



A COMPARATIVE STUDY OF SERUM CREATINE KINASE ACTIVITY IN LACTATING COWS AFTER INTRAMUSCULAR INJECTIONS OF TYLOSIN ALONE AND COADMINISTERED WITH BROMHEXINE

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ABSTRACT

Changes in the serum creatine kinase (CK) activity were studied in 12 Black and White breed cows following single intramuscular (i.m.) injection of Tylovet-200 and Bromhexotylosin-2 (BHT-2) at a dose rate of 0.5 ml/kg. Individual blood samples were collected from the jugular vein at 0 (pre-dose) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 h after dosing. It was established a time dependent moderate elevation of the serum CK activity after treatment with both tylosin products, reaching peak values at 12 h post-dose. The i.m. injection of BHT-2 caused a more intensive increase of serum CK levels, but it was not statistically significant compared to the effect of Tylovet-200.

Key words: creatine kinase, tylosin, cows

INTRODUCTION

The determination of the creatine kinase (CK) activity could be used as an indirect and specific indicator of muscle damage, including after intramuscular injections of different drugs (1). According to Braun *et al.* (2) the distribution of the CK activity in different organs in Holstein cows during lactation is as follows: 100 % – in muscles; 54 % – in heart; missing – in intestines, brain, rumen, uterus, erythrocytes, and pancreas; less than 1 % – in udder and kidneys.

Taking into consideration that CK is a sensitive, earlier and with a short half-life index, which might serve as a marker of muscle damage, our goal was to study if it could be used in a such way in cows. Previous investigations at our laboratory have showed that intramuscular (i.m.) injection of two tylosin dosage forms caused a significant elevation of CK values in pigs (3).

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MATERIALS AND METHODS

Animals

Experiments were performed on 12 clinically healthy Black and White breed cows, weighing 452.8 ± 16.4 kg. They were of mixed parity, in mid lactation and divided into 2 groups of 6 animals each (1 control and 1 experimental).

The cows were kept under conventional conditions of housing, feedings and milking.

Dosage forms and experimental procedures

Control group received tylosin tartrate (TT) (10 mg/kg) as a dosage form Tylovet-200 (containing 200 mg TT/ml) (Biovet Ltd, Bulgaria). Experimental group was treated with 10 mg/kg TT + 0.6 mg/kg bromhexine hydrochloride (BH) as Bromhexotylosin-2 (BHT-2) (200 mgTT/ml + 12 mg BH/ml). Administration was as a single i.m. injection (0.05 ml/kg) into the neck.

Blood samples were collected from the jugular vein at 0 (pre-dose) and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 h after dosing. After centrifugation at 3500 rpm serum was separated from clotted blood samples, and stored at 4 °C. The serum Ck activity was determined within 24 h after blood sampling.

Creatine kinase activity assay

CK measurement was performed by the method of Grinio & Konsistorum (4). The procedure was based on the method of Ennor and Rosenberg, involving the determination of the amount of creatine, liberated after incubation of the enzyme in the presence of creatine phosphate and ADP at 37 °C for 0.5 h. The CK activity values are given in U/l.

Kinetic and statistical analyses

The kinetic analysis was performed with the WinNonlin 4.0.1 (Pharsight Corporation) software. The following kinetic parameters were calculated: maximum serum CK activity (CK_{max} , U/l), time to CK_{max} (T_{max} , h) and the area under the serum CK activity to T_{last} ($AUC_{0-Tlast}$, U.l.h/l), where T_{last} was the last sampling time for each group (48 h for cows NoNo 1–3, and 24 h for NoNo 4–6).

Individual $AUC_{0-Tlast}$ was calculated according to the linear trapezoidal method, and CK_{max} and T_{max} were taken from the observed data. All kinetic parameters values and CK activity values were expressed as means \pm SEM. The data were evaluated using the general ANOVA/MANOVA procedure. Group and time post-injection were used as fixed effects within animals and CK activity values as a variable. Differences ($P < 0.05$) were evaluated by the least significant difference (LSD) (t -test).

RESULTS

The results obtained for the dynamics of the serum CK activity (mean \pm SEM) after i.m. use of Tylovet -200 and BHT-2 are presented in Table 1.

Table. 1. Serum creatine kinase activity (mean \pm SEM) in cows after a single i.m. injection of 2 tylosin dosage forms at a dose rate of 10 mg tylosin tartrate/kg (= 0.05 ml/kg)

Time post injection (h)	n	Serum CK activity (U/l) after i.m. injection of:	
		Tylovet-200	BHT-2
0	6	18 \pm 5.2	12 \pm 4.2
0.5	6	17 \pm 4.6	11 \pm 3.5
1	6	18 \pm 4.5	11 \pm 3.9
2	6	20 \pm 6.2	14 \pm 4.8
3	6	21 \pm 6.1	14 \pm 5.6
4	6	16 \pm 3.5	14 \pm 3.4
6	6	18 \pm 5.3	18 \pm 4.5
8	6	14 \pm 6.5	42 \pm 11.8*
10	6	38 \pm 11.1	47 \pm 11.2*
12	6	45 \pm 11.1*	53 \pm 13.7*
24	6	34 \pm 10.1	40 \pm 8.2*
48	3	36 \pm 12.5	34 \pm 6.2

* Significantly different at respective pre-dose level ($P < 0.05$).

The baseline CK levels before antibiotic administration varied from 12 \pm 4.2 U/l to 18 \pm 5.2 U/l. There was no essential difference in the basal activity values prior to Tylovet-

200 and BHT-2 treatment.

After Tylovet-200 injection the serum CK activity increased significantly, reaching peak values at 12 h, which were about 2.5 times

higher ($P < 0.05$) in comparison to the pre-dose period. Then was established a tendency of decrease of the serum enzyme activity. There were no differences (except for the 12th h) in the serum CK activity between post- and pre-dose. about serum CK activity at 8th, 10th and 12th h post-treatment compared to the pre-dose activity.

BHT-2 also caused an upward trend up to 12 h after i.m. injection, and the peak values were about 4.4 times higher than baseline values. After that a tendency for reduction of the serum CK activity was observed. Significant differences ($P < 0.05$) were found. Selected kinetic parameters (mean \pm SEM) of serum CK activity are given in Table 2.

Table 2. Kinetic parameters (mean \pm SEM) of serum creatine kinase activity in cows after a single i.m. injection of 2 tylosin dosage forms at a dose rate of 10 mg tylosin tartrate/kg (= 0.05 ml/kg)

Groups	n	Kinetic parameters		
		T_{\max} (h)	CK_{\max} (U/l)	$AUC_{0-T_{\text{last}}}$ (U.l/h/l)
Tylovet-200	6	16.5 \pm 6.5	51 \pm 9.1	1260 \pm 422.4
BHT-2	6	9.0 \pm 0.7	61 \pm 12.3	1418 \pm 404.8

There were no substantial differences in T_{\max} between Tylovet-200 (16.5 \pm 6.5 h) and BHT-2 (9.0 \pm 0.7 h) injected cows. The mean serum CK_{\max} and $AUC_{0-T_{\text{last}}}$ in the BHT-2 group (61 \pm 12.3 U/l and 1418 \pm 404.8 U.h/l, respectively) were higher than in the Tylovet-200 group (51 \pm 9.1 U/l and 1260 \pm 422.4 U.h/l, respectively), but the differences were not statistically significant.

No adverse reactions such as pain or swelling at the site of injection were recorded.

DISCUSSION

The skeletal musculature contains the largest CK amount among all other tissues and because of this the specificity of CK measurement to monitor muscle damage is very high (5). The increase of the serum CK activity in case of muscle damage caused by i.m. injections of different drugs is predominantly due to MM isoenzyme (6).

The morphological assessment at the injection site of different Tylan doses revealed that the tissue damage corresponded to injection volumes 0.25–1 ml in rabbits and 0.5–3 ml in pigs (7).

The increase of serum CK activity after i.m. tylosin dosage forms injections in comparison to pre-dose indicated that this enzyme activity in cows is influenced by the drugs in this study. The basal serum CK activities in our study varied between 2 and 39 U/l, showing that it differs substantially between individual cows. Similar fluctuation of serum CK activity is obtained in humans, pigs, cats, dogs and rabbits (3, 8–10).

Our findings for peak CK levels found at the 12th post-injection hour are similar to another study in pigs as they were established at 9th h post-dose (3). The presence of active

substances and vehicles at the injection site causes a prolonged effect on muscle and might explain the gradual CK increase and the variations in CK activity between animals, but there were no significant differences between Tylovet-200 and BHT-2 groups. On the other hand, the delay observed between drug administration and peak CK activity could be attributed to the fact that CK originating from skeletal muscle enter in the blood via the lymph (11).

The reduction of the serum CK activity after peak values could be explained both by the direct elimination and by the intravascular inactivation (12).

The medicinal products causing severe tissue irritation at injection site increased the serum CK activity in cows by up to 10–16 times (13).

In this study, the i.m. injection of tylosin alone (as Tylovet-200) or coadministered with bromhexine (as BHT-2) at therapeutic dose rates (10 mg TT/kg, and 10 mg TT/kg + 0.6 mg BH/kg, respectively) caused a mild muscle damage demonstrated as moderate elevation of serum CK activity – no more than 2.5 and 4.4 times higher than the respective baseline values. However, there were no statistically significant differences between these tylosin products related to the CK activity values at the corresponding post-dose intervals, and between the serum CK activity kinetic parameters, respectively.

It is possible that both pharmacologically active and inactive substances are involved in the effect of tylosin products on serum CK levels.

The results of this study are similar to those of the experiment in pigs conducted by Gueorgieva *et al.* (3). These data provide evidence that Tylovet-200 and BHT-2 caused

a slight local tissue damaging effect after i.m. injection in cows. It was manifested as a modest elevation of serum CK activity at several hours post treatment. Intramuscular injection of these two medicinal products is well tolerated by musculature.

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