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NON-INVASIVE EVALUATION OF LOCAL TISSUE TOLERANCE AFTER INTRAMUSCULAR INJECTION OF TOBRAMYCIN IN RABBITS

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Summary

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This study was performed to investigate the local tissue reaction in response to intramuscular (i.m.) injection of tobramycin in rabbits assessed by the determination of serum creatine kinase (CK) activity at several hours post-injection. Twelve clinically healthy cross breed rabbits (Wien blue x Chinchila) of both sexes were used. The rabbits were divided into 2 groups: control group (n=6), treated with saline to test the effect of injection volume and experimental group (n=6), treated with tobramycin, to test the effect of the active substance. Both tobramycin and saline were administered by a single intramuscular injection into the right site of the neck. Blood samples for CK analysis were drawn from *v. auricularis lateralis* by venfflon canule before injection (0 h) and 4, 6, 8, 12, 24, 30 and 48 h after injection. Serum CK activity increased markedly in tobramycin treated group, reaching peak values at hour 8. The postinjection values of serum CK activity in the experimental group remained significantly higher than in the pre-treatment period until 30th h. The i.m. administration of the same volume of saline caused a slight and transient increase of serum CK activity. In conclusion, this study provides evidence for a mild local tissue damaging effect of i.m. injection of tobramycin in rabbits manifested by a moderate elevation of serum CK activity at several post-injection hours.

Key words: creatine kinase, rabbits, tobramycin

INTRODUCTION

Intramuscular (i.m.) injection is an important route of administration for different veterinary drugs, including aminoglycoside antibiotics. The determination of plasma and serum creatine kinase (CK) activity was used as a specific and reliable marker for muscle tissue damage, caused by different factors, including i.m. injection of drug formulations (Cacace, 1972; Rasmussen and Svendsen, 1976; Lefebvre *et al.*, 1992; Ferre *et al.*, 2001). Muscle cells damage increases the permeability of the cell membrane and thus allows the enzymes to leak out into the extracellular spaces. The released material then enters the plasma via lymphatic drainage (Lindena and Trautschold, 1983; Lefebvre *et al.*, 1996). The increase of serum CK activity is proportional to the severity of the muscle lesion (Svendsen *et al.*, 1979; Diness, 1985; Lefebvre *et al.*, 1996; Verlinde *et al.*, 1996).

Aminoglycoside antibiotics are widely used for treatment of different infections in domestic animals. The pharmacokinetics of tobramycin that belongs to this group has been described in dogs, cats, humans (Pechere *et al.*, 1976; Jernigan *et al.*, 1988; Brown and Riviere, 1991) and more recently - in rabbits (Lashev and Dimitrova, 2002). To the best of our knowledge, no data on the local damaging effect after i.m.injection of tobramycin are available. Therefore, in this study we have investigated the effect of i.m. injection of tobramycin on serum CK activity in rabbits. The hypothesis that i.m. administration of tobramycin is well tolerated by muscle tissue was tested.

MATERIALS AND METHODS

Animals

Twelve clinically healthy cross breed rabbits (Wien blue × Chinchila) of both sexes, weighning 2-2.5 kg were used. The rabbits were housed in steel cages at room temperature (21°C), 55-80% humidity and 12 h daylight. They were fed a diet of good quality hay and grain and had free access to water. To avoid stress reaction one week adaptation and training period preceded the study.

Dosage form and experimental procedures

Tobramycin sulfate[1] (Tobramycin, Balkanpharma, Razgrad, Bulgaria) was administered as 4% solution at a dose rate of 5 mg/kg of body weight.

The rabbits were divided into 2 groups of 6 animals in each: control group, treated with saline, to test the effect of injection volume and experimental group, treated with tobramycin, to test the effect of the active substance. Both saline and tobramycin were administered by a single deep i.m. injection into the right site of the neck using a 7G disposable needle.

Blood samples (1mL) for CK analyses were drawn from *v. auricularis lateralis* by venfflon canule before injection (0 h) and 4, 6, 8, 12, 24, 30 and 48 hours after injection. The blood was allowed to clot for 3 h at room temperature and the serum was separated after centrifugation at 700g and stored at 4°C into polyethylene tubes. The serum enzyme activity was determined within 24 h after the collection of blood samples. Clinical reactions at the injection sites were followed up.

Creatine kinase activity assay

Serum CK activity was determined by the method of Grinio and Consistorum (1964). 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 ^{0}C for 30 min. The values of CK activity are given in U/L.

Kinetic and statistical analyses

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 vs time curve to 48 h ($AUC_{0\rightarrow48}$, U.h/L). The serum CK activity, CK_{max}, t_{max} and $AUC_{0\rightarrow48}$ values were expressed as means ± 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).

[1] Referred to as tobramycin in the text that follows

RESULTS

The dynamics of serum CK activity (means ± SEM) is presented on Fig. 1. There was no difference in baseline values prior to drug and saline administration. Serum CK activity increased markedly in tobramycin-treated group, reaching peak values at 8th h, which were about 3 times higher ($P < 0.01$) compared to the pre-treatment period. Then a gradual decrease of serum enzyme activity was established but the postinjection CK values in experimental group remained significantly higher ($P < 0.05$) than in the pre-treatment period until the 30th hour. The i.m. administration of the same volume of saline caused a slight and transient increase of serum CK activity.

Except for the 8th h, there were no differences in serum CK activity between experimental and control group.

The mean values of kinetic parameters (means ± SEM) of serum CK activity are given in Table 1. No significant difference in t_{max} between saline (9.66 ± 2.94 h) and tobramycin (10.66 ± 2.66 h) treated rab-

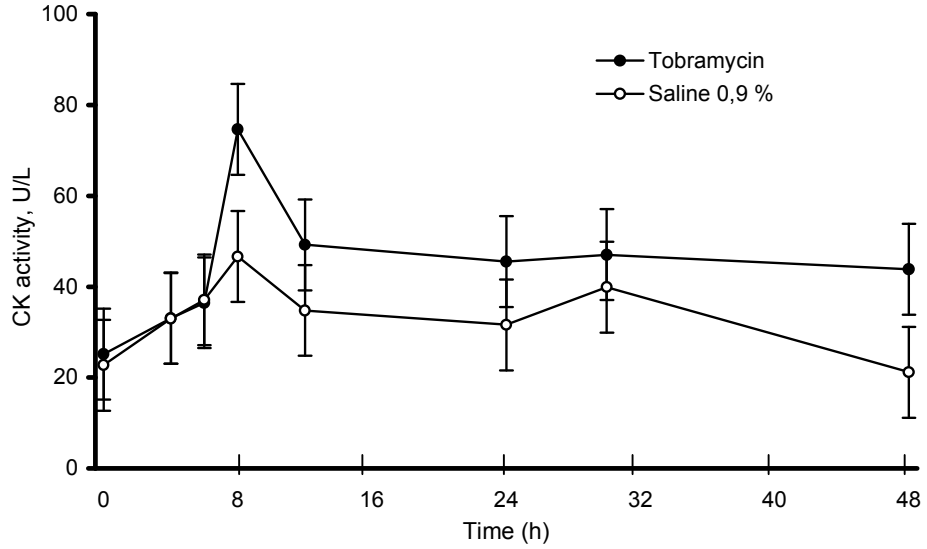

Fig. 1. Serum creatine kinase activity (means ± SEM) after i.m. injection of tobramycin and saline

Table 1. Kinetic parameters (means ± SEM) of serum creatine kinase activity in rabbits after i.m. injection of saline and tobramycin

Groups	n	Kinetic parameters		
		t_{max} (h)	CK_{max} (U/L)	$AUC_{0\rightarrow48h}$ (U.h/L)
0.9 % NaCl	6	9.66 ± 2.94	50.16 ± 6.46	969.00 ± 145.4
Tobramycin	6	10.66 ± 2.66	75.16 ± 10.0	1341.66 ± 94.88

bits was found. The mean serum CK_{max} and $AUC_{0\rightarrow48}$ values in experimental group (75.16 ± 10.0 U/L and 1341.66 ± 94.88 U.h/L, respectively) were higher than in control group (50.16 ± 6.46 U/L and 969.0 ± 145.4 U.h/L, respectively), but the differences between both groups were not statistically significant.

Clinical reactions such as swelling or pain at the injection site were not recorded.

DISCUSSION

The initial serum CK activities in our study ranged between 9 and 35 U/L, indicating that it may vary considerably between animals in rabbits. Similar fluctuations of serum CK activity have been described in humans, pigs, cats and dogs (Steiness *et al.*, 1978; Kaneko, 1989; Guerguieva *et al.*, 1998).

Our results for peak CK values found at the 8th post-injection hour are consistent with other studies in rabbits, showing that plateau of the elevated CK activity in blood is established between hours 4 and 12 (Hsu and Watanabe, 1983, 1984). Interestingly, there was no difference in t_{max} after saline and tobramycin administration, indicating that both the injection volume and the active substance could be involved in the elevation of serum CK activity since tissue damage was proportional to the injection volume (Diness, 1985). The marked increase of serum CK activities after i.m. administration of tobramycin compared to that after saline injection showed that serum enzyme activity was more affected by the active substance. Moreover, no circadian rhythm in serum CK activity has been reported (Noakes, 1989; Hortobagyi and Denahan, 1989). Therefore, the elevation of serum CK activities is due to the release of the enzyme from the site of injection in the experimental group. The decrease of serum CK activity, particularly at 48th post-injection hour, is dependent on the loss of enzyme activity from the injection site, due both to the direct elimination and intravascular inactivation (Lefebvre *et al.*, 1996).

Intramuscular injection of tobramycin at therapeutic dose rate in rabbits (5 mg/kg of body weight) caused a mild muscle damage seen as moderate elevation of serum CK activity. The peak post-injection levels were no more than 3 times higher as compared with the basal values. Drug formulations, causing severe tissue irritation at injection site increased the serum CK activity by up to 10 –16 times (Pyörälä, 1994).

In conclusion, this study provides evidence for a slight local tissue damaging effect of i.m. injection of tobramycin in rabbits seen as moderate increase of serum CK activity at several hours post-injection. Intramuscular injection of tobramycin at therapeutic dose rate is well tolerated by muscle tissue.

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