EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON ENDOTOXAEMIC STALLIONS

JANUSZ DANEK

Department of Animal Reproduction and Animal Health Protection
University of Technology and Life Sciences, 85-084 Bydgoszcz, Poland
jdanek@wp.pl

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Abstract

The effects of Escherichia coli endotoxin and human chorionic gonadotropin (hCG) on certain clinical and pathological variables and serum testosterone concentration were studied in the breeding stallions. The study was conducted on 10 stallions of Polish Primitive Horses divided into three groups: group I (n=3) - exposed to 0.3 μg/kg b.w. of LPS Escherichia coli; group II (n=3) - treated with physiological saline solution and human chorionic gonadotropin in the single dose of 6,000 IU; and group III (n=4) - given endotoxin and hCG. Rectal temperature, scrotal skin temperature, heart rate, and white blood cell count were determined. Serum blood samples were measured for testosterone concentrations. Comparing the group of stallions with the saline solution+hCG with endotoxin-treated stallions, there were significant clinical and haematological alterations (fever, with rise of rectal and scrotal temperature, tachycardia, and leukopenia). Endotoxaemia resulted also in changes of serum testosterone concentration (with early decrease and later increase). The hCG has not prevented the decrease in level of testosterone.

Key words: stallion, endotoxaemia, human chorionic gonadotropin, testosterone.

Lipopolysaccharide (LPS, endotoxin) is the outer component of the wall of Gram-negative bacteria and plays an important role in inflammatory reactions, which occur in response to these infections. The effects of endotoxaemia are mediated by inflammatory mediators synthesised and released by mononuclear phagocytes (21). These cells generate many proinflammatory mediators including cytokines (TNF-α, IL-1, IL-6), eicosanoids (arachidonic acid metabolites), and platelet activating factor, which are responsible for many effects of endotoxaemia in horses (27, 32, 33).

Endotoxins have been shown to cause various pathophysiological disturbances in reproduction and affect males of various animal species. On the basis of tests carried out on male animals, it has been accepted that after administration of LPS, a decrease in testosterone in the blood serum/plasma occurs as a result of impairment of androgen synthesis in Leydig cells (1, 3, 20, 23, 31).

Human chorionic gonadotropin (hCG) is a glycoprotein hormone, which is used to stimulate the Leydig cells to synthesise testosterone. The treatment with a single dose of hCG induces a plasma testosterone rise in the stallions (2, 7, 22, 25, 28). On the other hand, the testosterone response to hCG in endotoxaemic rats is negative (24). Reduced testosterone production by Leydig cells was also observed in the rams after infection with Trypanosoma congolense (19).

It was demonstrated that in response to Escherichia coli endotoxin infusion to breeding stallions, an increase in rectal and scrotal skin temperature, heart rate, and leukopenia with following leukocytosis was observed (8, 10, 11). Endotoxin influenced also the level of testosterone in the blood serum (significant decrease with later increase) (9-11).

The aim of the presented study was to evaluate the effect of a single dose of hCG on endotoxaemic stallions in the breeding season.

Material and Methods

Experimental procedures. The investigations were performed on 10 clinically healthy breeding stallions of Polish Primitive Horses. The study was carried out during the mating season (April-July). The stallions were divided into three groups: ENDO (three stallions aged 8-10 years and weighing 340-380 kg), PSS+hCG (three stallions aged 5-12 years and weighing 260-320 kg), and ENDO+hCG (four stallions aged 4-10 years and weighing 280-350 kg). Lipopolysaccharide from Escherichia coli serotype 055:B5 (Sigma Chemical Co., USA) dissolved in apyrogenic physiological saline solution (PSS, Polfa, Poland) was administered intravenously. The first group of the stallions was infused with 0.3 μg/kg b.w. of LPS (0.3 μg LPS in 1 ml of saline). The stallions of group PSS+hCG were administered PSS (1 ml of saline/kg b.w.) and a single intramuscular dose of hCG (6,000 IU, Chorulon, Intervet, Holland). Group ENDO+hCG was given 0.3
μg/kg b.w. of LPS and 6,000 IU of hCG. The hCG was injected directly after the infusion of PSS or LPS.

Clinical, haematological, and hormonal examinations. Clinical examination comprising observations of the animals, measurement of rectal temperature, scrotal skin temperature, and heart rate, was performed immediately before the infusion (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 scrotal skin temperature was measured using electronic thermometer, which was kept (1 min) in the middle, between left and right part of the scrotum. After the clinical examinations, the whole blood samples were taken (always at the same time) from the jugular vein into EDTA-vacutainer tubes and tubes for serum using a catheter (Secalon® Kathy 1, Viggo, Spectramed, UK). Blood in plain tubes was allowed to clot at room temperature, then, serum was collected by centrifugation. Serum samples were stored at -20°C until assayed. The white blood cells (WBC) count was determined using a Sysmex F800 analyser (TOA Medical Electronics Co., Japan). The level of testosterone was measured with the use of RIA kit (Spectria, Orion Diagnostica, 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 Fischer test. The differences were statistically significant at P<0.05.

Results

After administration of the endotoxin, all experimental stallions showed signs of endotoxaemia. In stallions receiving endotoxin (ENDO group), a significant increase in rectal temperature was observed within 2-7 h after administration of LPS. The maximum increase in the temperature (to 38.8°C, ΔTg = 2.0°C) was noted at hour 4. In the group of stallions with endotoxin and human chorionic gonadotropin (ENDO+hCG group), rectal temperature increased between the 3 and 8 h. The maximum increase in the temperature (to 38.6°C, ΔTg = 2.0°C) was noted also at hour 4 (Fig. 1).

In stallions from group ENDO, a statistically significant increase in scrotal skin temperature was observed within 2-5 h after administration of LPS. In this group of stallions, the maximum increase in the temperature (to 34.8°C, ΔTsc = 2.1°C) was noted at the 3rd h. In the ENDO+hCG group, the increase in scrotal skin temperature was observed within 1-4 h after LPS administration (maximum increase at 2 h, up to 35.3°C, ΔTsc = 2.2°C (Fig. 2).

The heart rate increased significantly in the ENDO and ENDO+hCG groups at hours 1-4 and maximum increase (to 65.3 beats/min, ΔHR = 28.7 beats/min, and to 68.5 beats/min, ΔHR = 26.2 beats/min, respectively) was observed at 2 h (Fig. 3).

Endotoxin-treated stallions had a significantly reduced number of white blood cells (Fig. 4). The counts of the cells decreased at 1-4 h after endotoxin administration in the ENDO and ENDO+hCG groups. The largest decrease in WBC was at the 2nd h of the experiment, and was characterised by a decrease in the number of WBC to 2.1 x 10^9/L, ΔWBC = -6.2 x 10^9/L (group ENDO), and at 1st h, to 2.5 x 10^9/L, ΔWBC = -5.7 x 10^9/L (group ENDO+hCG). The number of WBC increased again at 24–72 h (ENDO) and at 7 h and 24 h (ENDO+hCG).

Similar changes of clinical and pathological variables were not observed in the group of stallions treated with PSS and hCG.

After LPS administration, there was a statistically significant decrease in testosterone concentration at 4-24 h, after which there was an increase at 48-72 h. A maximum decrease (to 0.30 ng/mL, ΔT - 1.13 ng/mL) was noted at 6 h, and maximum increase (to 2.80 ng/mL, ΔT - 1.37 ng/mL) was observed at 48 h. In the ENDO+hCG stallions a rapid increase in testosterone concentration took place in the 1st h and then a statistically significant decrease at 5-8 h (maximum decrease at 7 h, to 0.16 ng/mL, ΔT - 1.27 ng/mL) was found, after which there was again a statistical increase at 48-72 h. In the PSS+hCG group, there was a significant increase in the testosterone concentration at the 1-8 and 48-72 h, with maximum increase at 48 h to 6.82 ng/mL, ΔT - 5.37 ng/mL (Fig. 5).

Discussion

In the presented study, the comparison of the groups of stallions with physiological saline solution+Chorulon (PSS+hCG) and the endotoxin treatments showed that there were significant clinical and haematological alterations, which were significant and characteristic of endotoxaemia. The stallions’ reaction to administration of endotoxin (groups ENDO and ENDO+hCG) was fever, with rise of the rectal and scrotal temperature, tachycardia and leukopenia. The results are close to the observations of other authors examining the reaction in horses (in general) to endotoxin (5, 13, 17, 26, 34) and especially in stallions in breeding season after administration of 0.3 μg/kg b.w. of Escherichia coli LPS (8, 10, 11).

These effects were observed due to the synthesis and release of many mediators and proinflammatory substances produced during endotoxaemia (21). Fever is one of the most consistent changes induced by LPS administration. Endotoxin and host products directly affect the hypothalamic thermoregulatory centre and initiate fever (6, 12). Endotoxin and other inflammatory mediators also promote adherence of neutrophils to the vascular endothelium (4, 15). The postendotoxin initial leukopenic response resulted mainly in a decrease in circulating neutrophil count, but later leukocytosis was the result of the next defence reaction caused by the neutrophil reserve released from the bone marrow into the circulating blood.
Fig. 1. Rectal temperature in the stallions (mean ± S.D).

ENDO – stallions treated with endotoxin (0.3 μg/kg b.w.), PSS+ hCG - stallions treated with physiological saline solution+Chorulon (6,000 IU), ENDO+hCG - stallions treated with endotoxin+Chorulon , * - compared with t=0 h, a:b - significant differences between groups at P<0.05.

Fig. 2. Scrotal skin temperature in the stallions (mean ±SD).

Symbols are explained in the footnote to Fig. 1.
Fig. 3. Heart rate in the stallions (mean ±SD).
Symbols are explained in the footnote to Fig. 1.

Fig. 4. White blood cell counts in stallions (mean ±SD).
Symbols are explained in the footnote to Fig. 1.
LPS stimulated haemodynamic changes in cardiovascular function and alterations in vascular stenosis and in blood pressure growth, which in turn produced higher heart rates (32, 33). Significant increase in scrotal skin temperature gives evidence for disturbances in the testicular thermoregulation in the stallions after endotoxin injection. The study on men indicated that intrascrotal temperature was compared with oral body temperature in subjects with fever. When the body temperature increased, the testicular thermoregulatory mechanism appeared to fail and the intrascrotal temperature has raised (18).

Treatment with LPS resulted in a statistically significant decrease in the testosterone concentration, reaching the lowest values at the 6th h, and a renewed increase at the 48-72 h. In previous studies (10, 11), similar changes of testosterone concentration were described in blood serum of stallions after administration of 0.3 μg/kg b.w. of *Escherichia coli* LPS. It was demonstrated in many studies that endotoxaemia resulted in depression of testosterone production, and the change in blood content of testosterone was observed after LPS infusion in the males. After *S. typhimurium* endotoxin administration, a decrease in testosterone concentration occurs in rams’ blood serum for about 12–24 h, while in boars an increase in the concentration of this hormone takes place especially at the 1–2 h with a later decreasing tendency in its concentration (29, 30). In adults rats (20), a single injection of low dose of LPS caused a biphase decrease in serum testosterone concentrations at 6 h (early phase) and at 18-24 h (late phase). In the mice, the inhibition of steroidogenesis (decrease in serum testosterone concentration) by endotoxin is in opposition to serum LH or Leydig cell LH receptor binding on Leydig cells (14). In an experimental endotoxaemia model utilising mice, serum testosterone was found to be decreased by 90% 2 h post intraperitoneal injection of endotoxin. The early depression of serum testosterone was shown to be associated with a decrease in steroidogenic acute regulatory (StAR) protein levels while the prolonged decrease corresponded to a decreased protein and transcript levels of steroidogenic enzymes in Leydig cells (3). In adult rats, there is an initiate expression of IL-1α and IL-1β in testicular macrophages and a decrease in plasma testosterone by 60%, at 2nd h after administration of endotoxin (16). Reduced testosterone production by Leydig cells was also observed in the rams after infection of *T. congolense* (19).

In the presented study, the human chorionic gonadotropin given in a single dose (6,000 IU) did not prevent (in spite rapid increase in testosterone concentration at 1st h of the experiment) a decrease in serum level of testosterone in the ENDO+hCG group of stallions. In contrast, in the PSS+hCG group of stallions, the serum testosterone concentrations showed a sharp rise after injection of a single dose of hCG and there was a biphase increase in testosterone concentration with maximum at the 1st and 48th h. A similar observation has

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**Fig. 5.** Concentration of testosterone in blood serum of the stallions (mean ±SD).

Symbols are explained in the footnote to Fig. 1.

a:b:c - significant differences between groups at P<0.05.
been reported in mature warm-blooded stallions, which received 5,000 IU of hCG (2).

It seems reasonable to speculate that the actions of testosterone responsiveness to hCG described in the presented study are related to inhibitory effect of LPS on biosynthetic capacity of the Leydig cell and blood accumulation of testosterone in the breeding stallions.

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References

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