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Local Tissue Damage in Cows after Intramuscular Administration of Preparations Containing Phenylbutazone, Flunixin, Ketoprofen and Metamizole

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Pyörälä S, Laurila T, Lehtonen S, Leppä S, Kaartinen L: Local tissue damage in cows after intramuscular administration of preparations containing phenylbutazone, flunixin, ketoprofen, and metamizole. Acta Vet. Scand. 1999, 40, 145-150. – Tissue irritation after intramuscular injections of 4 nonsteroidal anti-inflammatory agents was studied in 5 lactating cows. Preparations containing phenylbutazone, flunixin, metamizole (dipyrone) and ketoprofen were investigated; physiological saline was used as a control substance. Tissue reactions at the injection sites were examined by palpation and by determining serum creatine kinase. A kinetic method based on creatine kinase released from the injured muscle tissue was used, which allowed estimation of the amount of damaged muscle. The metamizole preparation clearly provoked signs of pain in all the cows. After flunixin and phenylbutazone injections slight reactions were observed, and ketoprofen and saline did not cause any clinical signs. Some palpatory findings after injections were found for all the preparations except saline. Based on serum creatine kinase, the 2 most irritating preparations were the ones containing flunixin and phenylbutazone. After injections of these 2 substances, the estimated amount of damaged muscle was about 80 grams. The statistical difference between flunixin and phenylbutazone and the other 2 preparations was significant. Physiological saline had no effect on serum creatine kinase. For preparations containing phenylbutazone and flunixin, intravenous administration is recommended.

bovine; creatine kinase; nonsteroidal anti-inflammatory agents; muscle damage; local tolerance.

Introduction

Nonsteroidal anti-inflammatory agents are widely used in cattle. For practical reasons they are usually administered to cattle intramuscularly (i.m.). After i.m. injections of various medicinal preparations, injection-site lesions are possible (*Rasmussen* 1978). Post-injection muscle injury may be caused by the vehicle, the active ingredient, or both. Repeated injections and large injection volumes promote tissue damage (*Kern* 1987). Prolonged persistence of drug residues is possible at the injection site (*Rasmussen & Svendsen* 1976). Losses to the beef industry due to injection-site lesions have probably been underestimated (*George et al.* 1995).

The use of serum creatine kinase (CK) provides an organ-specific method for determining muscle damage (*Lefèbvre et al.* 1994, 1997). Intramuscular injections increase serum CK activity owing to local areas of muscle damage or

necrosis. A general equation for quantitative estimation of skeletal muscle damage, based on CK release after injections, has been developed for some animal species including cattle (*Lefébvre et al.* 1996).

Most of the nonsteroidal anti-inflammatory drugs are known to be tissue-irritating (*Adams* 1995, *Bishop* 1996). Specific studies on their tissue irritation when injected by extra-vascular routes to cattle have not been published. The aim of this investigation was to study tissue irritation of 4 nonsteroidal anti-inflammatory agents.

Materials and methods

The product names, companies, active substances, concentrations, dose levels and approved routes for administration in Finland for the 4 examined anti-inflammatory preparations are listed in Table 1. At the time of the study, all 4 substances were approved for food animals in the European Union. Phenylbutazone and metamizole have been prohibited in the European Union for food animal use from the beginning of 1998 as no maximum residue limit values could have been set for them (*Anon.* 1997).

Except for ketoprofen preparation, there was no information about pH in the labelling of the investigated preparates; thus the pH of each product was measured. The products were administered at the dose level recommended by the manufacturers of the products (Pharmaca Fennica Veterinaria 1994-95). The dose used for metamizole was 40 mg/kg. Physiological saline (0.1 ml/kg) was used as a control substance. Five clinically healthy 2-6-year-old Ayrshire cows in their mid to end-lactation period, weighing from 440 to 660 kg, were used as experimental animals. Cows were housed in a stanchion barn, milked twice daily, and fed according to the national standards.

Experimental design

Each preparation was administered to all animals using a cross-over design. The interval between injections was 6 weeks. Drugs were administered as deep intramuscular injections into the neck (*M. trapezius, M. serratus cervicis ventralis*), with an 18 G disposable needle. The maximum dose at one injection site was 20 ml for all drugs except phenylbutazone preparation, for which, according to the manufacturer's instructions, the dose should not exceed 10 ml per site.

All reactions of the animals to pain and discomfort immediately following the injections were registered. The injection sites were inspected and palpated during the experimental period by one person who was unaware of the treatment details. Clinical findings (pain, high skin temperature, oedema) were recorded using a scoring system where 0 = no reaction and 3 = severe reaction. Blood samples for CK analysis were drawn from the jugular vein via a permanent cannula before the administration and 4, 8, 12, 24, 32, 48, and 72 h later. The blood was allowed to clot for 4 h at room temperature and the serum was then separated and frozen at –20 °C until CK analysis was done. CK activity was assessed by routine methods (*Szasz et al.* 1979). The normal reference value for adult cows used was 50-220 U/l (*Radostits et al.* 1994). The area under the time-CK activity curve (AUC) was calculated using a pharmacokinetic software package. The amount of injured muscle tissue (Q) was estimated according to *Lefébvre et al.* (1994).

Statistical analyses

The clinical scores in different groups were statistically compared using a non-parametric method. The AUC and Q values were compared between groups using analysis of variance and Student's *t*-test.

Table 1. The trade names, manufacturers, active substances, concentrations, dose levels and approved routes of administration to cattle in Finland, of the 4 anti-inflammatory products investigated.

Trade name	Manufacturer	Active substance and concentration	Dose and route of administration
Reumuzol® vet[1]	Orion-Farmos, Finland	Phenylbutazone 200 mg/ml	10 mg/kg i.v., i.m.
Finadyne® vet	Schering-Plough, USA	Flunixin 50 mg/ml	2.2 mg/kg i.v., i.m.
Romefen® vet	Rhone-Mérieux, France	Ketoprofen 100 mg/ml	3 mg/kg i.v., i.m.
Novalgin® vet[1]	Hoechst, Germany	Metamizole 500 mg/ml	20-50 mg/kg i.v., i.m.

[1] Phenylbutazone and metamizole have been prohibited in the European Union for food animal use from the beginning of 1998 as no maximum residue limit values could have been set for them.

Results

The measured pHs for the preparations were as follows: phenylbutazone 9.3, flunixin 8.0, ketoprofen 6, and metamizole 6.6. The basal CK activity level before drug injections ranged between 86 and 136 U/l. The clinical reactions following injection varied between drugs. All 5 animals clearly showed signs of pain after injection of metamizole preparation for several min. After flunixin and phenylbutazone injections slight reactions were seen, and ketoprofen and saline did not provoke signs of pain in any of the animals. Some palpatory findings after injections were found for all the preparations except saline; at 72 h after injections palpatory changes had disappeared in all the groups. There were no statistical differences between groups in this respect.

All products provoked an increase in serum CK activity, but the magnitude of the rise varied greatly (Fig. 1). Administration of physiological saline solution had no effect on serum CK activity. Mean maximum concentrations (C_{max}) for serum CK, mean time points for maximum concentration (Tmax), mean areas under curve (AUC) values calculated from the serum CK concentration-time curves (0-72 h), and mean amounts of estimated damaged muscle tissue (Q) for different drugs are given in Table 2. The estimated amounts of damaged muscle tissue after injections of flunixin and phenylbutazone were significantly greater ($p<0.05$) than those provoked with the other agents.

Discussion

Non-steroidal anti-inflammatory agents are widely used for treatment of various inflammatory conditions in cattle and other food animals (*Kopcha et al.* 1992). They are generally considered tissue-irritating, but to our knowledge, no comparative studies between different drugs in this respect exist. Nonsteroidal anti-inflammatory agents are, by nature, generally acids, but some solutions of injectable preparations have been formulated to be highly alkaline. This is suggested to be one reason for necrosis caused by these substances when injected by perivascular route (*Adams* 1995). The pH of the formulations is, however, a minor factor if it is between 3 and 10 (*Lefèbre et al.* 1997); for all the preparations studied here it remained within those limits and thus probably did not significantly contribute to the post-injection tissue damage.

The approved route of administration for cattle and horses for nonsteroidal anti-inflammatory preparations varies between formulations and countries. In treatment of horses, the only recommended route of administration for most nonsteroidal anti-inflammatory agents is intravenous (*Bishop* 1996). However, according to

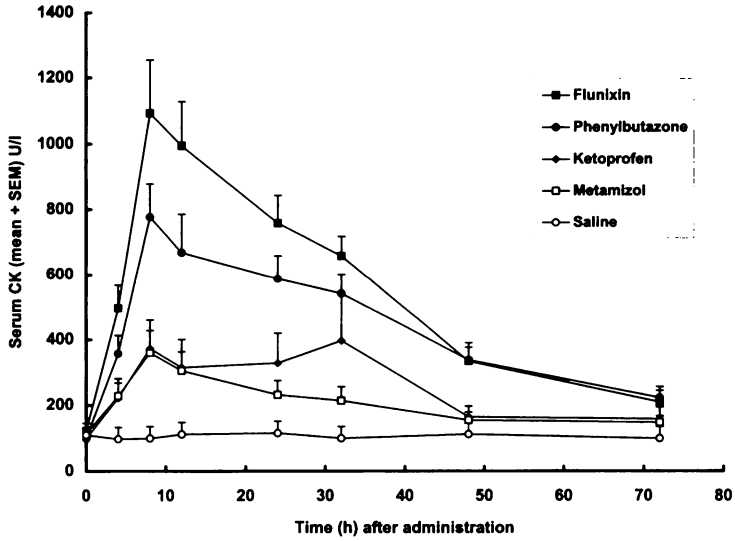

Figure 1. Time-concentration curves of serum creatine kinase (CK) after intramuscular administration of 4 nonsteroidal anti-inflammatory products or saline (mean SEM of 5 cows). Doses are given in Table 1.

labels of the preparates studied here, for all except the ketoprofen preparation i.m. and i.v. route was allowed for both cattle and horses.

Phenylbutazone is considered to be the most tissue-injuring substance among the nonsteroidal anti-inflammatory agents (*Bishop* 1996). According to *Adams* (1995) phenylbutazone precipitates in the neutral pH of muscle, decreasing absorption of the substance. This could also cause additional tissue irritation. I.m. injections of a phenylbutazone preparation (8.8 mg/kg) into horses destroyed 22-56 grams of muscle (*Toutain et al.* 1995). However, results from 2 older studies on horses were not consistent based on observations of post-injection muscle injury due to phenylbutazone (*Landuyt et al.* 1993, *Sullivan & Snow* 1982); this may be explained by differences in vehicles between different formulations. In the horse, there is evidence that even i.v. administration of phenylbutazone includes risk of necrotizing phlebitis (*Adams* 1995). In our study, the preparation containing flunixin caused even more tissue damage than the one containing phenylbutazone but the difference was not statistically significant. Up to ten-fold increases in levels of serum CK activity were found after administration of these 2 drugs, and the estimated amount of destroyed muscle tissue was about 80 grams per animal. Local tissue damage caused by these 2 agents was comparable to that caused by some antimicrobial drugs known to be tissue-damaging, such as enrofloxacin, tylosine, trimethoprim-sulphadoxin or long-acting oxytetracycline (*Pyörälä et al.* 1994, *Chavez Moreno & Bickhardt,* 1997). The flunixin preparation contains propylen glycol, which is known to cause lesions when injected i.m. (*Rasmussen* 1978); this may be one reason for the high levels of muscle damage caused by this preparation. Clinical signs provoked by the preparations did not fully agree with the CK results, as the metamizole preparation caused the most serious clinical reactions. It is possible that some ingredients in the solutions may cause immediate post-injection pain without

Table 2. Maximum concentration (C_{max}; mean and range) for serum creatine kinase (CK), area under curve (AUC; mean and SEM) calculated from the serum CK concentration-time curves (0-72 h), and mean amount of estimated damaged muscle tissue (Q; mean and SEM) for different products.

Product (active substance	Cmax for serum CK (U/L)		AUC (U/L/h)		Q (g)	
	Mean	Range	Mean	SEM	Mean	SEM
Reumuzol® vet (phenylbutazone)	777[a]	469-1262	31656	4362	78[a]	15.6
Finadyne® vet (flunixin)	1093[a]	769-1340	36650	3679	91[ab]	8.7
Romefen® vet (ketoprofen)	396[bd]	143- 908	16866	3784	42[bd]	12.1
Novalgin® vet (metamizole)	360[cd]	249-6429	14706	2452	35[cd]	5.0

Values with different superscripts differ significantly within columns.

producing serious muscular damage. Pain and discomfort after i.m. injections, however, are not acceptable due to animal welfare reasons.

Residues from nonsteroidal anti-inflammatory agents after treatment may not be a big problem, as these drugs are generally used in conjunction with antimicrobials, which have longer withdrawal periods. Besides, half-lives of nonsteroidal anti-inflammatory agents in cattle are relatively short; the only exception is phenylbutazone, which is eliminated very slowly (*Backer et al.* 1980). If severe tissue reactions occur after administration, the lesions may persist for months (*George et al.* 1995). Animals treated with nonsteroidal anti-inflammatory agents may also go to slaughter sooner than planned if they do not recover from the disease they were treated for, and there may be a risk of inadequate meat quality at the i.m. injection site. Repeated administrations may lead to multiple sites of lesions and considerable losses of meat.

Local tolerance of injectables in the target species should be carefully monitored during the drug marketing authorization process of the products. Very irritating preparates should not be approved for extravascular administration to any animal species. For the 2 most irritating nonsteroidal anti-inflammatory preparations examined in the present study the intravenous route of administration is to be preferred. The approach using kinetic method makes it possible to determine a quantitative equivalent of destroyed muscle, which gives an indication of the size of the actual muscle lesion. The method is useful e.g. in comparisons of different formulations.

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Sammanfattning

Lokal vävnadsirritation i kor efter intramuskulära injektioner med fenylbutazon, flunixin, ketoprofen, och metamizol.

Vävnadsirritation orsakad av intramuskulära injektioner med fyra non-steroidala anti-inflammatoriska agenser studerades på fem lakterande kor. De undersökta preparaten innehöll fenylbutazon, flunixin, ketoprofen, och metamizol (dipyron). Fysiologisk saltlösning användes som kontroll.

Injektionsställena palperades noggrant för att hitta möjliga lokala reaktioner, och kreatinkinas koncentrationen i serum bestämdes före och efter injicieringen av läkemedlen. En kinetisk metod grundad på kreatinkinas avsöndrad från den förstörda muskelvävnaden användes. Med denna metoden var det möjligt att uppskatta mängden av förstörd muskelvävnad. Metamizol-preparatet orsakade tydliga smärtsymptom hos alla kor; flunixin och fenylbutazon orsakade lindriga symptom medan ketoprofen och saltlösningen inte förorsakade kliniska symptom. Vissa palpatoriska reaktioner efter injektionerna kunde konstateras efter alla preparat utom saltlösningen. Enligt bedömning baserad på serumets kreatinkinasnivå förorsakade de preparat som innehöll flunixin eller fenylbutazon de kraftigaste reaktionerna. Efter injektioner med dessa två läkemedel, var den beräknade mängden av förstörd muskel 80 gram. Den statistiska skillnaden mellan flunixin samt fenylbutazon och de andra läkemedlen var signifikant. Fysiologisk saltlösning påverkade inte serumhalten av kreatinkinas. Vi rekommenderar att fenylbutazon och flunixin skulle ges intravenöst.

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