

THE INFLUENCE OF DIAZEPAM ON THE NUMBER AND MORPHOLOGICAL CHANGES OF CHOSEN CELLS AND TISSUES OF ORGANS IN TRANSPORTED CALVES*

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The aim of the present work was to assess the effect of diazepam supplementation on the chosen blood cells and cells of organs tissues of calves that were exposed to transport stress. The studies were performed on 28 calves in the age of 14 days. The calves were fed *per os* with low quantities of milk, diazepam in the dosage 1mg/kg b.w., half hour before transportation and once a day in the same dosage for the subsequent 5 days. In the case of calves fed with diazepam, decrease of the total leucocyte and lymphocyte count as well as decrease in changes in neutrophil counts were encountered. In the tissues of lungs, liver and thymus elevated level of mastocytes was observed in various functional states beginning from the state of graininess formation to their full degranulation.

Key words: calves, diazepam, stress, leucocytes, lungs, liver, thymus

Introduction

In the literature there are found reports on the application of various anti-stress and pro-adaptive factors on animals, and their advantageous effect on the condition and behavioral reactions of animals [5, 6]. Various preparations from the group of neuroleptics and tranquilizers were studied when considering their applicability in attenuation of stress situation of animals [5, 6, 7, 8]. The mostly advantageous results were obtained when diazepam (Relanimal) was used. This preparation has an advantageous effect on the condition of transported animals, favors quicker normalization of physiological parameters, however in the area of behavioral reactions significantly decreases aggressiveness. Supplemented before or after transportation diminishes adaptation-feed stress, morbidity and mortality, facilitates transition of the period of regrouping and dislocation, periodically diminishes fighting predispositions [5, 9]. In the present study, clinical and zootechnical parameters were considered and in the lower extent the effect of this preparation on immune system cells as well as tissues of organs was studied [8].

The aim of the present work was to assess the effect of supplementation of diazepam on the chosen cells of blood and tissues of organs in calves that were exposed to transportation stress.

Materials and methods

The studies were performed on 28 and 14-days old cross-bred calves cb + hf (25+75) from one of 4 groups, 7 animals in each, from which 2 groups: D and D-D were calves that were transported to calf house distant 10 km, two: K and K-D calves kept in a cowhouse during the experiment, in the place of birth. The calves from the group K-D and D-D were fed individually *per os*, with slight quantity of milk, diazepam in the dosage 1mg/kg b.w., 0.5 h before transportation of D-D group and once per day, in the same dosage for the subsequent 5 days. Calves from the groups K and D did not consume any preparation. Blood was sampled from all the calves before and after transportation and afterwards after 24 and 72 h and after 7, 14 and 21 days after transportation of calves to calf house. In the full blood, leucocytes count was determined as well as counts of all the cellular fractions: lymphocytes, neutrophiles, eosinophiles, basophiles and monocytes. On the last day of experiment, for the reason of histological examination, 2 calves from each group underwent slaughter, from which the samples of segments from: tonsils, lungs, liver, spleen, thymus, mediastinal lymph nodes and ilium intestine. Histological preparations, prepared according to the method given by Hultquist and Karlsson [1], after own modification [2], underwent ultrastructural assessment with electron microscope Tesla BS 500.

Results and discussion

The obtained results are presented in Table 1. In the case of animals from all the groups, during the experiment, slight changes in leucocytes counts were observed. In transported calves (D and D-D) systematic decrease in leucocyte counts was denoted until 1 week after transportation. In calves' blood in both groups fed with diazepam (K-D and D-D) it was found that during the whole experimental period, lower concentration of white blood cells was observed. The analysis of changes in lymphocytes counts in all groups, showed that only in group D, significant changes between samplings were detected: 24 hours after transportation, when the lowest concentration was reached and the subsequent until the last day of the experiment, when decrease in the number of these cells was denoted ($p \leq 0,05$). In the case of blood of calves fed with diazepam (K-D and D-D), during the experiment, lower concentration of lymphocytes was denoted when compared with the group that did not receive diazepam.

Also, only in the case of the group D, significant differences in the concentration of neutrophils in all the samplings were observed. Directly after transportation the highest level of these cells was denoted and the lowest on the 7th day of the experiment ($p \leq 0.05$). In the remaining calves, the changes in the concentration of granulocytes were not significant.

The comparison of the concentrations of residual cells of the immune system showed irregular fluctuations in their counts. It is difficult to relate these changes with the effect of introduced diazepam.

Histological analysis of internal organs of calves showed slight changes in the structure of lungs, liver, thymus and ileum intestine [1, 2, 3, 4]. The reported changes concerned mainly mobilization of cells of the immune system.

In the case of calves used in the experiment, the changes in the tissues of: lungs, liver and thymus were detected.

In lungs (Fig. 1, 2) the cells of macrophages in parafollicular septum and in close vicinity of respiratory epithelium, individual and in some cases clustered in groups were encountered. In the connective endothelial tissue of precapillary blood vessels and in adventitia of capillaries individual mastocytes were observed.

In liver tissue (Fig. 2, 3) around intralobular vessels, individual macrophages were detected. Moreover, around the central vein, individual mastocytes were observed. We concluded that this was an effect of mobilization of specific cells of the immune system of liver, so-called stellate cell. The mastocytes present in perivascular zone of lungs and liver might be used to measure activation of cell of the immune system since they constitute an indirect link of immune response.

In thymus (Fig. 3, 4) the process of degradation of cells of stroma of thymus, was observed due to the presence of thymus cells (Hassall)

Conclusion

We found that the supplementation of diazepam to the transported calves had the influence on decrease of the total leucocytes and lymphocytes counts. We assumed that diazepam had also an influence on the reduction of changes in the concentration of granulocytes that occurred in transported calve that did not receive this pharmacological preparation.

In the performed histological analysis, changes in tissues of lungs, liver and thymus were observed. The mentioned processes were more intense in tissues of calves that were transported and received diazepam. The performed histological analysis enabled to detect

increase in the number of mastocytes. Different functional state was detected, from the state of formation of graininess to their full degranulation. Described mastocytes represented the type of connective tissue mastocytes.

Table 1. Mean concentrations of cells of blood in transportation (D, D-D) and non transportation (K, K-D calves.

Parameters	Groups	Bleeding						
		Directly after transp.	Directly after transp.	24 h after transp.	72 h after transp.	7 days after transp.	14 days after transp.	21 days after transp.
Leucocytes (T/l)	D	X±SD 12,27±0,93	11,97±1,23	11,49±2,84	10,26±4,77	9,99±3,47	10,41±3,13	9,21±3,48
	K	X±SD 9,40±1,70	11,24±5,13	10,89±2,27	11,37±2,96	10,20±2,63	10,37±1,17	11,03±1,82
	D-D	X±SD 9,29±2,46	8,79±2,39	8,83±2,87	8,24±2,51	7,70±1,67	8,36±2,82	8,26±2,68
	K-D	X±SD 9,58±4,09	10,17±3,82	9,50±3,24	9,00±2,64	7,52±1,31	10,27±2,87	8,88±2,53
Lymphocytes (T/l)	D	X±SD 7,55±1,58	7,13±1,26	8,24 ^{ab} ±1,20	6,19±1,11	7,34 ^{cd} ±0,85	5,89 ^{ac} ±1,74	5,58 ^{bd} ±1,32
	K	X±SD 6,11±0,93	6,81±1,95	5,52±1,13	6,46±2,04	6,21±1,44	5,98±1,80	6,92±1,42
	D-D	X±SD 5,85±0,20	5,42±0,27	5,45±0,34	5,13±0,73	5,17±0,47	3,99±0,57	4,96±0,63
	K-D	X±SD 4,689±0,02	5,45±0,06	5,53±0,06	5,04±0,14	4,42±0,09	5,85±0,04	4,21±0,06
Granulocytes (T/l)	D	X±SD 3,72±1,32	4,07 ^a ±1,57	2,53±1,01	3,49 ^b ±0,87	1,97 ^{abc} ±0,67	3,44 ^c ±1,55	2,91±1,12
	K	X±SD 2,82±0,76	3,39±1,78	4,65±0,91	3,98±1,95	3,07±1,47	3,60±1,63	3,56±1,12
	D-D	X±SD 2,80±0,18	2,81±0,24	2,78±0,33	2,55±0,71	2,04±0,40	3,33±0,59	2,29±0,47
	K-D	X±SD 4,43±0,02	4,19±0,06	3,66±0,06	4,68±0,14	2,68±0,09	3,96±0,04	4,28±0,06
Basophiles (T/l)	D	X±SD 0,12±0,12	0,02±0,04	0,07±0,13	0,10±0,10	0,11±0,07	0,03±0,08	0,03±0,07
	K	X±SD 0,08±0,09	0,16±0,09	0,11±0,01	0,05±0,09	0,06±0,05	0,21±0,20	0,02±0,04
	D-D	X±SD 0,11±0,01	0,09±0,01	0,23±0,04	0,12±0,06	0,09±0,03	0,12±0,06	0,16±0,06
	K-D	X±SD 0,09±0,02	0,06±0,04	0,15±0,02	0,06±0,02	0,07±0,02	0,14±0,02	0,04±0,01
Eosinophiles (T/l)	D	X±SD 0,04±0,06	0,02±0,05	0,03±0,06	0,01±0,04	0,07±0,08	0,03±0,05	0,01±0,03
	K	X±SD 0,01±0,04	0,02±0,04	0,02±0,04	0,02±0,04	0,01±0,04	0,03±0,08	0,02±0,04
	D-D	X±SD 0,04±0,01	0,02±0,01	0,02±0,01	0,01±0,02	0,01±0,01	0,03±0,01	0,01±0,01
	K-D	X±SD 0,02±0,01	0,07±0,05	0,02±0,01	0,02±0,01	0,02±0,01	0,04±0,01	0,04±0,04
Monocyte (T/l)	D	X±SD 0,21±0,18	0,30±0,21	0,17±0,07	0,17±0,06	0,25±0,21	0,14±0,06	0,12±0,05
	K	X±SD 0,24±0,12	0,51±0,08	0,49±0,33	0,26±0,16	0,20±0,08	0,17±0,13	0,18±0,09
	D-D	X±SD 0,20±0,01	0,23±0,02	0,30±0,05	0,18±0,04	0,18±0,04	0,20±0,04	0,19±0,03
	K-D	X±SD 0,10±0,02	0,04 ^{ab} ±0,05	0,36 ^a ±0,12	0,09±0,03	0,027 ^b ±0,04	0,18±0,02	0,11±0,03

ab – the same small letters of the alphabet – significance of difference between breeding $p \leq 0,05$ for particular parameters

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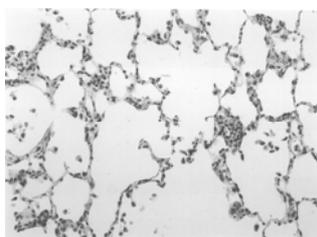


Fig.1. Histopathological image of the lungs of calves without diazepam (250x)

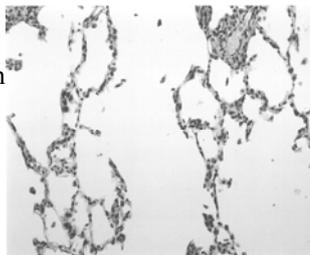


Fig.2. Histopathological image of the lungs of calves with diazepam (250x)

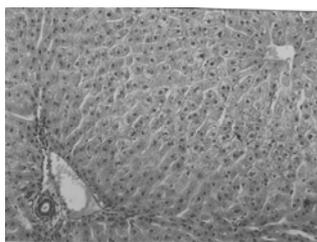


Fig. 3. Histopathological image of the liver of calves without diazepam (250x)

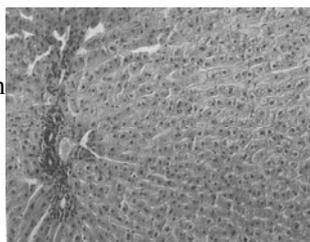


Fig. 4. Histopathological image of the liver of calves with diazepam (250x)

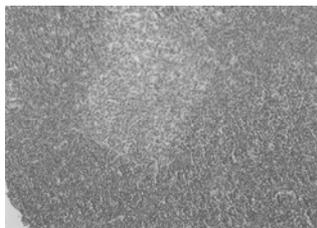


Fig. 5. Histopathological image of the thymus of calves without diazepam (250x)

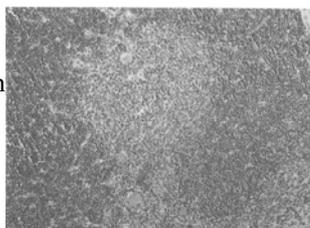


Fig. 6. Histopathological image of the thymus of calves with diazepam (250x)