Praca oryginalna *Original paper*

Comparison of the serum level and pharmacokinetic parameters of oxytetracycline after administration of long action preparations in sheep*)

JOZEF NEUSCHL, PETER POPELKA, JOZEF NAGY, JOZEF SOKOL, SLAVOMIR MARCINČÁK

University of Veterinary Medicine, Department of Pharmacy, Pharmacology, Toxicology, Komenského 73, 041 81 Košice, Slovak Republic

Neuschl, J., Popelka, P., Nagy, J., Sokol, J., Marcinčák, S.

Comparison of the serum level and pharmacokinetic parameters of oxytetracycline after administration of long action preparations in sheep

Summary

Blood serum concentrations and biological half-life of oxytetracycline (OTC) after long action preparations were investigated in adult sheep of the Slovak Merino breed. A the same time their local tolerance has also been observed. In the first group oxytetracycline was administered in the form of preparation Tetraxyl L. A. inj. a.u.v. and in the second group in the form of preparations Alamycin L.A. inj. a.u.v. The blood serum concentrations of OTC were studied at intervals of 1, 3, 6, 24 hours and 2, 3, 4 and 5 days after single administration of the preparations. Oxytetracycline has been determined by high HPLC chromatography.

Therapeutically concentrations of OTC (above 0.5 μg.ml^{-1}) produced by Tetraxyl were detected within 74 hours and after Alamycin administration within 67–68 hours. Detectable concentrations of OTC (under MIC) of Tetraxyl (0.28 μg.ml^{-1}) and Alamycin (0.14 μg.ml^{-1}) were recorded in 96 hours. On the 5th day all samples in both groups were negative. Biological half-life of Tetraxyl was determined at 36.5–38.5 hours and Alamycin at 36.0–37.0 hours. Short-time palpation hyperaesthesia was recorded in 1 sheep (within 13 hours) after Tetraxyl administration and in 2 sheep (within 15 hours) from the Alamycin group. On the basis of the results it was concluded that in selected pharmaceutically parameters and also in local tolerance Tetraxyl L.A. is more favorable in comparison with Alamycin L.A.

Keywords: oxytetracycline, sheep, Tetraxyl L. A., Alamycin L. A.

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 (6, 10, 14). Equivalent drugs can differ also in general or local tolerance (4). These findings were the basis for introduction of problem solving concerning bioavailability of drugs – level determination of drug applicability. Due to pharmaceutically studies reasons of this non-equivalence, which is called as endogenous and exogenous factors were resolved. Among exogenous factors on the first place are included physical and chemical properties of drug, drug and its form, concentration, and also technological procedures, vehicula and auxiliary substances. These findings conduced to increasing of interest related to drug form from aspect of its importance for drug effectiveness (16).

The result of this effort is development of drug forms of higher generations. Drugs of the second generation are preparations with controlled release of drug. Adjuvant substances which are added slow up the release of drug, what makes possible to keep the concentration of drug in blood at desired level for longer time (7, 17). Tetracycline preparations with protracted effect (long action – L.A.) are also the result of above mentioned biopharmaceutical and pharmacological studies. The advantages of tetracycline 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 usage 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

*) Supported by grant VEGA (Ministry of Education of the Slovak Republic) No. 1/0618/03.

changes in site of i.m. application of L.A. preparations may be influenced by the type of solvent (9, 13). Preparations used in this experiment, Tetraxyl L.A. inj. and Alamycin L.A. inj., are on the basis of oxytetracycline in the 20% concentration. In both preparations oxytetracycline is used in form of dehydrate and the same vehicula (polymer dimethylacetamid) and auxiliary substances are included.

On the basis of above mentioned the kinetics of serum levels of oxytetracycline and biological half-life in sheep after one application of mentioned long action preparations (Tetraxyl L.A. inj. and Alamycin L.A. inj.) were compared. The aim of the work was to judge local reaction in the site of application and tolerance of the medications.

Material and methods

Preparations characteristics. Tetraxyl L.A. inj. is pure dark-brown or orange-brown liquid. According to producers data the preparation contains 20.0 million U.I. (expressed as 21.57 g of OTC) of oxytetracycline dihydricum per 100 ml and from auxiliary substances: magnesii oxidum leve, natrii formaldehydsulfoxylas, olaminum, aqua pro injectione. Dimethylacetamide is indicated as a solvent.

Alamycin L.A. inj. is pure brown-yellow liquid. According to producers data it contains 21.6 g of oxytetracycline dihydricum per 100 ml. Solvents and auxiliary substances are the same as for Tetraxyl; however the information is without quantification.

Experimental set. The experiment was realized with set of 11 adult sheep, breed Slovak merino with average weight 51 kg. Experimental set was divided into two groups. In the first group (n = 6) oxytetracycline was administered in the form of Tetraxyl L.A. inj. a.u.v. (Biotika, Slovenská Ľupča, Slovak Republic). In the second group (n = 5) oxytetracycline was administered in the preparation form of Alamycin L.A. inj. a.u.v. (Norbrook Laboratories Limited, North Ireland). Oxytetracycline was administered to animals in a single dose of 20 mg.kg^{-1} of live weight i.m. into cervical muscle. Tetraxyl L.A. and Alamycin L.A. were administered in volume 1 ml/10 kg of live weight. At one site of application was Tetraxyl and Alamycin administered in volume to 5 ml. Concentrations of oxytetracycline in blood serum was observed at 1, 3, 6 and 24 hour, then on day 2, 3, 4 and 5 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^{-1}. Concentration of oxytetracycline we stated in µg.ml^{-1}. Constants of elimination were calculated using a method of the smallest squares according to one-compartment pharmacokinetic model. Constants of elimination from serum levels were counted from decreasing curve from interval 1 up to 96 hours. Biological half-life was calculated like this: mean constant of elimination ln2/ke. Statistical evaluation was provided by unpaired Student 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 general behaviour of animals, food reception and droppings consistence.

Results and discussion

Figures 1 and 2 demonstrate dynamics of changes of oxytetracycline serum levels in sheep after a single Tetraxyl L.A. inj. and Alamycin L.A. inj. application. It is seen from figures that in serum concentrations of oxytetracycline, in selected period (with exception of 96 hours) are not statistically significant differences between Tetraxyl and Alamycin. In period of 1, 3, and 6 hour non significant higher serum concentrations of OTC were produced after Alamycin administration. On the other hand, in following period (24, 48, 72,

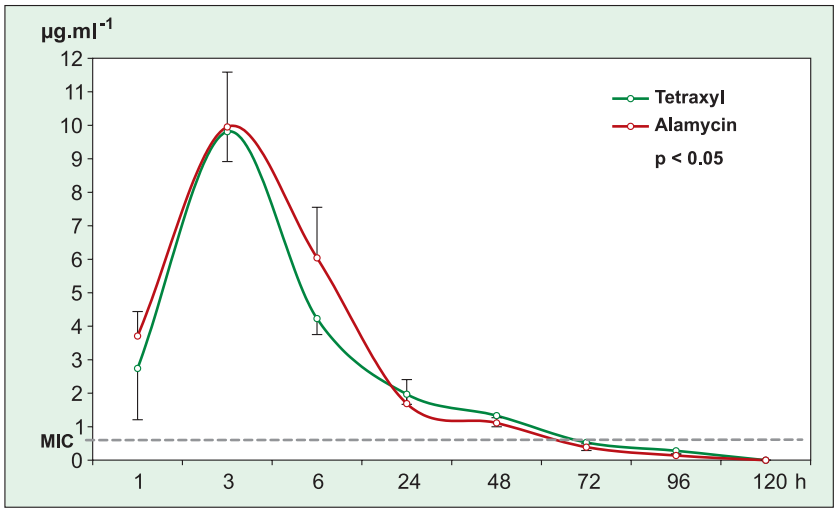

Fig. 1. Blood serum concentrations of oxytetracycline (20 mg.kg^{-1} b.w.) in sheep after simple administration of preparations Tetraxyl L.A. inj. and Alamycin L.A. inj. (mean ± SD)

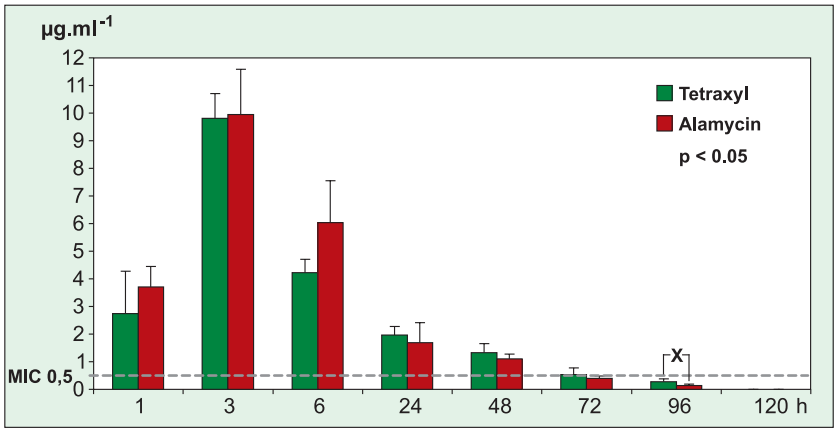

Fig. 2. Blood serum concentrations of oxytetracycline (20 mg.kg^{-1} b.w.) in sheep after simple administration of preparations Tetraxyl L.A. inj. and Alamycin L.A. inj. (mean ± SD)

and 96 hours) higher concentrations of OTC were recorded after Tetraxyl administration. These differences were non significant in time of 24, 48, and 72 hours; however on 96 hours they become significant ($p < 0.05$).

Maximum serum concentrations are recorded in 3rd hour after application of both preparations. In this time interval the level of oxytetracycline is after Tetraxyl application 9.81 and 9.95 $\mu g.ml^{-1}$ after Engemycin application. Therapeutic concentrations of oxytetracycline (MIC above 0.5 $\mu g.ml^{-1}$) are still recorded on hours 72 after Tetraxyl (0.51-0.52 $\mu g.ml^{-1}$), in hours 72 (0.53 $\mu g.ml^{-1}$) and in hours 67-68 after Engemycin (0.50 $\mu g.ml^{-1}$) application. Measurable concentrations (under MIC) after Tetravet application were still perceivable on day 4 (0.28 $\mu g.ml^{-1}$) and after Engemycin application on day 4 (0.14 $\mu g.ml^{-1}$). On the 5 day all samples, in both groups, were negative. Half-live of oxytetracycline was registered after Tetraxyl as 36.5--38.5 hours and after Alamycin as 36-38 hours.

In tab. 1 survey of serum oxytetracycline levels in observed time intervals is presented.

Changes in general behaviour, food intake and changes in droppings consistence we have not registered after any preparation. We did not record any local changes at the site of puncture in 5 animals but short-time hyperaesthesia for palpation (within 13 hours) was registered in one sheep after Tetravet application and in 2 sheep (within 15 hours) from Alamycin group (5 animals).

Because pharmacotherapeutical 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 bio phase (i.e. in the environment, in which drug comes into direct contact with receptors (5, 17), the aim of study was to find out if and to what extent various equivalent preparations will be also equivalent in selected pharmacokinetic parameters. In regard to presented information the kinetics of serum levels of oxytetracycline and its biological half-life and local a total tolerance in sheep after one application of the mentioned long--action (L.A.) preparations (Tetraxyl L.A. and Alamycin L.A) were compared.

Dosage, drug form, means of application, rate of metabolism, absorption processes, chemical properties of drug influence biological availability and persistence of drug in organism, and important place take also pharmaceutically auxiliary substances (adjuvant). Parenteral repository preparations are possible to gain by more ways. One of the ways of their preparation is realized by using modern adjuvant. Various synthetic polymers belonging to adjuvant pharmaceutical substances have important position in their preparation. These polymers are dissociated into non-toxic components in organism by hydrolytic or enzymatic way (11). Biodegradable polymers are the carriers of so

Tab. 1. Significant differences between concentrations of oxytetracycline in blood serum in sheep after single administration of preparations Tetraxyl L.A. and Alamycin L.A. (mean ± SD)

Drugs	Hours						
	1	3	6	24	48	72	96
Tetraxyl	2.74 1.53	9.81 0.89	4.23 0.48	1.97 0.30	1.33 0.33	0.53 0.24	0.28 0.10
Alamycin	3.71 0.73	9.95 1.64	6.04 1.51	1.69 0.72	1.11 0.16	0.39 0.10	0.14 0.05
P	–	–	–	–	–	–	< 0.05

Explanation: $p < 0.05$ significant difference

called long action effect. Long action preparations on the basis of oxytetracycline tested by us also contain biodegradable polymers. It comes from the fact that producers indicate dimethylacetamide in Tetraxyl L.A. and also in Alamycin L.A. as a solvent. Other adjuvants contained in preparations are also identically. However, adjuvants contained in Alamycin L.A. are quantified contrary to Tetraxyl L.A. At single i.m. oxytetracycline application in dose 20 $mg.kg^{-1}$ of live weight they indicate its therapeutically levels (over 0.5 $\mu g.ml^{-1}$) within 3 days in Tetraxyl L.A. and without indication by producer in Alamycin L.A. They also indicate minimal irritability and good tolerance in the site of i.m. application.

It is concluded from these comparative studies that there are not quantitative differences in serum levels of oxytetracycline between Tetraxyl L.A. and Alamycin L.A. (with exception in 96 hours, $p < 0.05$). In the observed time interval (1, 3, and 6 hour) none significantly higher oxytetracycline concentration after Alamycin against levels after Tetravet application was recorded. In all other time intervals (24, 48, 72, and 96 hours) on contrary higher concentrations after Tetraxyl were recorded. In 24, 48, and 72 hours the differences were statistically non significant while in 96 hour of observing they were statistically significantly higher ($p < 0.005$) after Tetraxyl application compared to Alamycin. The highest levels after application of both preparations were recorded in 3rd hour. In this time interval measured oxytetracycline level after Tetraxyl application was 9.81 and after Alamycin 9.95 $\mu g.ml^{-1}$.

Therapeutic concentrations after Tetraxyl application were still recorded on 74 hour (3rd day) and after Engemycin in 67-68 hours. Measurable concentrations were perceivable still on the day 4 after Tetraxyl (0.28 $\mu g.ml^{-1}$) and on the day 4 also after Alamycin application (0.14 $\mu g.ml^{-1}$). On the 5th day were determined zero levels of oxytetracycline after both preparations. Course of serum levels in individual time intervals is not significantly different. Course of serum levels is in accordance with other parameter – pharmacokinetic index – biological half-life. It is 36.5-38.5 hours in Tetraxyl and 36.0-38.0 hours in Alamycin.

Higher serum level of oxytetracycline registered in the first hour after Alamycin shows its faster absorption from application site compared to Tetraxyl. But steeper decrease of oxytetracycline serum levels recorded from 24 hours after Alamycin and its survival in therapeutically concentrations for shorter time than after Tetraxyl shows at faster start of eliminative phase in Alamycin. Tested preparations are the same in content of effective substance, form of basis, vehiculum (diacetylamid), and also other adjuvants are equivalent (magnesii oxidum, natrii formaldehydsulfoxylas, olaminium, *aqua pro injectione*) and the most probable conclusion on not significant difference in oxytetracycline kinetics (in phase of absorption and elimination) is impact of different quantitative content of vehicular and adjuvants or technological processes respectively. There are not studies concerning with oxytetracycline kinetics observation in oxytetracycline long action preparations depending on the type of polymer and other adjuvants contained in them. Previous studies (8, 12) confirmed different kinetics of oxytetracycline and different local tolerance of long action preparations in dependence on the type of polymer. From results of studies dealing with pharmacokinetic problems of long action preparations is seen that differences in altitude of oxytetracycline serum levels or in its survival in blood there are differences also in the same species of animals depending on used long action preparation (1, 2, 3, 15). Above mentioned studies substantially correspond to our findings.

General side reactions were not observed after using tested preparations and they ere very well tolerated. In local irritability we recorded little differences between preparations. Tetraxyl induced short-time palpation hyperaesthesia in 1 sheep from six animals (within 13 hours) in the site of puncture, while Alamycin induced short-time palpation hyperaesthesia in 2 sheep from five animals (within 15 hours). In general 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. Forasmuch as both preparations used in this study were applied in the same concentration, and volume and they contained equivalent vehiculum and other adjuvants, little differences in local irritability can be caused by individual sensitivity of animals, or different quantity of adjuvants in preparations.

The findings obtained suggest that Tetraxyl L.A. appears more beneficial in observed pharmacokinetic parameters as well as in tolerance compared to Alamycin L.A.

References

1. *Arqum I. A., Nawaz M., Khan F. H., Khalid R.*: Bioavailability and disposition kinetics of two long acting oxytetracycline after a single intramuscular administration in goats. Pakistan Vet. J. 1995, 15, 51-53.
2. *Cohen R. O., Ziv G., Soback S., Glickman A., Saran A.*: The pharmacology of oxytetracycline in the uterus of post parturient dairy cows with retained foetal membranes. Israel J. Vet. Med. 1993, 48, 69-79.
3. *Escudero E., Carceles C. M., Serrano J. M.*: Pharmacokinetics of oxytetracycline in goats: modifications induced by a long-acting formulation. Vet. Rec. 1994, 135, 548-552.
4. *Hirtz J.*: La disponibilité biologique des médicaments. Labo-Pharma, Problémes et techniques 1972, 208, 63-68.
5. *Holomáň J.*: Vplyv chronických ochorení pečene na farmakokinetiku liečivých látok. Farmako-terap. Zprávy Spofa 1984, 30, 265-284.
6. *Chalabala M., Mandák M.*: Mikrotobolky a mikrodraže. Katedra galenickej farmácie. Jbid Chem. (Suppl III) 1977, 57-72.
7. *Chalabala M., Burelová A., Rak J.*: Vztah lékové formy k účinku léčivé látky při tvorbě a formulaci léku nových generací. Českoslov. Farm. 1983, 32, 182-186.
8. *Neuschl J., Nagy J., Sokol J., Popelka P., Čonková, E., Šutiak V.*: 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. Vet. Med.-Czech 2000, 45, 83-87.
9. *Nouws J. F. M., Smulders A., Rappalini M.*: A comparative study on irritation and residue aspects of five oxytetracycline formulations administered intramuscularly to calves, pigs and sheep. Vet. Quart. 1990, 12, 129-138.
10. *Rak J., Chalabala M.*: Pokroky vo výrobe a kontrole tabliet. XIX. Rýchlosť rozpúšťania. Českoslov. Farm. 1984, 33, 295-301.
11. *Rak J., Ford J. L., Rostron C., Walters V.*: The preparation and characterisation of poly-(D,L)-lactic acid for use as biodegradable drug carrier. Pharm. Acta Helv. 1985, 60, 162-169.
12. *Sokol J., Matisová E.*: Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction. J. Chromatogr. A 1994, 669, 75-80.
13. *Svendsen O.*: Studies of drug injuries caused by intramuscular injection of drugs and vehicles. Thesis Fac. Vet. Med., Copenhagen 1989, 16-21.
14. *Švec P.*: Na margo dynamického vývoja liekových foriem a rozvoja galenickej farmácie. Slovakofarma Rev. 1998, 8, 82-83.
15. *Vyhnálek J., Hera A.*: Použití Terramycinu L.A. v podmínkách ČR – účinnost i snášanlivosť. Veterinářství 1994, 45, 164-165.
16. *Zathurecký L., Chalabala M., Janku I., Modr Z.*: Biopharmacy and Pharmacokinetics (In Slovak). Osveta , Martin 1989a, 12.
17. *Zathurecký L., Chalabala M., Janku I., Modr Z.*: Biopharmacy and Pharmacokinetics (In Slovak). Osveta , Martin 1989b, 13.

Author's address: Assoc. Prof. Jozef Neuschl, DVM, PhD., University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovak Republic; e-mail: neuschl@uvm.sk