A COMPARISON OF THE SERUM LEVEL KINETICS AND BIOLOGICAL HALF-LIFE OF OXYTETRACYCLINE AFTER LONG ACTION PREPARATIONS (TETRAVET 20% L.A. INJ. AND ENGEMYCIN 10% L.A. INJ.) IN SHEEP AND THEIR TOLERANCE*  

POROVNANIE KINETIKY SÉROVÝCH HLADÍN A BIOLOGICKÝ POLČAS OXYTETRACYKLÍNU U OVIEC PO PRÍPRAVKOCH S PREDLŽENÝM ÚČINKOM (TETRAVET 20% L.A. INJ. A ENGEMYCIN 10% L.A. INJ.) A ICH ZNÁŠANLIVOSŤ


University of Veterinary Medicine, Košice, Slovak Republic

ABSTRACT: Blood serum concentrations and biological half-life of oxytetracycline after long action preparations were investigated in adult sheep of the Slovak Merino breed. Contemporarily their local tolerance has also been observed. In the first group oxytetracycline was administered in form of the preparation Tetravel 20% L.A. inj. a.u.v. (Sanofi, Ltd., France) and in the second group in form of the preparation Engemycin 10% L.A. inj. a.u.v. (Intervet, Ltd., The Netherlands). Oxytetracycline was administered intramuscularly at a single dose of 20 mg per kg of live weight. The blood serum concentrations of oxytetracycline were studied in the intervals of 1st, 6th, 24th hours and 2nd, 3rd, 4th, 5th, 6th, 7th and 8th days after single administration of preparations. Oxytetracycline was determined by high HPLC chromatography (Sokol and Matisová, 1994). The significantly higher serum concentrations of oxytetracycline (p < 0.05) were recorded after preparation Tetravel L.A. from 24th hour to 5th day. Concentrations of oxytetracycline were detected on the 6th day after Tetravel L.A. preparation and in the case of Engemycin L.A. on the 5th day. Longer half-life was registered after Tetravel L.A. preparation (40-42 hours). After Engemycin L.A. this time was registered as 37-39 hours. Tetravel L.A. does not induce any local reactions at the site of administration. In contrast to Tetravel L.A., Engemycin L.A. caused inflammatory edematisation in 50% of animals which persisted 3 days only, without other complications.


INTRODUCTIONS

Equivalent drugs, i.e. drugs containing identical medical substance with the same purity (quality) and the same mass (dose) turned out not to have necessarily the same efficiency (Chalábala and Mandák, 1977; Rak and Chalábala, 1984; Švec, 1998). Equivalent drugs can differ also in general or local tolerance (Hirtz, 1972). These findings were the basis for introduction of the problem concerning bioavailability of drugs-level

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determination of drug applicability. Successive studies aimed at clarification of factors affecting this important pharmacokinetic phenomenon contributed to the fact that an increased attention started to be paid to the drug form because of its special importance for drug effectiveness (Zathurecký et al., 1989). The result of this effort is development of drug forms of higher generations. Drugs of the second generation are preparations with a controlled release of drug. Adjuvant substances which are added slow up the release of drug, which makes it possible to keep the concentration of drug in blood at a desired level for a longer time (Zathurecký et al., 1989; Chalabala et al., 1983). Tetracycline preparations with protracted effect (long action – L.A.) are also the result of the above mentioned biopharmaceutical and pharmacological studies. The advantages of tetracyclines L.A. against classical ones are in general known. The advantages of preparations with protracted effect (long action – L.A.) come from changed pharmacokinetics of tetracycline antibiotic as a result of the use of viscous vehicle on the basis of modern polymers (polyvinylpyrrolidone, dimethylacetamide, glycerol-formaldehyde, N-methylpyrrolidone, aluminium monostearate), or other adjuvant substances. Not only pharmacokinetic parameters, but also irritability, oedema, or possible occurrence of necrotic changes at the site of i.m. application of L.A. preparations may be influenced by the type of solvent (Nouws et al., 1990; Svendsen, 1989). Concentration of preparations also plays an important role. Tetravet 20% L.A. inj. and Engemycin 10% L.A. inj. often used in practice are on the basis of oxytetracycline. The former contains the vehicle dimethylacetamide and Engemycin 10% L.A. polyvinylpyrrolidone. On the basis of the above mentioned fact we started to compare kinetics of serum levels of oxytetracycline and biological half-life in sheep after one application of the mentioned long action preparations: Tetravet 20% L.A. inj. and Engemycin 10% L.A. inj. The aim of the work was to judge local reactions at the site of application and tolerance of the medications.

MATERIAL AND METHODS

Characteristics of preparations

Tetravet 20% L.A. inj. is a pure dark brown liquid. According to the producer’s data the preparation contains 200 mg of oxytetracycline per 1 ml and 1.85 g of magnesium oxide per 100 ml of preparation. Dimethylacetamide is indicated as a solvent.

Engemycin 10% L.A. inj. is a pure yellow liquid. According to the producer’s data it contains 100 mg of oxytetracycline per 1 ml. Polyvinylpyrrolidone is indicated as a solvent.

Experimental set

The experiment was conducted with a set of 8 adult sheep, Slovak merino breed weighing 40–55 kg. The experimental set was divided into two groups. In the first group oxytetracycline was administered in the form of Tetravet 20% L.A. inj. a.u.v. (Sanofi Ltd., France). In the second group oxytetracycline was administered in the preparation form of Engemycin 10% L.A. inj. a.u.v. (Intervet Ltd., The Netherlands). Oxytetracycline was administered to animals in a single dose of 20 mg/kg of live weight i.m. to thigh muscle. Engemycin was administered in a volume of 2 ml/10 kg of live weight and Tetravet in a volume of 1 ml/10 kg of live weight. At one site of application Engemycin was administered in volume to 10 ml and Tetravet in volume to 5 ml. Concentrations of tetracycline in blood serum were in the 1st, 6th and 24th hour, then on day 2, 3, 4, 5, 6, 7 and 8 after application of preparations. Oxytetracycline was determined on the liquid chromatograph of Hewlett Packard firm (Avondale, PA, USA) series 1050, at wave length 360 nm with sensitivity 0.05 µg/ml. Concentration of oxytetracycline was given in µg/ml. Constants of elimination were calculated using the least squares method according to one-compartment pharmacokinetic model. Constants of elimination from serum levels were computed from a decreasing curve from the interval 1 up to 120 hours. Biological half-life was calculated like this: mean constant of elimination ln 2. Statistical evaluation was provided by Student’s t-test. Local reaction in the site of drug application was evaluated according to painful reaction of animals for palpation, presence of oedema and increased local temperature. Tolerance of preparations was evaluated according to the general behaviour of animals, feed intake and droppings consistency.

RESULTS

Fig. 1 demonstrates dynamics of changes in oxytetracycline serum levels in sheep after a single Tetravet 20 % L.A. inj. and Engemycin 10 % L.A. inj. application. It is seen from the figure that there are differences in serum levels height (quantitative differences), but dynamics of level changes in the individual observed time periods is substantially the same. In the first observed time interval (first hour) we notice unimportant higher concentrations of oxytetracycline (3.34 µg/ml) after Engemycin application compared to Tetravet (3.09 µg/ml). In all other time intervals we observe, on the contrary, higher concentrations after Tetravet application. These differences are not statistically significant in 6th hour, but in 24th hour and on day 2, 3, 4, and 5 of observations they are statistically significantly higher (p < 0.05) after Tetravet application compared to Engemycin. Maximum serum concentrations are recorded in 6th hour after application of both preparations. In this time interval the level of oxytetracycline is 5.52 after Tetravet application and 5.28 µg/ml after Engemycin application. Therapeutic concentrations of oxytetracycline (MIC above 0.5 µg/ml) are still recorded on day 4 after Tetravet (0.63 µg/ml) and on day 3 after Engemycin (0.60 µg/ml) application. Measurable concentrations after Tetravet
I. Blood serum concentrations of oxytetracycline in sheep after simple administration of preparations Tetravet 20% L.A. inj. and Engemycin 10% L.A. inj.

<table>
<thead>
<tr>
<th>Administration of preparations</th>
<th>Values in μg per ml of blood serum in time intervals</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Tetravet 20% L.A. inj.</td>
<td>3.09 ± 0.38</td>
</tr>
<tr>
<td>Engemycin 10% L.A. inj.</td>
<td>3.34 ± 0.44</td>
</tr>
</tbody>
</table>

x = p < 0.05

application were still perceivable on day 6 (0.06 μg/ml) and after Engemycin application on day 5 (0.26 μg/ml).

Tab. I shows a survey of serum oxytetracycline levels in the observed time intervals. No changes in general behaviour, feed intake and in droppings consistence elevated registered after any preparation. We recorded painful reactions for palpation and raised temperature at the site of puncture in all animals one hour after application of Engemycin. Significant warm oedema was gradually developed in two of animals and it persisted for 3 days. We did not record any local changes at the site of puncture in three animals but short-time hyperaesthesia for palpation (within 12 hours) was registered in one sheep after Tetravet application.

**DISCUSSION**

As the pharmacotherapeutic effect of drugs depends mainly on their bioavailability (relative drug amount which can get from the site of application to systemic circulation in unchanged form in a certain time) and on drug concentration in biophase (i.e. in the environment, in which drug comes into direct contact with receptors (Holomán, 1984), we decided to find out if and to what extent various variables comprised in Tetravet L.A. and Engemycin L.A. influence oxytetracycline kinetics and local tolerance of tested preparations. Pharmaceutical adjuvants have also an important function in biological accessibility and drug survival in the organism besides dose, drug form, the way of application, metabolism speed, resorptive processes and chemical properties of the drug.

Parenteral repository preparations are possible to gain by more ways. One of the ways of their preparation is realized by using modern adjuvants. Various synthetic polymers belonging to adjuvant pharmaceutical substances have an important position in their preparation. These polymers are dissociated into nontoxic components in the organism in a hydrolytic or enzymatic way (Rak et al., 1985). Biodegradable polymers are the carriers of so called long action effect. Long action preparations on the basis of oxytetracycline tested by us also contain biodegradable polymers. It follows from the fact that producers indicate dimethylaceticamide in Tetravet L.A. and polyvinylpyrrolidone in Engemycin L.A. as a solvent. At single i.m. oxytetracycline application in dose 20 mg/kg of live weight they indicate its therapeutic levels (over 0.5 μg/ml) from 30 minutes after application for 4–5 days in Tetravet L.A. and at least for 3 days in Engemycin L.A. They also indicate minimal irritability at the site of i.m. application. It results from our comparative studies that there are quantitative differences in serum levels of oxytetracycline between Tetravet L.A. and Engemycin L.A., but the dynamics of level changes in individual observed time periods is basically the same. In the first observed time interval (first hour) we recorded a nonsignificantly higher oxytetracycline concentration (3.34 μg/ml) after Engemycin against levels after Tetravet application (3.09 μg/ml). In all other time intervals, on the contrary, we recorded higher concentrations after Tetravet. In 6th hour the differences were statistically nonsignificant while in 24th hour and on day 2, 3, 4, and 5 of observations they were statistically significantly higher.
(p < 0.005) after Tetravit application compared to Engemycin. The highest levels after application of both preparations were recorded in 6th hour. In this time interval the measured oxytetracycline level after Tetravit application was 5.52 μg/ml and after Engemycin 5.28 μg/ml.

Therapeutic concentrations after Tetravit application were still recorded on 4th day (0.63 μg/ml) and after Engemycin on 3rd day (0.60 μg/ml). Measurable concentrations were perceivable still on day 6 after Tetravit and on day 5 after Engemycin application. Different course of serum levels accords with different levels of another pharmacokinetic index – biological half-life. It is 40–42 hours in Tetravit and 37–39 hours in Engemycin. Higher serum level of oxytetracycline registered in the first hour after Engemycin shows its faster resorption from the application site compared to Tetravit. But a steeper decrease of oxytetracycline serum levels recorded from 6th hour after Engemycin and its survival in therapeutic concentrations for a shorter time than after Tetravit shows a faster start of eliminative phase in Engemycin. The stated statistically significantly higher serum levels of oxytetracycline and its survival in therapeutic concentrations one day longer after Tetravit L.A. application compared to Engemycin L.A. at the same dose in the form of basis, witnesses about the probably different influence of polyvinylpyrrolidone and dimethylacetamide on the kinetics of oxytetracycline especially in the phase of resorption. It is not possible to exclude a factor of higher oxytetracycline concentration in Tetravit from probable causes, mainly quantitatively different kinetics of oxytetracycline besides different vehicles between two compared preparations. Considering that a higher drug concentration in a smaller volume of injected solvent rather accelerates resorption, it is interesting that in the first time period (1st hour) a lower serum concentration of oxytetracycline after Tetravit application was recorded. It is not possible to unambiguously deduce from these facts what is the contribution of different oxytetracycline concentrations in preparations or different vehicles in diverse oxytetracycline kinetics. We do not know studies concerning with oxytetracyline kinetics observation in tetracycline long action preparations depending on the type of polymer contained in them. From the results of studies dealing with pharmacokinetic problems of long action preparations it is seen that differences in oxytetracyline serum levels or its survival in blood there are differences also in the same species of animals depending on the used long action preparation (Cohen et al., 1993; Vyhnaček and Hera, 1994; Escudero et al., 1994; Arqum et al., 1995). The above mentioned studies substantially correspond to our findings. No general side reactions were observed after using the tested preparations and they were very well tolerated. In local irritability we recorded differences between the preparations. Tetravit did not cause many reactions at the site of puncture, while Engemycin induced inflammatory oedema persisting for 3 days without other complications in 50% of animals. In general a higher drug volume especially at i.m. application causes the development of painful reaction. But also injected solution with higher concentration induces higher local irritability. Our results suggest that the type of vehicle contained in preparations participated in higher level in differential degree of local irritability of tested preparations than factor of applied volume. It is confirmed by the fact that at one site of application Engemycin was used in higher volume (up to 10 ml) than Tetravit (up to 5 ml) but in lower concentration (10%) compared to Tetravit (20%). Irritability degree of tested preparations found by us is not the same as findings of Nouws (1990).

Our findings suggest that Tetravit L.A. appears more beneficial in observed pharmacokinetic parameters as well as in tolerance compared to Engemycin L.A. It was proved that after Tetravit L.A. application oxytetracycline serum concentrations (p < 0.05) in all observed time intervals, except in 1st and 6th hours, were statistically significantly higher compared to Engemycin L.A. The same was proved by finding that Tetravit L.A. MIC survives one day longer and is also locally better tolerated.

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Contact Address:

MVDr. Jozef Neschl, Csc., Univerzita veterinárskeho lekársctva, Komenského 73, 041 81 Košice, Slovak Republic
Tel. +421 95 633 21 11–15, fax +421 95 632 36 66, e-mail: sutia@uvm.sk