EFFECT OF DEXAMETHASONE ON A MODEL ENDOTOXAEMIA IN THE STALLION

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Abstract

The effects of intravenous infusions of Escherichia coli endotoxin and dexamethasone on certain clinical variables and serum testosterone were studied in breeding stallions of Polish Primitive Horses. Three stallions were given Escherichia coli LPS at a dose of 0.3 µg/kg b.w. and 4 horses were treated with dexamethasone (DEX), given intravenously at a single dose of 0.069 mg/kg b.w., 10 min before the infusion of LPS. Rectal (Tr) and scrotal skin temperature (Tss), heart rate (HR), white blood cell (WBC) and platelet (PLT) counts, and serum testosterone (T) were determined. In endotoxin-treated stallions clinical and haematological alterations were significant and characteristic of endotoxaemia. In response to endotoxin administration an increase in Tr, Tss and HR values was observed. A statistically significant decrease in WBC (with following increase), and PLT counts was noticed. The endotoxaemia resulted also in decrease, and then increase in serum T concentration after LPS administration. DEX treatment somehow prevented an endotoxin-induced increase in Tr, with no influence on the decrease in WBC and PLT counts in whole blood and level of testosterone in serum. Only slight changes in Tss and HR were noted. A positive influence of administration of DEX was noticed on changes in WBC number and serum T concentration.

Key words: stallion, endotoxin, dexamethasone, clinico-pathologic variables, testosterone.

Pathophysiological effects of Gram-negative organisms and their endotoxins or lipopolisaccharides (LPS) are similar. Lipopolysaccharide is a strong stimulator of many specific and non-specific organism reactions (7, 30, 40). Bacterial endotoxin can initiate a variety of clinico-pathological responses in the horses (4, 6, 27, 33, 39). In the previous paper (8, 11, 13), it was demonstrated that in response to Escherichia coli, endotoxin infusion breeding stallions reacted with an increase in rectal and scrotal skin temperature and heart rate, and leukopenia with following leukocytosis. Administration of endotoxin altered also the level of testosterone (significant decrease with following increase) in the blood serum (9, 13).

Dexamethasone (DEX) is a long-acting, synthetic analogue of hydrocortisone. Although the drug is used in a variety of ways, in general, it reduces inflammation and depresses the immune system. DEX is widely used in various equine disorders, including allergic, cutaneous, circulatory, intestinal, and musculoskeletal diseases (22). Apart from many other characteristic effects of anti-inflammatory steroid drugs, DEX prevents some adverse effects of endotoxin (especially during endotoxin-induced shock) on body systems in horses (15, 16, 32, 34). Co-incubation with 100 µM DEX suppresses the production of TNF-α by lipopolysaccharide-stimulated equine peritoneal macrophages (28).

Mice treated in vivo with DEX before LPS challenge showed dramatic reductions in TNF production, within the ascites and plasma (31). Glucorticoids suppress also the production of interleukin-1 and expression of interleukin-1 receptor antagonist (IL-1ra) by LPS stimulated peripheral human monocytes (2).

The previous paper (13), demonstrated that non-steroidal anti-inflammatory drug - flunixin meglumine (FM) prevented an endotoxin-induced increase in rectal and scrotal skin temperature, and heart rate and had significant effect on changes of serum testosterone concentration after addition of endotoxin. The aim of present study is an evaluation of selected clinical variables and serum testosterone concentration in stallions being in breeding season, and treated with endotoxin and dexamethasone.

Material and Methods

Experimental procedures. Seven clinically healthy breeding stallions of Polish Primitive Horses were investigated. The study was carried out during the mating season (April-July). The stallions were divided into two groups: E (3 stallions aged 8-12 years, weighing 370-400 kg, and E+ DEX (4 stallions aged 4-
14 years, and weighing 280-400 kg). The stallions of group E were infused intravenously with 0.3µg/kg b.w of lipopolysaccharide (LPS), from *E. coli*, serotype 055:B5 (Sigma Chemical Co., St Louis, MO, USA), dissolved in 500 mL of apyrogenic physiological saline solution (Pollà, Poland). The stallions from group E+DEX were administered intramuscularly a single dose (0.069 mg/kg b.w.) of DEX in the form of Dexafort (Intervet, Holland) 10 min before the infusion of 0.3 µg of LPS. Dexafort contains 2 mg of dexamethasone phenylpropionate, and 1 mg of dexamethasone sodium phosphate. The presence of bi-esters of dexamethasone causes Dexafort to work immediately and at least 7 days after its administration.

**Clinical, haematological, and hormonal examinations.** Clinical examinations comprised observations of animals and measurement of rectal (Tr) and scrotal skin (Tss) temperature, and heart rate (HR), before the infusion of LPS (marked as time 0) and 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 24 h, 48 h, and 72 h thereafter. The Tss was measured using electronic thermometer, which was kept (1 min) in the middle, between left and right part of the scrotum. The study was made in a stable, where the mean temperature amounted to 18.2°C, humidity - 75.2% and pressure - 778.2 mmHg. After the clinical examinations, blood samples were taken (always at the same time for each stallion) from the jugular vein using a catheter (Secalon® Kathy 1, Viggo, Spectramed, UK) and collected into EDTA-vacutainer tubes and tubes for serum. Blood in plain tubes was allowed to clot at room temperature, and EDTA-vacutainer tubes and tubes for serum. Blood in EDTA-vacutainer tubes and tubes for serum. Blood in plain tubes was allowed to clot at room temperature, and serum was obtained by centrifugation. Serum samples were stored at -20°C until assayed. The white blood cells (WBC) and platelets (PLT) counts were determined using a Sysmex F800 analyser (TOA Medical Electronics Co., Japan). The level of testosterone (T) was measured with the use of RIA kit (Spectria, Orion Diagnostica, and Finland). Sensitivity of the assay was 0.03 ng/ml.

**Statistical analyses.** The data were analysed statistically using the Statistica StatSoft PL programme with ANOVA variance analysis. The mean values were compared using Fisher test. Unpaired Student's t-test was used to determine statistical significance level of differences in variables between the investigated groups. The differences were statistically significant at P<0.05.

**Results**

In stallions receiving LPS (group E), a significant increase in Tr was observed within 2 h to 8 h after administration of the endotoxin. The maximum increase in the temperature (up to 38.8°C, ∆T=2.0°C) was noted at the 4th h. In stallions treated with endotoxin and DEX (E+DEX), Tr increased between the 2nd and 6th h. The maximum increase in Tr (to 38.2 °C, ∆T=1.4°C) was noted also at the 4th h (Fig. 1).

In stallions from group E, a statistically significant increase in Tss was observed within 2-5 h after administration of LPS. In this group the maximum increase in the temperature was noted at the 3rd h (34.8°C, ∆Tss=2.2 °C). For group E+DEX, the increase in Tss was observed within 2-4 h after LPS administration (maximum increase at the 2nd h, up to 34.1°C, ∆Tss=1.2 °C) (Fig.2).

The HR of the stallions was markedly increased. A significant increase was noted in group E at hours 2-4 and in group E+DEX at the 2nd h, (with maximum increase to 65.3 beats/min., ∆HR -28.7 beats/min, and 65.7 beats/min, ∆HR -20.2 beats/min, respectively) (Fig. 3).

Endotoxin-treated stallions had significantly reduced number of WBC (Fig. 3). The counts of WBC decreased after endotoxin administration at hours 1-4 and 1-3, in groups E and E+DEX, respectively. The highest decrease in WBC count in group E, was at the 2nd h after the treatment (2.2 x 10^9/l, ∆WBC -6.2 x 10^9/l), and in group E+DEX at the 1st h (2.8 x 10^9/l, ∆WBC -6.7 x 10^9/l). In both groups the WBC count increased again at hours 24 - 48 (group E) and 6-72 (group E+DEX). Counts of platelets in group E, decreased between the 2nd and 4th h. The maximum decrease took place at the 3rd h of the experiment (95.0 x 10^9/l, ∆PLT -155.3 x 10^9/l). For group E+DEX, the number of PLT decreased between the 1st and 4th h. The maximum decrease took place at the 1st h (138.0 x 10^9/l, ∆PLT -173.0 x 10^9/l) (Fig. 5).

There was a statistically significant decrease in T level between hours 3 and 24 after endotoxin administration (group E). The maximum decrease was observed at the 6th h (to 0.30 ng/ml, ∆T=0.9 ng/ml). Then there was an increase in T concentration between hours 48 and 72, with maximum value at the 48th h (2.8 ng/ml, ∆T=1.6 ng/ml). In E+DEX stallions, there was still a slight increase in the T concentration at the 1st h and then a statistically significant decrease between hours 3 and 24, after which there was a not significant increase between hours 48 and 72 (Fig. 6).

**Discussion**

The present study indicated that stallions’ reaction to the administration of endotoxin was fever, with rise of the rectal temperature. The results are close to the observations made by other authors examining a reaction of horses to a single dose of *E. coli* endotoxin (4, 8, 32, 41). The endotoxin infusion caused significant increase in the stallions scrotal skin temperature (13). This indicates the presence of disturbances in the testicular thermoregulation after endotoxin injection. Administration of the endotoxin resulted also, in an increase in heart rate. LPS stimulated haemodynamic changes in cardiovascular function, and alterations in vascular stenosis, and in blood pressure growth, which in turn produced higher heart rates (30, 39). In response to endotoxin infusion horses reacted with leukopenia. The results are convergent with the data obtained previously in stallions (11, 12), and generally in horses (23, 32).
**Fig. 1.** Rectal temperature in the stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX

* -compared with t=0 h, a:b - significant differences between groups (P<0.05)

**Fig. 2.** Scrotal skin temperature in the stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX

* -compared with t=0 h, a:a - no significant differences between groups (P<0.05)
Fig. 3. Heart rate in the stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX
* -compared with t=0 h, a:a – no significant differences between groups (P<0.05)

Fig. 4. White blood cell numbers in whole blood of stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX
* -compared with t=0 h, a:b - significant differences between groups (P<0.05)
Fig. 5. Platelet count in whole blood of the stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX
* -compared with t=0 h, a:a - no significant differences between groups (P<0.05)

Fig. 6. Concentration of testosterone in blood serum of the stallions (mean ± S.D).

E-group stallions with LPS, E+DEX-group stallions with LPS+DEX
* -compared with t=0 h, a:b - significant differences between groups (P<0.05)
The post-endotoxin leukopenia mainly resulted from decreases in circulating neutrophil count. Endotoxin promotes margination of circulating neutrophils along the vessel wall; and causes sequestration of these cells in the hepatic, spleen, and lung vasculature (5, 19). Neutrophilia is the next defence reaction, caused by the neutrophil reserve released from the bone marrow into the circulation. This, of course, produced an increase in the WBC counts, and caused leukocytosis. Thrombocytopenia was noticed in horses in other studies, as a result of administration of different doses of endotoxin (6, 33). In stallions there was maximal decrease in platelet count at the 3rd h after infusion of 0.3 µg/kg b.w. of E. coli LPS (11, 12), whereas in mares a significant decrease in PLT number was ascertained at the 48th h, after three times administration of E. coli endotoxin at the total dose of 0.5 µg/kg b.w. (14). The decrease in the platelet count in blood seems to be related mostly with disturbances of vascular haemostasis - DIC (disseminated intravascular coagulation) (26). In studies in vitro, it has been found that endotoxin by PAF (platelet-activating factor) induces platelet aggregation in horses (20).

The administration of endotoxin to the stallions; causes changes in the level of testosterone in the blood serum, which can probably result from disturbances of synthesis and blood accumulation of this hormone. Treatment with LPS resulted in a statistically significant decrease in the T concentration, maximum at the 6th h, and again an increase between the 48th and 72nd h. In previous works (9, 13), similar changes of testosterone concentration were described in blood serum of stallions after intravenous administration of 0.3 µg/kg b.w. of E. coli LPS. Based on tests carried out on male laboratory animals, it was demonstrated that after administration of LPS a decrease in T in the blood serum occurs as a result of androgen synthesis impairment in Leydig cells (1, 3, 17, 18). In adult rats, a high dose of LPS caused reduction in serum testosterone concentration and vascular changes, accompanied by an increase in endothelial permeability, microhaemorrhages, and inflammatory cell infiltration in the testes (29). In rams, a decrease in testosterone concentration in blood serum occurs for about 12-24 h, while in boars an increase in the concentration of this hormone takes place 1-2 h after Salmonella Typhimurium endotoxin administration, with a later tendency towards a decrease in its concentration (35, 36). Other studies in boars (37, 38) showed that endotoxin induced infiltration of polymorphonuclear leukocytes into the testicular interstitium, and morphological changes of Leydig cells.

DEX belongs to a group of drugs with immunosuppressive and anti-inflammatory properties. The multiple mechanisms responsible for the anti-inflammatory effects of glucocorticoids (GC) include the inhibition of phospholipase A₂, causing suppression of the production of arachidonic acid and its metabolites (24). The effect of DEX to inhibit COX (cyclooxygenase enzyme) synthesis is selective to the LPS-induced enzyme (25). Indeed, co-incubation with 100 µM DEX suppresses the production of TNFα by lipopolysaccharide-stimulated equine peritoneal macrophages, but this concentration of DEX greatly exceeds therapeutic dose of DEX for horses (28). The effects of corticosteroids, have been assessed in endotoxaemia, particularly during endotoxin shock, in horses. In anesthetised ponies of both sexes, the treatment with DEX (2 mg/kg b.w.) after infusion of E. coli endotoxin (125 µg/kg b.w.), indicated that DEX does not counteract changes evoked by LPS (lactic acidosis, prolonged coagulation times, leukopenia, haemoconcentration, and elevated blood biochemical values) (15). The injection of DEX (1.1 mg/kg b.w.), 30 min before E. coli endotoxin (0.1 µg/kg b.w.) infusion prevented partially the rectal temperature increase and attenuated the significant leukopenia, but had no effect on the heart rate (32).

It is necessary to add that treatment with DEX alone had negative effect on the stallions blood serum testosterone concentration (10). There was noted a statistically significant decrease in the T values (maximum at 5 h, ΔT - 0.48 ng/ml).

In the present study, DEX treatment in a slight degree prevented an endotoxin-induced increase in rectal, and scrotal skin temperature, and heart rate, with no influence on the decrease in platelet and leukocyte number in the blood. Administration of DEX had significant effect on the changes of leukocyte count and testosterone concentration after treatment with of endotoxin. For example, flunixin meglumine (FM, non-steroidal anti-inflammatory drug) at a dose of 1.1 mg/kg given 5 min after the injection of Escherichia coli LPS (0.3 µg/kg b.w.), had also diverse effects on certain pathological responses and concentration of blood serum testosterone in the stallions (13). It exerted the slight changes in rectal and scrotal skin temperature and heart rate in stallions treated with endotoxin+FM, in comparison with those treated with endotoxin alone.

Other research (12) showed no significant changes in the PLT count in stallion blood after administration of endotoxin+FM. That and the present study, indirectly indicate that products of cyclooxygenase (inhibited by anti-inflammatory drugs) do not have conclusive influence on endotoxin-induced aggregation of platelets in the horse. The (PAF) is a critical mediator of this process (21).

In conclusion, DEX had different effects on changes in LPS-treated stallions. A positive influence of the administration of DEX was noticed in the rectal temperature, changes in the WBC number, and the serum testosterone concentration. There were noted also smaller changes in the scrotal skin temperature and heart rate. The range and level of these changes should be taken into consideration in the evaluation of bacterial toxemaia status, and effects of steroidal anti-inflammatory drugs in stallions during reproductive season.
References


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