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RESEARCH ON INFLAMMATORY ANEMIA INDUCED BY CORTICOSTEROIDS

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Abstract

Research was conducted on 10 CD1 laboratory mice, non-consanguineous strains, divided into two groups of 5 individuals per group. The body weight of individuals ranged from 20 to 30 grams. Both groups were given the same favourable climate, humidity and light conditions. The forage diet consisted of pelleted rodent feed, with feed and water administered at libitum. The control group was injected at the beginning of the experimental period with 1 ml NaCl subcutaneously and the experimental group with 1 ml dexamethasone i.e. 4 mg subcutaneously. The duration of the experiment was 14 days. At the end of the experiment, decreases in erythrocyte count, haemoglobin, haematocrit and increases in derived erythrocyte constants were observed. The results of the leukocyte formula showed an increase in the number of polymorphonuclear cells and a decrease in the other categories of leukocytes in the experimental group.

Key words*: dexamethasone, glucocorticosteroid, anti-inflammatory, mouse.*

INTRODUCTION

Dexamethasone is a synthetic glucocorticosteroid that is widely used to treat various types of conditions, but the same glucocorticosteroids are associated with many side effects (Alexandru et al, 2020). Therapeutic indications for glucocorticosteroids are mainly inflammatory conditions, autoimmune conditions, and hypersensitivity conditions, but the same glucocorticosteroids are also used as an anesthetic.

Although the practical applications of these types of substances are obvious, in practice it is also necessary to take into account the adverse effects that their administration may have.

Glucocorticosteroids can suppress the immune system 20-30 times greater than the ability of hydrocortisone. They have an anti-inflammatory role by suppressing the action of cytokines as well as nitric oxide.

The anti-inflammatory mechanism of these substances may be due to their anti-inflammatory effects, such as inhibiting leukocyte diapedesis and thus stopping the inflammatory reaction, and thus the algic component of inflammation (Cotor et al, 2021).

Glucocorticoids are synthesis and release moderators for prostaglandins, leukotrienes, PAF, various cytokines as well as for several enzymes such as collagenase

Among the negative effects of the administration of glucocorticoids in animals of economic interest, the decrease in milk production can be mentioned. This decrease in milk production is reported by several researchers and can be manifested by a decrease in the amount of fat, total protein, casein, but also of different minerals in milk (Oprea et al., 2019; Oprea et al., 2020; Petcu et al., 2020).

MATERIALS AND METHODS

The experiment was carried out in the biobase of the Faculty of Veterinary Medicine in Bucharest, on two groups of laboratory mice, a control group and an experimental group.

The mice were CD1 non-consanguineous strains and were divided into 5 individuals per group.

The experimental animals had body weights between 20 and 30 grams and were kept under favourable climate, humidity, and lighting conditions at all times.

Feed was administered ad libitum and consisted of combined granulated rodent feed, also water was administered at discretion.

Mice in the control group were injected subcutaneously with 1 ml NaCl 0.9% at the beginning of the experiment and mice in the experimental group were injected with 1 ml dexamethasone, i.e. 4 mg. Injection of the mice in the experimental group was performed twice, the first time at the start of the experiment and the second time at 7 days.

The duration of the experiment was 14 days. On the last day of the experiment blood was taken on anticoagulant. Erythrocyte count, leukocyte count, hemoglobin, hematocrit and derived erythrocyte constants were determined for each individual in both groups. Investigations were performed using a 5 DIFF LaserCyte haematology analyser from Idexx Laboratory.

The leukocyte formula was performed by the classical method under the light microscope and smear staining was performed by the May Grunwald Giemsa method. Interpretation of the results was performed using the T (Student) test.

RESULTS AND DISCUSSIONS

Decreases in erythrocyte count, haemoglobin and haematocrit were observed in the experimental group. Slight increases in MCV MCH and MCHC were recorded.

The leukocyte count decreased significantly in the dexamethasone-injected group (Table 1).

Erythrocyte count determination showed a slight decrease in the experimental group, the mean value being 2.36% lower than the control group.

Haemoglobin in the group that was inoculated with dexamethasone was 0.29% lower than in the control group.

Haematocrit also showed a slight downward trend, being 0.69% lower.

The secondary erythrocyte constants, MCV, MCH, and MCHC had slightly increased values compared to the control group: 1.72%, 2.15%, and 0.44%, respectively.

A marked decrease could be observed in the dexamethasone-injected mice group, the mean leukocyte count was 36.21% lower than the mean in the control group (Figure 1 and Figure 2).

As far as primary erythrocyte constants are concerned, a slight downward trend can be observed but without major significance. In contrast, the secondary erythrocyte constants showed an increasing trend (Ganz T., 2019; Jamela J., 2016).

Table 1. Mean values of haematological investigations

Parameter	Control group	Experimental group	Percentage (%)
E x 10^6 / µl	8.48	8.28	↓2.36
Hb g/dl	13.78	13.74	↓0.29
HTC %	43.28	42.98	↓0.69
MCV μ^3	51.1	51.98	↑1.72
MCH pg Hb/E	16.26	16.61	↑2.15
MCHC g Hb/dl E	31.83	31.97	↑0.44
Leucocyte x 10^3/µl	11.02	7.03	↓36.21

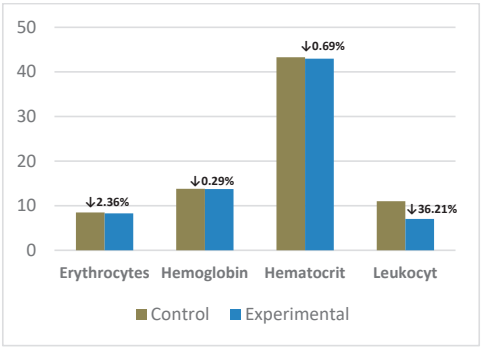

Figure 1. Mean values of haematological investigations (primary erythrocyte constants) and leucocytes

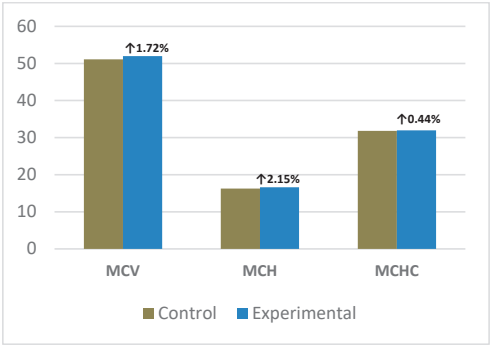

Figure 2. Mean values of haematological investigations (secondary erythrocyte constants)

Glucocorticosteroids may have a positive effect on circulating haemoglobin by delaying the phenomenon of erythrophagocytosis (Cringanu D. et al., 2021). The marked decrease in circulating leukocytes is a consequence of

dexamethasone administration in the experimental group.

White circulating cellular elements are affected by corticosteroids. An increase in polymorphonuclear cells is known following treatment with glucocorticoids, due to the increase in their release from the hematoforming marrow and also due to the decrease in the percentage of their removal from the circulatory sector (Ghiță M. et al., 2015).

In contrast, lymphocytes, eosinophils, monocytes, and basophils decrease in number after glucocorticoid administration.

Glucocorticoids prevent or suppress the entire inflammatory response to infectious, physical or immunological agents by inhibiting early inflammatory events such as oedema, cellular exudation, fibrin deposition, capillary dilation, leukocyte migration, and phagocytic activity. Later events such as capillary and fibroblast proliferation, collagen deposition, and scarring are also inhibited. The anti-inflammatory mechanism of glucocorticoids, although not fully understood, is of great therapeutic relevance and is the subject of intense scientific investigation (Forbes N. et al., 2015). Following the administration of dexamethasone to the mice in the experimental group, the following results were observed in the leukocyte count: the percentage of neutrophils was 4.84% higher and the other white cell populations showed decreases: eosinophils 50%, lymphocytes 8.76%, monocytes 50% (Table 2).

Similar results, but in postpartum dairy cows were also found by Jitkamol in 2004. In that paper it is mentioned that the animals received a single dose of dexamethasone. Dexamethasone being used in the respective experiment because it is used very often in practice to treat fatty liver syndrome and ketosis in ruminants (Shamay et al., 2000).

Following the administration of dexamethasone to lactating cows, the authors were able to observe the onset of a slight leukopenia. After performing the leukocyte formula, they observed the percentage increase of neutrophils.

No circulating basophils were recorded in the smears examined (Figure 3 and Figure 4).

Table 2. Mean leucocyte formula values

Category	Control group	Experimental group	Percent
Neutrophil %	25.64	26.88	↑4.84
Eosinophil %	0.4	0.2	↓50
Bazophile %	0.2	0	↓100
Lymphocytes %	50.2	45.8	↓8.76
Monocytes %	0.4	0.2	↓50

The increase in the percentage of neutrophils is attributed to the action of dexamethasone, a hormone glucocorticosteroid.

It stops the diapedesis of polymorphonuclears and they remain confined to the blood circulation (Ionita F., 2021; Alexandru D., 2020).

Glucocorticoids attenuate the ability of neutrophils to adhere to capillary endothelial cells by a dual mechanism.

They block the normal increase in expression of endothelial adhesion molecules (i.e., ELAM-1) and intercellular adhesion molecules (i.e., ICAM-1) and induce lipocortin, a protein inhibitor of phospholipase A2 (PLA2) (Marx J. et al., 2015).

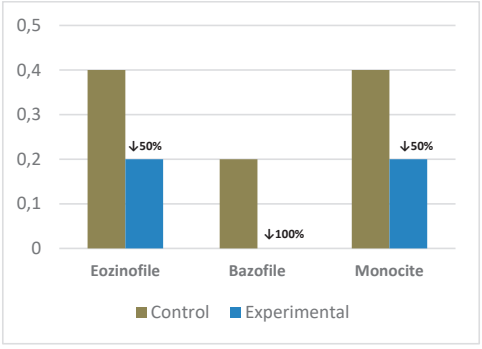

Figure 3. Average leukocyte counts (eosinophils, basophils, monocytes)

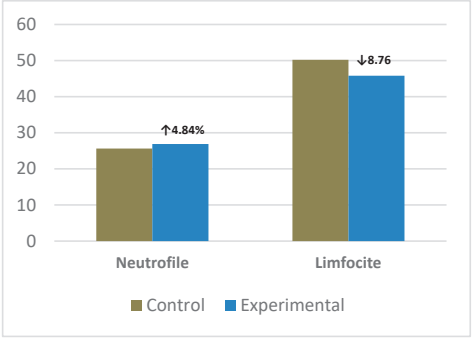

Figure 4. Mean values of leukocyte counts (neutrophils and lymphocytes)

The plasminogen activator and the inhibitory factor for cell movement are inhibited by glucocorticoids, which leads to a low release of hydrolytic enzymes and histamine. The specialty literature also mentions the decrease in chemokines, which attract white cells (Mittelstad et al., 2018; Salehzadeh M. et al., 2022).

Other researchers have demonstrated that the increased number of neutrophils in the blood circulation, after stress or after glucocorticoid treatments, is caused by several factors: first of all, an increased supply of neutrophils from the hematoforming bone marrow reserve, then a reduced diapedesis of neutrophils. The decrease in margination and diapedesis of neutrophils could be caused by the known ability of corticosteroids to reduce the adhesion of neutrophils to the vascular endothelium.

In the macrophages and monocytes from the inflamed tissue sectors, the production of free oxygen radicals and phagocytosis are inhibited. As the inflammation progresses, fibroblasts are inhibited, resulting in poor scarring. This inappropriate scarring affects the healing of the animals and thus implicitly the food safety (Petcu et al., 2007; Petcu, 2013; Petcu, 2015).

This impairment of neutrophil kinetics and margination may increase susceptibility to infectious diseases such as bovine mastitis.

Glucocorticoids slow normal wound healing by blocking inflammatory. collagen breakdown and disorganisation reaction (Leica I. et al, 2020; Curca D., 2008).

So basically there is an apparent increase in circulating neutrophils, they are just prevented from leaving the circulatory system (Cotor G. et al., 2017).

Mononuclear cells decrease numerically after the administration of the experimental substance. A single dose can lead to a decrease of up to 70% of lymphocytes and up to 90% of monocytes (Supeanu et al., 2020).

The redistribution of mononuclear cell populations leads to their numerical decrease in the blood. It should also be mentioned that glucocorticoids can lead to the death of some lymphocytes, an aspect shown by several researchers.

The most sensitive to apoptosis induced by these anti-inflammatories are T-lymphocytes. B-lymphocytes are a little more resistant. Different subpopulations of T-lymphocytes differ among themselves in their sensitivity to glucocorticoids. The decrease of basophils occurs through an incompletely elucidated mechanism (Vagnerová K. et al., 2023).

Corticosteroids in lactating cows can lead to lower milk production. This is the glucose sparing phenomenon. Following a marked hyperglycemia, the body's response is to secrete a larger amount of insulin. But glucocorticoids lead to the inhibition of the neoglucogenesis phenomenon by insulin and facilitate the installation of resistance to it in the peripheral areas, further ensuring hyperglycemia. Also from the category of negative effects of corticosteroids, it should be remembered that they exert some effects on the water and electrolyte balance, increasing potassium excretion and sodium retention. This aspect is explained by the activity of corticosteroids at the renal level.

Fluorinated corticosteroids do not have mineral and corticoid activity. They have an effect of polyuria and polydipsia due to the antidiuretic hormone that is inhibited as a result of a low renal sensitivity to this type of hormone.

In the specialized literature it is mentioned that they lead to the drastic decrease of calcium reserves through a low absorption of it and by increasing the renal threshold Depletion of systemic calcium reserves has negative effects on milk production but also on young bovine infants.

Also related to calcium metabolism, glucocorticoids inhibit osteoclasts and increase parathyroid secretion, which could structurally affect the bone but also bone healing. Affections at the skeletal level are not compatible with an industrial animal breeding system, the economic yield being strongly affected.

CONCLUSIONS

Haematological investigations in both groups of mice show that dexamethasone injection leads to a slight decrease in erythrocyte count (2.36%), haemoglobinemia (0.29%) and haematocrit (0.69).

Derived erythrocyte constants (MCV MCH and MCHC) showed increasing trends in

individuals of the experimental group (1.72%, 2.15% and 0.44%).

Results of leukocyte formula showed a higher percentage of circulating polymorphonuclear cells in the experimental group (4.48%).

Lymphocytes, eosinophils, basophils and monocytes had lower values than in the control group following dexamethasone administration. The percentage of lymphocytes was 8.76% lower, eosinophils 50% lower and monocytes 50% lower. No circulating basophils were found in blood smears from mice in the experimental group at the end of the research.

REFERENCES

Alexandru D. M., Dobre I.R., Crivineanu M. (2020). Diagnosis and treatment of canine appendicular osteosarcoma. A case report. *Scientific Works. Series C. Veterinary Medicine*. Vol. LXVI(1), ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Cotor G., Zagrai G., Gâjâilă G., Ghiță M., Ionescu A.M., Damian A., Zagrai (Măierean) A.M., Dragosloveanu S, Cotor D.C. (2021). The evolution of some blood parameters in hypovolemia conditions in rabbits. *Polish Journal of Veterinary Sciences*, 24(4), 589–594, ISSN:1505-1773,doi 0.24425/pjvs.2021.139984.

Cotor, G., Bălăceanu, R., Ghiță, M. (2017). *Laboratory exercises in animal pathophysiology, a clinical and experimental approach*. Ed. Printech, București, ISBN: 978-606-23-0793-6

Curcă Dumitru C. (2008). *Tratat de Fiziopatologie*. Ed. Printech, București.

Cringanu D., Negreanu R., Garjoaba I, Cringanu I., (2021). Therapeutic approach in veterinarian oncological emergencies. *Scientific Works. Series C. Veterinary Medicine.* Vol. LXVII(2), ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Forbes et al. (2015). P39. Comparative Performance of Two Bench-Top Hematology Instruments for Macaques and Mice. *JAALAS*, 54(5): 568-668. PROCYTE + HEMAVET.

Ganz T. (2019). Anemia of inflammation, *The New England Journal of Medicine,* London, 3; 133(1): 40-50. doi: 10.1182/blood-2018-06-856500.

Ghiță, M., Cotor, G., Vițălaru, A., Brăslașu, D. (2015), Comparative study on the effect of prednisone and dexamethasone on leucocytes, in rabbit. *Journal of Biotechnology*, Volume 208, Supplement, Page S92, ISSN 0168-1656.

Ionita F., Ancuta D., Coman C. (2021). Evaluation of induced metabolic syndrome of obesity by administering a purified diet in mice. *Scientific Works. Series C. Veterinary Medicine*. Vol. LXVII (1), ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Jamela Jouda (2016) The effect of long-term oral dexamethasone on blood cells counts and brain regions of young mice. *AENSI Publication,* pages 63-71 ISSN: 1995-0772 EISSN: 1998-1090.

Leica L., Mitrea I.L., Mariana I. (2020). Coagulopathy as a complication of babesiosis in a dog with hemothorax: clinical case report. *Scientific Works. Series C. Veterinary Medicine*. Vol. LXVI(1), ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Marx, J. O., et al. (2015). The Effects of Acute Blood Loss for Diagnostic Bloodwork and Fluid Replacement in Clinically Ill Mice. *Comp Med*, 65(3): 202-216. C57BL/6J.

Oprea, O.D., Petcu, C.D., & Ciobotaru-Pîrvu, E. (2019). A study concerning quality assessment and processing particularities in certain dairy products. *Scientific Works. Series C. Veterinary Medicine,* LXV(1), 121-126.

Oprea, O.D., Răduță, A., Călin (Nicolae), S., Andrei, C. (2020). Research on percentage variation concerning cow's milk protein, lactose and fat, depending on the season. *Scientific Papers. Series D. Animal Science,* LXIII(1), 182-186.

Petcu, C.D., Savu, C., Mitrănescu, E., & Chirilă, S. (2007). The implementation of the integrated quality and food safety management system in the food industry units. *Lucrări Ştiinţifice Medicină Veterinară, XL*, 545-51.

Petcu, C.D., Ciobotaru-Pîrvu, E., Oprea, O.D., & Ghimpețeanu, O.M. (2020). Ecological dairy products: healthy or just a trend? *Scientific Works. Series C. Veterinary Medicine*, LXVI(1), 87-95.

Petcu, C.D. (2013). Researches concerning some meat products control in a specialized unit. *Scientific Papers. Series D. Animal Science*, LVI, 323-325.

Petcu, C.D. (2015). *Meat quality and technology. Bucharest*, RO: Granada Publsihing House.

Salehzadeh, M. et al. (2022). Glucocorticoid production in lymphoid organs: Acute effects of lipopolysaccharide in neonatal and adult mice. *Endocrinology,* 163, bqab244.

Shamay A., Shapiro F., Barash H. (2000), Israel Bruckental, Nissim Silanikove. Effect of dexamethasone on milk yield and composition in dairy cows. *Annales de zootechnie*, 49(4), pp.343-352. 10.1051/animres: 2000125.hal-00889900.

Supeanu T.D., Supeanu A., Lucica S., Roman V., Cobzariu D., Baraitareanu S., Menchetti L., Danes D. (2020). A comparative clinical and paraclinical evaluation of the effects of polyspecific avian immunoglobulin y in Fiv+/- cats. *Scientific Works. Series C. Veterinary Medicine*. Vol. LXVI (1), ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Vagnerová K., Jágr M., Mekadim C. Ergang P. , Sechovcová H., Vodička M., Fliegerová K., V. Dvořáček , Mrázek J., Pácha J. (2023). Profiling of adrenal corticosteroids in blood and local tissues of mice during chronic stress. *Scientific Reports*, 13: 7278, https://doi.org/10.1038/s41598-023-34395-2