Acta Veterinaria (Beograd), Vol. 50. No. 5-6, 331-338, 2000.

UDK 619:615.33:615:034

BIOLOGICAL AVAILABILITY OF CHLORTETRACYCLINIUM CHLORIDE IN SHEEP AFTER ADMINISTRATION OF VUBIVET C prm.a.u.v. IN MILK OR WATER

NEUSCHL J, SOKOL J, NAGY J, ČONKOVA E, KREMEN J, ŠUTIAK V and ŠALY J.

University of Veterinary Medicine, Komenskeho 73, 041 81, Košice, Slovak Republic

(Received 12. September 2000)

The standard chlortetracyclinium chloride biological availability and other pharmacokinetic parameters were observed in adult sheep of the Slovak merino breed after administration of Vubivet C prm. in milk and in water. Both groups were given chlortetracyclinium chloride in Vubivet C prm. a.u.v. preparation (Biotika, Slovenska Lžupča, Slovak Republic) in a single dose of 20 mg/kg live weight accounting for 6.66 g of preparation per 10 kg live weight. The preparation was administered per os in 2 % suspension by probe. Chlortetracyclinium chloride concentrations in blood serum were determined chromatographically after 1, 3, 6, 12 and 24 hour, according to Sokol and Matisova (1994) with liquid chromotograph. After administration of Vubivet C prm. in milk, significantly lower serum concentrations of chlortetetracyclinium chloride (p.05) were detected at 1, 3, 6 and 12 hours as compared with the comparison standard in water. The ratio between the total area under the curve of chlortetracyclinium chloride concentration in the blood serum after Vubivet C prm. administration in milk and water indicated that biological availability of Vubivet C prm. administered in milk is 33.79 % lower than in water. When the preparation was applied in water the biological half-time of chlortetracyclinium chloride was 8.64 hr and 8.82 hr when applied in milk. If antimicrobial therapy of sucklings fed milk is necessary with this preparation, or other peroral preparations on the basis of classical tetracyclines, we recommend them to be administered in water or other liquid. After application or before administration (at an interval of 2-3 hours) milk or milky feed mixture may be given. The above mentioned recommendation is supported by the finding that serum concentrations of chlortetracyclinium chloride after administration in milk are higher than the MIC but the obtained values would most likely not be effective in medium susceptible strains.

Key words: chlortetracycline, serum, sheep, Vubivet C, biological availability

INTRODUCTION

Pharmacokinetic and biopharmaceutic studies have shown that not the dosage but the concentration of a drug in systemic blood and in the biophase, is decisive for a therapeutical effect. From this knowledge the term biological availability is used to mark the efficiency level of an administered drug. For objective evaluation of this new parameter, drug movement in the body in the stage of its liberation, absorption, distribution, metabolism in the organism and elimination from it began to be observed (Zaturecku *et al.*,1989a). The former definition of biological availability (Wagner, 1961) was amended by the Food and Drug Administration in 1973. Subsequently, Zaturecku (1977;1978) suggested a new definition of biological availability. According to this accepted proposition, biological availability is defined as the relative amount of drug contained in the administered medicinal preparation that gets into the systemic blood circulation and the rate at which this process takes place. When drugs are administered extravascularly, especially orally, a certain amount of the administered dose will never get into the systemic circulation, i.e. the biological availability is not complete. In relation to incomplete biological availability, besides increased systemic clearance, insufficient absorption plays a significant role which is affected by various endogenic or exogenic factors including food (Barr and Riegelman,1970; Smyth,1964; Barr,1969; Jeminet.1969). Even though the amount, composition and time interval between food intake and drug treatment more often influence velocity than the quantitative feature of absorption, it is presumed that enteral absorption with ingested food remains unaffected only for a small number of drugs (Jaroç *et al.*,1987). The amount and composition of food, or enteral chyme may slow down not only the disintegration of solid drug forms, so reducing dissolution of effective curative substances in the enteral fluid but may also bind the drug physically or chemically (Scheler and Blanck, 1977; Brodie,1964).

Significant chemical interaction between tetracycline antibiotics and calcium caseinate, or ions of bi-or trivalent metals (calcium, aluminium, manganese, magnesium, iron, copper, nickel, bismuth) resulting in production of very slowly absorbable and antibacterially ineffective chelate complexes is generally known (Zaturecku, 1989; Netter, 1972; Remmer, 1972; Hlavka and Boothe,1973 *et al.*).

With this in mind the following captured our attention: the instructions for administration of premix Vubivet C prm. a.u.v. which contains tetracyclinium chloride recommended administration not only in water or tea but also in milk and milky feed mixture. This stimulated us to start observations of the degree of biological availability and other pharmacokinetic parameters of tetracyclinium chloride in sheep after single application of this preparation in water or milk.

MATERIALS AND METHODS

Characteristic of the preparation. Vubivet C prm. a.u.v. is a powdered mixture, brown in colour, with a characteristic smell, contains 3 % of chlortetracyclinium chloride.

Experimental group. The experiment was carried out in 8 adult sheep of the Slovak merino breed, weighing 40-50 kg. The experimental sheep were divided into two groups. Both groups were given chlortetracyclinium chloride in Vubivet

C prm. a.u.v. preparation (Biotika, Slovensk Lžupča, Slovakia) in a dose 20 mg/kg of live weight equivalent to 6.66 g of preparation per 10 kg of live weight. The preparation was administered per os as 2% suspension by probe. Individuals from the first group were given Vubivet C prm. in 333 ml water, the second group in 333 ml milk. Vubivet C prm. administered in water represented a comparison standard. Chlortetracyclinium chloride concentrations in blood serum were observed at 1, 3, 6, 12 and 24 hours after application of the preparation. We determined chlortetracyclinium chloride chromatographically, according to Sokol and Matisova (1994), using a liquid chromatograph Hewlett Packard, Avondale, PA, USA (1050 series), at a wavelength of 360 nm with a sensitivity of 0.05 $\mu g.ml^{-1}$. The elimination constants were calculated according to a mono-compartmental pharmacokinetic model from the descending curve over an interval of 3-24 hours. Biological half-time was calculated as follows: ln 2/ke. The area under the curve (AUC) of chlortetracyclinium chloride was determined by numerical integration of data using the linear trapezium rule. We did not use the transferring data principle. The following parameteres of biological availability were observed: C max (maximum concentrations), T max (time of maximum concentration onset) and AUC (area under the curve) of chlortetracyclinium chloride concentrations. Statistical significance was estimated by Student's t-test.

RESULTS

Figure 1 illustrates the dynamics of changes in serum chlortetracyclinium chloride level in sheep after single application of Vubivet C prm. preparation in water and in milk. The figure shows that there are significant differences in the height of serum levels in serum chlortetracyclinium chloride concentrations

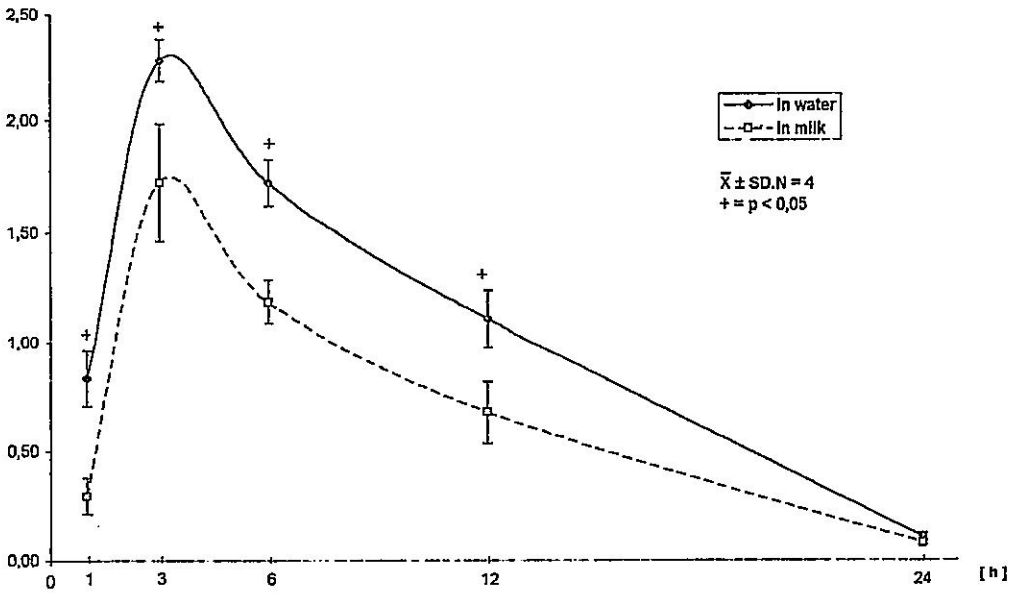

Figure 1. Blood serum concentrations of chlortetracycline chloride (20 mg/kg) in sheep after single administration of preparation Vubivet C prm. a.u.v.

between Vubivet C prm. administered in water and in milk (quantitative differences), but the pattern of change over single time sections was substantially similar. Similarity in the dynamics of serum level changes were recorded in the ascending as well as in the descending part of the curve. At all observed time intervals (1, 3, 6, 12, and 24 hr.) we registered higher serum levels of tetracyclinium chloride after administration of Vubivet C prm. in water then after administration of the same preparation in milk. Except for 24 hours, the differences between the measured values were statistically significant ($p<0.05$). Maximum serum concentrations were recorded 3 hours after administration of the preparation in water (2.2 $\mu g ml^{-1}$) as well as in milk 1.72 $\mu g\ ml^{-1}$). Therapeutic concentrations of chlortetracyclinium chloride (MIC over 0.5 $\mu g\ ml^{-1}$) were recorded even 12-14 hours after application in both water and milk.

The biological half-time of chlortetracyclinium chloride after application of Vubivet C prm. in water was 8.64 hr and after application in milk 8.82 hr.

DISCUSSION

Classical tetracyclines compared with the modified compounds (demeclocycline, metacycline, clomocycline, lymecycline, rolitetracycline) or tetracyclines of the second generation (doxycycline, minocycline), and particularly of the third generation (thiacycline), have lower stability, solubility, less perfect absorption from the gastrointestinal tract, or possibly weaker penetration into tissues. Despite this, these preparations remain in veterinary medicine partly because any differences in antimicrobial spectrum among tetracyclines detected in vivo are slight and it is not clear whether these differences are significant even *in vivo* (Wenke *et al.*,1984).

Out of the three classical tetracyclines, chlortetracycline is absorbed most incompletely and irregularly. It is thought that the digestive tract may substantially influence the biological availability of tetracyclines particularly by limiting the absorptive capacity of the intestinal mucosa concerning the particular tetracycline antimicrobicum. Vonderbank (1956) showed that after a single peroral therapeutic dose of tetracycline, maximum serum values of 1.0-1.5 $\mu g\ ml^{-1}$ are reached or 2.0-3.0 $\mu g\ ml^{-1}$ after repeated administration; after single administration of chlortetracycline these are approximately the same but after repeated administration there are lower values (1.5-2.5 $\mu g\ ml^{-1}$). Higher serum levels may occur (Schatz,1973; Vesell, 1975. Heilmayer *et al.*,1969; Neuschl, 1988; Neuschl 1989; Neuschl, 1991; Kietzmann *et al.*,1995).

Previous favourable reports of studies directed towards improvement of classical tetracycline absorption from the gastrointestinal tract with simultaneous citric acid or phosphate administration were not confirmed in later studies and did not bring the expected effect (Wenke *et al.*,1984; Pollet *et al.*,1983). These authors found that if this combination is to have definite importance, it is necessary to add citric acid or phosphates in high doses (5 times more than classical the tetracycline antimicrobicum). The affinity of tetracyclines to calcium and other ions is very high.

Therefore it is not surprising that food containing calcium and ions of other bi-, or trivalent metals (aluminium, manganese, magnesium, iron, copper,

nickel, bismuth) significantly influences absorption of classical tetracyclines predominantly. Zaturecku et al. (1989) and Jaroš et al.(1987) showed that after administration of equal dose of tetracycline antibiotics, 50-80 % lower plasmic concentrations were detected in individuals who took tetracyclines after meals compared with those who took them in the fasting state. They also observed that absorption of tetracycline antibiotics is lower, especially when milk or antacids containing calcium, magnesium and aluminium cations are used simultaneously. There is no doubt that milk can improve gastrointestinal tolerance of tetracyclines, but on the other hand, as a consequence of generating very poorly absorbed complexes of tetracyclines with calcium caseinate and calcium ions (with the exception of doxycycline and minocycline) serum tetracycline levels may remain low and their effect decreased. In an extreme case the treatment might fail (Jaroç et al., 1987). The instructions for administration of Vubivet C prm. preparation containing chlortetracyclinium chloride, recommended administration also in 0.2-0.4 % concentration in milk. We did not apply recommended concentration (0.2-0.4 %) because at a chlortetracyclinium chloride dose of 20 mg kg^{-1} live weight, it would be necessary to administer large amounts (as much as 1.665 l per 1 0 kg of live weight in 0.4 % concentration) that would inevitably influence the elimination of chlortetracyclinium chloride.

There were quantitative differences in serum chlortetracyclinium chloride levels but the dynamics of changes over certain time intervals were similar for water and milk as the diluent. We recorded significantly higher serum concentrations ($p<0.05$) of chlortetracyclinium chloride after administration of Vubivet in water, with the exception at 24 hours. Based on the ratio between the total area under the curve of chlortetracyclinium chloride concentration in the blood serum after Vubivet administration in milk and water biological availability of Vubivet administered in milk was 33.79% lower than in water. The longer biological half-time of chlortetracyclinium chloride after administration of the preparation in milk indicates, at the same time, its slightly delayed enteral resorption. Considering our findings and the fact that average bacteriostatic concentrations for most gram-positive microbes range between 0.2-1.5 μg ml^{-1} and for most gram-negatives between 0.4-3.1 μg ml^{-1} serum (Garrod and Waterworth, 1960; Ritzerfeld and Müller, 1962) we do not recommend giving Vubivet C prm. in milk not even in a low concentration. The recorded serum levels of chlortetracyclinium chloride after administration of Vubivet C prm. in milk (at 1 hr - 0.30; at 3 hr - 1.72; at 6 hr - 1.18; at 12 hr - 0.67 μg ml^{-1} of serum) as compared with the significantly higher (p.05) levels after administration of the preparation in water (at 1 hr - 0.83; at 3 hr - 2.28; at 6 hr - 1.72; at 12 hr - 1.10 μg ml^{-1} of serum) would most likely guarantee a good therapeutic effect only for susceptible strains. This is supported by the fact that chlortetracycline MIC in very susceptible strains is generally lower than 1 μg ml^{-1} but in medium susceptible strains it ranges from 1 to 5 μg ml^{-1} of serum (Vonderbank, 1956; Wenke *et al.,* 1984). If antimicrobial therapy of sucklings fed milk is necessary with this preparation or other peroral preparations formulated on the basis of classical tetracyclines, we recommend them to be administered in water or other liquid. After application or before administration (with an interval of 2-3 hours) milk or milky feed mixture may be given.

REFERENCES

1. *Barr WH,* 1969; Factors involved in the assessment of systematic or biologic availability of drug products. *Drug Inform Bull, 3,* .27-45.
2. *Barr WH, Riegelman S,* 1970; Intestinal drug absorption and metabolism:I Comparison of methods and models to study physiological factors of in vitro and in vivo intestinal absorption. *J Pharm Sci. 59,*154-163.
3. *Brodie BB,* 1964; Physico-chemical factors in drug absorption.In:Absorption and Distribution of Drugs. Ed.T.E. Binns, Edinburgh, Livingstone Ltd., 3-62.
4. *Garrod LP, Waterworth PN,* 1959-1960; *Antibiot. Ann,* 440.
5. *Heilmayer L, Otten H, Plempel M,* 1969; Antibiotika-Fibel. Stuttgard, G.Thieme Verlag.
6. *Hlavka JJ, Boothe JH ,* 1973; The tetracyclines. *Progr Drug Res,17,* 210-240.
7. *Jaroš Z, Loučka B, Geršl V.*1987; Faktory ovlyvnujci učinky lek.... Martin, Vydavatelstvo Osveta, 10;13;27.
8. *Jeminet F,* 1969; Resorption des m,dicaments. *Pharm Acta Helv., 44,* pp.261-289.
9. *Kletzmann M, Nolte M, Mischke R.* 1995; Pharmakokinetik und Biovertugbarkiet von oral verabreichten Tetracyklin bei Hunden und Katzen, *Kleintierpraxis,* 40,4,.253—258.
10. *Neuschl J,* 1988; Concentrations of Some Tetracyklines in the Blood Serum and Soft Tissues (In Slovak). *Vet Med (Praha), 33, 9,* 561-568.
11. *Neuschl J,* 1989; The Effect of Differentiated Doses of Some Tetracyklines on their Serum Levels and Concentrations in Soft Tissues (In Slovak). *Vet Med (Praha), 34, 6,* 371-380.
12. *Neuschl J,* 1991; Vergleich einiger pharmakokinetischer Parameter der in der Veterln„rmedizin am h„ufigsten angewandten Tetracykline. Arch exper Vet med, (Leipzig) 45,1,105-112.
13. *Netter KJ,* 1972; Grundlagen des Arzneimitelstoffwechsels. *Arzneimittel-Forsch., 22,* 285-295.
14. *Pollet RA, Glatz CE, Dyer DC, Barnes HJ,* 1983: Pharmacokinetics of chlortetracycline potentiation with citric acid in the chicken. *Am J Vet. Res, 44, 9,* 1718-1721.
15. *Remmer H,* 1972; Der Einfluss des Stoffwechsels von Arzneimitteln auf ihre Wirkungssterke und Wirkungsdauer. *Arzneimittel - Forsch, 8,* 118-141.
16. *Ritzerfeld W, Muller N.* 1962; Compilation of regulations for test and methods of assay of antibiotic drugs. *Arzneimittel-forschung, 12, 30.*
17. *Schatz F,* 1973: Fluorometric determination of tetracycline in serum and urine, *Arzneimittlforschung, 23, 3,* 426-428.
18. *Scheler W, Blanck J.* 1977; Physico-chemical fundamentals and thermodynamics of the membrane transport of drugs In: Kinetics of drug action. Ed.JM van Rossum, Berlin, Springer-Verlag, 3-62.
19. *Sokol J, Matisov E,* 1994: Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction, *J Chromat, A,* 669, 75-80.
20. *Smyth DH,* 1964; Allmentary absorptions of drugs: physiological consideration. In: Absorption and distribution of drugs. Ed.T.E. Binns, Edinburgh, Livingstone Ltd. 1-17.
21. *Vesell ES,* 1975; Pharmacokinetik. Der individualle Factor bei der Reaktion auf Pharmaka Triangel,14, 3-4, 126 -130.
22. *Vonderbank H,* 1956; Aureomycin and Achromycin. *Arzneimittelforschung 6* (Beiheft).
23. *Wagner JB,* 1961; Biopharmaceutics; Absorption Aspects. *J Pharm Sci.* 50,.359-387.
24. *Wenke M, Mraz M, Hynie S.* 1984; Farmakol¢gie pro lekare II. Praha, Avicenum, Zdravotnick, nakladatelstvi 1960-962.
25. *Zaturecky L,* 1977; Progress in developing a standard terminology in biopharmaceutics and pharmakokinetics. *Drug Intell Clin Pharm, 11,.* 281-296.
26. *Zaturecky L,* 1978; Terminologia a definicia z kladnych biofarmaceutickych pojmov. Farmaceutick, prehlady 1977- 1978, Martin, Vydavatelstvo Osveta, 47-61.
27. *Zaturecky L, Chalabala M, Jank... I, Modr Z.,* 1989; Biofarm cia a farmakokinetika. Martin, Vydavatelstvo Osveta, 12;74.
ACKNOWLEDGEMENT: Supported by Scientific Grant Agency of the Ministry of Education of the Slovak Republic and Slovac Academy of Sciences (grant No. 1/51/50/98).

BIOLOŠKA RASPOLOŽIVOST HLORTETRACIKLIN HLORIDA KOD OVACA POSLE APLIKACIJE PREPARATA VUBIVET C U MLEKU ILI VODI

NEUSCHL J. SOKOL J. NAGY J. ČONKOVA E. KREMEN J. ŠUTIAK V i ŠALY J.

SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja biološke raspoloživosti hlortetreciklin hlorida kao i drugi farmakokinetički parametri kod odraslih ovaca slovačke merino rase posle aplikacije preparata Vubivet C u mleku ili vodi. Obe eksperimentalne grupe ovaca su dobijale hlortetraciklin hlorid u preparatu Vubivet C (Biotika, Slovenska Lupča, Slovačka) u jednostrukoj dozi od 20 mg/kg odnosno ukupno 6.66 g preparata na 10 kg telesne mase. Preparat je aplikovan u obliku 2% suspenzije *per os* putem sonde. Koncentracija hlortetraciklin hlorida u krvnom serumu je određivana tečnom hromatografijom posle 1, 3, 6, 12 i 24 sata. Posle aplikacije preparata Vubivet C u mleku, koncentracija hlortetraciklin hlorida u serumu je bila značajno manja ($P<0.05$) u odnosu na aplikaciju istog preparata u vodi. Odnos između ukupnih površina koje ograničavaju krive koncentracija hlortetraciklin hlorida u serumu posle aplikacije u mleku ili vodi ukazuje da se biološka raspoloživost smanjila za 33.79 % zbog mleka. Kada se preparat aplikuje sa vodom biološko poluvreme je 8.64 časa a 8.82 časa posle aplikacije u mleku. Ako je neophodno da se sprovodi antimikrobna terapija mladih koji se hrane mlekom, mi preporučujemo da se preparati tetraciklina aplikuju rastvoreni u vodi. Mleko treba dati ovim mladuncima 2-3 sata pre ili posle aplikacije leka.