teff and given water *ad libitum*.

Endotoxaemia was induced by the intravenous injection of 10 µg/kg commercially available *Escherichia coli* 055:B5 lipopolysaccharide[1], according to the sublethal model proposed by Moore (1981a).

The observation and collection of samples were carried out at 24 h, 12 h and immediately prior to, as well as 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h after endotoxin administration.

The clinical parameters evaluated included general behaviour, rectal temperature, heart rate, respiratory rate and quality, pulse quality, the colour of the mucous membranes, capillary refill time and consistency of the faeces.

Blood was collected in 5 m*l* vacuum tubes containing ethylene diamine tetra-acetic acid (EDTA)[2] for haematological determinations. A microhaematocrit centrifuge[3] was used to determine the haematocrit. The total white cell count was determined by a coultercounter model FN[4].

Blood for the determination of the total serum protein level was collected in 10 m*l* vacuum tubes containing no anticoagulant[2]. The level was determined according to the biuret method with a R.A. 1000 automatic analyzer[5].

The blood lactate level was determined according to the enzymatic UV 365 mm method of Boehringer Mannheim (Catalogue No. 149993) on blood that had been collected in 5 m*l* vacuum tubes containing Anderson's anticoagulant[2].

Blood for arterial acid base determinations was collected from the translocated left arteria carotis externa in heparinized 5 m*l* syringes[6]. An ABL3 semi-automated acid base analyzer[7] was used to determine the pH, pO_2, pCO_2 and HCO_3 levels.

RESULTS

Within 5 min of the administration of endotoxin, Horse A went down. When he got up he was ataxic. Horse B was depressed after 30 min. At about 1 h after administration both horses developed shock, went down, and did not get up again.

The results in Table 1 indicate that both horses developed a subnormal temperature, a biphasic increase in heart rate and an increase in the respiratory rate. The quality of the respiration of both cases changed and they developed severe dyspnoea. Within 15–30 min the mucous membranes of both horses started to become congested and then assumed a muddy, toxic colour. At the same time the capillary refill time increased to 5–6 s. Both horses developed a bounding peripheral pulse which weakened and became impalpable after 6 h.

The faecal consistency of both decreased and often faeces rather liquid in consistency with little fibrous material was passed.

The results of the haematocrit, total white cell count, total serum protein and blood lactate determinations are shown in Table 2.

Both the haematocrit as well as the total serum protein level of both cases increased, while the total white cell count dropped dramatically before it started to rise again slowly. The blood lactate level increased dramatically in both cases.

It is evident from Table 3 that both horses became acidotic, hypoxic and hypocapnic.

Received 9 May 1989—Editor

[1] Lipopolysaccharide. Sigma Chemical Company, St. Louis, USA.
[2] Venoject Tube. Terumo Co. Ltd, Japan.
[3] Microhematocrit Centrifuge. Hawksley, Sussex, England.
[4] Coulter Electronics (Pty) Ltd, Hialeah, Florida, USA.
[5] Technicon Instruments Corporation, Tarrytown, New York.
[6] Terumo Syringe. Terumo Co. Ltd, Japan
[7] Radiometer, Copenhagen

TABLE 1 Rectal terperature, heart rate and respiratory rate before and after administration of lipopolysaccharide

Parameter	Horse	Time								
		−24 h	−12 h	0 h*	0h15	0h30	1 h	2 h	4 h	6 h
Rectal temperature °C	A	37,1	37,7	37,0	37,4	37,4	36,6	37,4	36,8	36,4
	B	37,0	37,8	37,0	37,4	36,8	36,8	37,4	36,7	35,6
Heart rate per min	A	36	16 (AV block)	28	48	76	60	36	72	88
	B	36	33	32	36	48	36	64	61	56
Respiratory rate per min	A	18	12	9	36	36	36	20	20	24
	B	21	12	12	28	32	36	36	30	25

* Immediately prior to administration

TABLE 2 Haematocrit, total white cell count, total serum protein and blood lactate before and after administration of lipopolysaccharide

Parameter	Horse	Time								
		−24 h	−12 h	0 h*	0h15	0h30	1 h	2 h	4 h	6 h
Haematocrit *l/l*	A	36	32	34	42	46	48	50	49	50
	B	38	36	33	39	40	45	51	52	51
Total white cell count × 10^9/*l*	A	8,7	8,8	8,5	3,9	4,6	3,0	2,8	3,6	3,7
	B	6,7	8,5	7,8	2,4	2,9	2,3	2,4	2,6	2,8
Total serum protein g/*l*	A	78,4	79,3	79,8	85,1	86,3	89,5	92,2	90,0	91,3
	B	76,9	78,4	73,0	78,1	77,7	78,4	83,0	82,4	80,7
Blood lactate mmol/*l*	A	0,98	0,69	0,64	5,16	4,80	5,32	7,21	7,22	14,14
	B	0,87	0,55	0,46	1,29	1,93	2,40	11,77	16,23	12,55

* Immediately prior to administration

TABLE 3 Arterial acid base changes before and after administration of lipopolysaccharide

Parameter	Horse	Time								
		−24 h	−12 h	0 h*	0h15	0h30	1 h	2 h	4 h	6 h
pH	A	7,460	7,453	7,453	7,379	7,388	7,351	7,307	7,340	7,320
	B	7,421	7,449	7,433	7,434	7,427	7,359	7,280	7,223	7,332
pO_2	A	78,0	85,1	106,9	88,6	96,9	77,9	66,6	60,7	68,0
	B	84,6	77,1	99,6	98,3	76,7	85,0	72,8	122,6	44,4
pCO_2	A	33,3	36,9	36,6	30,8	30,4	29,9	32,1	28,4	27,8
	B	41,9	35,6	39,2	35,5	34,3	37,8	35,7	40,3	30,6
HCO_3^-	A	23,6	25,5	25,5	17,8	18,0	16,3	15,6	15,1	14,1
	B	27,0	24,3	26,0	23,4	22,5	21,0	16,2	16,1	16,2

* Immediately prior to administration

Six hours after administration of the endotoxin it became apparent that the prognosis for survival was hopeless and that it would be inhumane to keep the horses alive. Euthanasia was therefore applied.

Discussion

Some of the changes that occurred were similar to those described by workers like Moore, Garner, Shapland & Hatfield (1980), Moore (1981 a & b), Ewert, Fessler, Templeton, Bottoms, Latshaw & Johnson (1985) and Spurlock, Landry, Sams, McGuirk & Muir (1985) after the intravenous injection of 10 µg/kg of endotoxin. Some of the results in our study did however differ from those described by these workers, although we used the same dose and route of administration of endotoxin. Both horses in our study went down initially into sternal recumbency. As their conditions deteriorated, they went into lateral recumbency and could not get up at all. The rectal temperature of both fell to subnormal levels, while Moore (1981c) described a biphasic increase during sublethal endotoxaemia. This drop in temperature was probably caused by the shock which ensued in these animals.

Another important finding in this study was the dramatic rise in the blood lactate levels. Based on the prognostic value of lactate in equine colic cases, as described by Donawick, Ramberg, Paul & Hiza (1974), both cases developed levels which were

much higher than the minimum of 7 mmol/ℓ which is considered to be consistent with a lethal condition.

Moore (1981a), using 10 µg/kg of endotoxin intravenously, described a sublethal endotoxin model in equines. The same model was used in this study, but both horses were humanely killed, because in our opinion the prognosis for survival was hopeless, and it would have been inhumane to carry on with the experiment. The very high blood lactate levels were consitent with a lethal condition and indicate that these animals would have died had they not been disposed of. The reason why the sublethal model proposed by Moore (1981a) appeared to be lethal in our study is not clear. It may be one or a combination of the following factors; firstly, it has been shown by Baker, Gaffin, Wells, Wessels & Brock-Utne (1988) that the anti-lipopolysaccharide IgG levels in horses may vary. It may therefore be possible that low antibody levels in these 2 horses caused them to react more severely to the endotoxin. Secondly, as both horses were quite old, age may have played a role. A third possibility is that the specific batch of lipopolysaccharide may have been responsible for the differences found.

In our opinion this study indicated that an intravenous dose of 10 µg/kg of endotoxin could be potentially lethal. Further research would be necessary to confirm this finding and to establish why different animals apparently react differently to a standard dose of endotoxin.

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