P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2011,(5), (1) :07-12

Pharmacokinetics and tissue and milk disposition of Tilmicosin in sheep after single administrations

Arooba M.S. Ibrahim

University of Baghdad - College of Vet. Med.

ARTICLE INFO

Received: 2 / 12 /2010
Accepted: 4 / 5 /2011
Available online: 14/6/2012
DOI: 10.37652/juaps.2011.15503

Keywords:
Pharmacokinetics ,
tissue ,
milk disposition ,
Tilmicosin ,
sheep.

ABSTRACT

Tilmicosin was administered to sheep intravenously and subcutaneously to determine its concentration in blood, tissue and milk and its kinetic behaviour. After a slow intravenous and subcutaneous injection, the serum concentration-time curve indicated a two compartment open model , elimination half-life (t1/2β) of (4·36 ± 0·04) hours. After a subcutaneous injection the peak plasma drug concentration was (22.54 ± 0.62) mg/ml (Cmax) at 0.5h (Tmax) and the drug was rapidly absorbed and slowly eliminated (t1/2β: 5·02 ± 0·08) h. The apparent volume of distribution of tilmicosin was more than (1.89 ± 0.041). Tilmicosin residues found in liver, kidney and tissue after the 14 and 28 day of withdrawal after intravenous and subcutaneous respectively. Tilmicosin was extensively secreted into milk, Tilmicosin was detectable in milk for 5 and 28 days after a single dose intravenous and subcutaneous respectively.

Introduction

Tilmicosin is a macrolide antibiotic synthesized from tylosin for veterinary use. broad spectrum activity against gram-positive bacteria and mycoplasmas, some gram-negative organisms and members of the chlamidia group. It has an antibacterial spectrum similar to tylosin with enhanced activity against *Pasteurella multocida* and *Pasteurella haemolytica* (1,2). It is recommended for treatment and prevention of pneumonia in cattle, sheep, and swine, associated with *Pasteurella multocida* and *Pasterurella haemolytica*, *Actinobacillus pleuropnemoniae*, mycoplasma species and other microorganisms found sensitive to this compound. Tilmicosin is a 16-membered ring, with a large distribution volume and long serum half-life. Tilmicosin can easily accumulate in pulmonary alveolar macrophages (PAM) because of the ion-trapping process (3,4). Indeed, the great pH difference between the intracellular compartment and plasma allows Tilmicosin to attain higher concentrations in PAM than in plasma.Tilmicosin is available as an injectable formulation, administered subcutaneously in cattle and sheep for the treatment of respiratory diseases and as a medicating ingredient for swine feeds for control and treatment of respiratory diseases(5,6,7). The purpose of the present investigation was to study the plasma kinetics , half – life and volume distribution of Tilmicosin in sheep and determine its concentration in various tissues and milk after intravenous or subcutaneous injection.

* Corresponding author at: University of Baghdad - College of Vet. Med. E-mail address:

Material and Methods

Experimental animal and drug administration

The experiment was conducted in (12) healthy adult female sheep weighting 35-55 kg. The animals were kept under constant observation for two weeks before commencement of the experiment. The animals were examined clinically to evaluated health status and to rule out the possibility of any diseases. All animals divided to two groups (6 animal/group) to receive either intravenous or subcutaneous injection (of tilmicosin (Elanco Animal Health) at the dose rate of 10 mg/kg.

Collection of samples

Blood samples (3ml each) were collected through the intravenous catheter fixed in the jugular vein into heparinized glass tubes before administration and at 2, 5, 10, 15, 30 min. and 2, 4, 6, 8, 10, 12, 24, 36 and 48 hours after intravenous administration while blood samples were collected before administration and at 5, 10, 15, 30 min. and 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours after subcutaneous administration of

the drug. Plasma was separated by centrifugation at 3.000 rpm for 10 min. at room temperature and stored at -20oC and assayed with in 24h.

Tissue sample (Liver, Kidney, Muscle and Fat) days post-dosing were collected from animals treatment prior to dosing and at intervals of 3, 7, 21 and 28, after killing the animals. All samples were analyzed using microbiological assay method. Extrapolation of this elimination curve indicates the plasma concentration at zero time. (8,9).

Milk sample were collected from animals treatment prior to dosing and at intervals of 8, 24, 48, 72 hr and 4, 5, 6, 7, 10, 14, 21 and 28 days post-dosing. Milk samples were analyzed using by B. stearothermophilus (Delvo test).(10)

Bioassay of tilmicosin and pharmacokinetic analysis

Plasma tilmicosin concentration was determined by microbiological assay most of the earlier studies used biological assays involving inhibition of bacteria growth (typically Micrococcus lutea). Tilmicosin standards (0.19, 0.39, 0.78, 1.5, 3.12, 6.25, 12.51, 25.03, 50.06, 100.12 mg/ml) were prepared by serial dilutions of stock solution of the pure tilmicosin in drug –free plasma of sheep. Tilmicosin concentration was plotted against time in a semilogarithmic system and correct curve was drawn by using the statistical calculation according to the standard methods. Calibration curve was prepared for drug concentrations ranging from 0.19 to 100.12 mg/ml and was used to quantify the drug concentration in sample. Different pharmacokinetic parameters were calculated using following equations as described by (11 ,12).

a) half-life: distribution, elimination and absorption phase.
   (i) $t_{1/2}\alpha = 0.693/\alpha$ (ii) $t_{1/2}\beta = 0.693/\beta$ (iii) $t_{1/2}k(a) = 0.693/k(a)$
b) AUC (0 - ∞), the total area under the serum drug concentration – time curve and AUMC, the area under the first moment of the serum drug concentration – time curve were calculated by trapezoidal rule.
c) $Vd_{(area)}$, the apparent volume of distribution.
   $$Vd_{(area)} = \text{Dose (mg/kg)} / \beta \times (AUC)$$
d) $Vd_{(ss)}$, the apparent volume of distribution of drug at steady state.
   $$Vd_{(ss)} = \text{Dose} \times AUMC / (AUC)^2$$
e) Cl_B, the total body clearance of drug.
   $$Cl_B = \beta \times Vd_{(ss)} \times 1000$$
f) MRT, the mean residence time.
   $$MRT = AUMC / AUC$$
g) F, the fraction of drug absorbed after non-vascular administration.
   $F = t_{1/2}\beta$ (I.V.) x AUC (S.C.) / $t_{1/2}\beta$ (S.C.) x AUC (I.V.)

Results

Following intravenous and subcutaneous administration, the data were fitted to two-compartment and one compartment open model, respectively. The drug was detected in plasma up to 10 to 24 h. following intravenous and subcutaneous administration, respectively. Comparative disposition of tilmicosin following single dose intravenous and subcutaneous administration in sheep is shown on semilogarithmic scale in (Fig.1.) The pharmacokinetics intravenous administration of tilmicosin is typical for the macrolide class of antibiotics, the drug was rapidly distributed (t1/2α : 0.14 ± 0.01 h, the apparent volume of distribution Vd(area) of tilmicosin was large volume (2.12 ± 0.036 L/kg). and elimination half-life (t1/2β: 4·36 ± 0·04 h) from the body with a clearance rate of 4.50 ± 0.43 ml/min/kg). Following subcutaneous administration, the peak plasma drug concentration was 22.54 ± 0.62 mg/ml (Cmax) at 0.5h (Tmax) and the drug was detected up to 24 h. The drug was rapidly absorbed t1/2 k(a) : (0.08 ± 0.02), widely distributed (Vd(area): 1.89 ± 0.04 l/kg) and slowly eliminated (t1/2β: 5·02 ± 0·08 h; ClB : 2.98 ± 0.44 ml/min/kg) following subcutaneous injection. The bioavailability of tilmicosin was 68.0±6.0% following subcutaneous injection. Various pharmacokinetic parameters calculated from plasma concentration of tilmicosin after single dose intravenous and subcutaneous administrations are summarized in table 1.

Tilmicosin concentrations in tissues sample (Liver, Kidney, Muscle and Fat) of animals treatment by intravenous or subcutaneous injection as reported in (Table 2 & 3). The major persistent residues found in the liver, kidneys and rapid depletion of residues in muscle and fat tissues collected. Total residues remained above 0.5 mg/kg in the injection site at 28 days post-treatment for subcutaneous injection.

Tilmicosin residues detected in milk sample of animals treatment by intravenous or subcutaneous injection using by B. stearothermophilus (Delvo test)

as reported in Table 4. Tilmicosin residues found in milk sample at period for 4 and 21 days post-treatment for intravenous and subcutaneous injection respectively.

Table 1: Pharmacokinetic parameters of Tilmicosin after single dose intravenous and subcutaneous administration (10 mg / Kg) in sheep

Parameter	Unit	Intravenous (Mean ± SE, n=6)	Subcutaneous (Mean ± SE, n=6)
$t_{1/2}\alpha$	h	0.14 ± 0.01	*****
$t_{1/2}\beta$	h	4·36 ± 0·04	5·02 ± 0·08
$t_{1/2}$ k(a)	h	-----	0.08 ± 0.02
AUC	µg.h/ml	78.42 ± 7·28	35.78 ± 3·21
AUMC	µg.h^2/ml	72.52 ± 4·20	122.88 ± 18·84
$Vd_{(area)}$	L/kg	2.12 ± 0.036	1.89 ± 0.041
$Vd_{(ss)}$	L/kg	1.24 ± 0.04	0.95 ± 0.08
Cl_B	Ml/min/kg	4.50 ± 0.43	2.98 ± 0.44
MRT	h	1.01 ± 0.12	3.43 ± 0.13
F		-----	62.0 ± 4.0
C_{max}	µg/ml	-----	22.54 ± 0.62
T_{max}	h	-----	0.70

$t_{1/2}\alpha$: half-life of distribution, $t_{1/2}\beta$: half-life of elimination $t_{1/2}$ k(a): half-life of absorption, AUC: total area under plasma drug concentration curve, $Vd_{(area)}$:, apparent volume of distribution, $Vd_{(ss)}$:volume of distribution of steady state, Cl_B: total plasma clearance, MRT:mean residence time, F: bioavailability, C_{max}: maximum drug concentration, T_{max} : time of maximum observed concentration in plasma

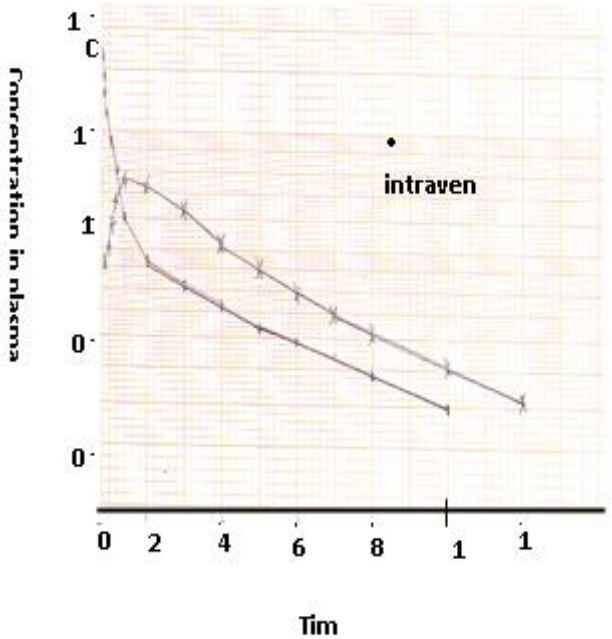

Fig1: Semilogarithmic plot of Tilmicosin in plasma versus time following single dose intravenous and subcutaneous administration at the dose of rate 10mg/kg of body weight in sheep. Each point represents mean ± S.E. of six animals

Table 2. Residues of total tilmicosin in tissues of sheep treated with a single I.V injection at a dosage of 10 mg/kg BW.

Withdrawal (days)	Mean Tilmicosin (mg/kg)			
	Liver	Kidney	Muscle	Fat
3	5.89	7.99	1.18	1.23
7	4.10	3.24	0.32	0.68
14	1.12	1.12	0.28	0.24

Table 3. Residues of total tilmicosin in tissues of sheep treated with a single S.C injection at dosage of 10 mg/kg BW.

Withdrawal (days)	Mean Tilmicosin (mg/kg)				
	Liver	Kidney	Muscle	Fat	Inj. Site
3	9.87	24.18	1.49	1.44	42.10
7	5.90	4.55	1.22	1.25	15.40
21	4.18	2.12	0.62	1.55	7.25
28	2.45	0.45	0.38	1.42	0. 85

Table 4. Residues of tilmicosin in milk samples of sheep treated with a single I.V & S.C injection at dosage of 10 mg/kg BW.

Withdrawal	I.V injection	S.C injection
8 hr	+	+
24 hr	+	+
72 hr	+	+
4 day	+	+
5 day	+	+
6 day	-	+
7 day	-	+
10 day	-	+
14 day	-	+
21 day	-	+
28 day	-	+

Discussion:

Following intravenous administration of the drug, the drug concentration in plasma (0.32 ± 0.03 mg/ml) was measured at 10 hr (13) reported similar observation in calves, however () reported lower trough concentration of 0.1 mg/ml at 12h post intravenous injection in neonatal calves. Peak plasma concentration (Cmax) of 22.54 ± 0.62 mg/ml was observed at 0.5 h following subcutaneous injection, which is in agreement with observations reported in calves (24.3 ± 0.72 mg/ml), goats (22.4 ± 1.3 mg/ml) and sheep (23.1 ± 2.2 mg/ml) (14).

Following intravenous administration , faster distribution (α : 5.77 ± 2.00 h-1) and slow elimination (β : 0.46±0.02 h-1) indicated that the drug is rapidly distributed and then relatively slowly eliminated in sheep. The elimination half-life of tilmicosin was 4·36 ± 0·04 h.) It is in agreement with the half life of tilmicosin in reported in calves (4.18 ± 0.12h.) , sheep

(4.01± 0.04 h.) and goats (3.98 ± 0.01 h.) (10 , 12 , 13 ,14) However longer elimination half-life of (5.31 ± 0.7 h.) was reported in camel (14) may be due to slower body clearance (1.72 ± 0.18 ml/min/kg) of the drug in camel. The total body clearance of tilmicosin following intravenous administration in sheep was 4.50 ± 0.43 ml/min/kg. which supports faster elimination of the drug in claves , goats and sheep and horses (15 ,16). Elimination half life and total body clearance suggests faster elimination of timicosin in sheep. The apparent volume of distribution of the drug following intravenous administration was 1.24 ± 0.03 L/Kg, which is lower than the apparent volume of distribution of 2.01 ± 0.17 L/Kg reported in cow (17).

Following subcutaneous administration, the elimination half – life of tilmicosin (5·02 ± 0·08 h.) is similar to elimination half – life of tilmicosin reported in cow (4.25 ± 0.22), goats (5.32 ± 0.04) (16). The subcutaneous bioavailability of tilmicosin was 62.0 ± 4.0 percent. However, higher bioavailability of tilmicosin following intramuscular administration has been reported in cow calves (85%), buffalo calves (82.4%), goats (87%) and sheep (84.6%) (17,18).

The success of antimicrobial therapy can be largely determined by integrating the pharmacokinetic and pharmacodynamic parameters like AUC/MIC and Cmax/MIC (19). The effective use of the antibacterial drugs against clinically important pathogens depends on designing dosages that attain a Cmax/MIC ratio > 8-10 and an AUC/MIC ratio > 100-125 (Lode et al, 1998). Thus values of AUC/MIC and Cmax/MIC after subcutaneous administration were calculated using MIC90 (0.2 mg/ml) of Tilmicosin against Salmonella, Escherichia coli and Pasteurella multocida isolated (18). The calculated value of Cmax/MIC (107.55) and AUC/MIC (332.9) indicates that tilmicosin may have excellent clinical and bacteriological efficacy against gram negative infections in sheep. In conclusion, tilmicosin can be used at the dose of 10 mg/kg to treat various susceptible infections in sheep (19).

Macrolides become widely distributed in tissues, and concentrations are about the same as in plasma, or even higher in some instances (12). They actually accumulate within many cells, including macrophages, in which they may be > or = 20 times the plasma concentration. This accumulation accounts in part for the long dosing interval that characterizes some macrolides (tilmicosin). Tilmicosin tend to concentrate in the spleen, liver, kidneys, and particularly the lungs. They enter pleural and ascitic fluids. They concentrate in the bile and milk (20). Tilmicosin was excreted in milk and a slow depletion rate maintaining detectable concentrations more than 5 & 21 days after intravenous and subcutaneous administration respectively this result like (21) which found

Tilmicosin was excreted in milk with a mean concentration peak of 24.6µg/mL and a slow depletion rate maintaining detectable concentrations more than 5 days after intravenous administration, and Radio-labeled tilmicosin was administered subcutaneously at a single dose of 10 mg/kg bw. The animals were managed as dry cows until parturition and milk samples collected after this time. In colostrums, tilmicosin represented 89 % of the total radioactive residue, which means that the administered dose remained largely unchanged for a long period since the interval between dosing and calving was around 50 days (22,23).

References:

1. Womble A, Giguère S, Murthy YVSN, Cox C, Obare E (2006) Pulmonary disposition of tilmicosin in foals and in vitro activity against *Rhodococcus equi* and other common equine bacterial pathogens. J Vet Pharmacol Ther 29:561–568.
2. Honeybourne D (1997) Antibiotic penetration in the respiratory tract and implications for the selection of antimicrobial therapy. Curr Opin Pulm Med 3:170–174.
3. Gallina G, ; Lucatello L,; Drigo I. & Cocchi M.(2010) Kinetics and intrapulmonary disposition of tilmicosin after single and repeated oral bolus administrations to rabbits. Vet Res Commun 34 (Suppl 1):S69–S72
4. Lacoste, E. (2003). Residue study of tilmicosin in bovine milk following a single subcutaneous administration of Micotil® 300 in dairy cattle. Unpublished Study No.A03586/T5CCFF0301 from Avogadro Labs, Fontenilles, France conducted for Elanco Animal Health, Eli Lilly and Company, Suresnes Cedex, France.
5. Montesissa, C., Capolongo, F., Santi, A., Biancotto, G., and Dacasto, M. (2004). Metabolism of tilmicosin by rabbit liver microsomes and hepatocyte. The Veterinary Journal, 167, 87-94.

6. Modric, S. Webb, A.I., and Derendorf, H. (1998). Pharmacokinetics and pharmacodynamics of tilmicosin in sheep and cattle. J.vet. Pharmacol. Therap., 21, 444- 452.
7.Modric, W. (1998). Pharmacokinetics and pharmacodynamics of tilmicosin in sheep and cattle, J. of Veterinary Pharmacology & Therapeutic, Vol.22, P.444- 452.
8. Ramadan, A.(1997). Pharmacokinetics of tilmicosin in serum and milk goats. Research in Veterinary Science 62, 48-50.
9. Riviere, J. E.(1999). Comparative pharmacokinetics. Principles, techniques and applications. Ames, IA, Iowa State University Press.
10. Iveta, K., Bernadetta, H., (2003) Interactions of Antimicrobials in Milk and Their Detection by the Disk Diffusion Method and Delvotest SP. Journal of AOAC International. 86: P. 529-533
11. Stoev, G., and Nazarov, V. (2008). Identification of the related substances of tilmicosin by liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry, 22, 1993 – 1998.
12. Lombardi K. R.; Portillo, K. ; T, Hassfurther, R. & Hunter, R (2011) Pharmacokinetics of tilmicosin in beef cattle following intravenous and subcutaneous administration.
13. Chan W., Gerhardt G. C., Salisbury C. D., (1994). Determination of tylosin and tilmicosin residues in animal tissues. J.vet. Pharmacol. Therap., 18, 144-152.
14. Thomson, T.D., (1989). Serum tilmicosin profiles following a single 10 mg/kg administration of the proposed tilmicosin bovine parenteral formulation in neonatal calves in several anatomical sites. Research Report T5C768804, Sponsor Submitted.
15. Kiehl D.E., Kennington A.S. (1995). Analysis of tilmicosin in swine liver extracts by liquid chromatography / atmospheric pressure chemical ionization mass pectrometry. Rapid Commun Mass Spectrom. 9, 1297-301.
16. Mark E.P. Hows, Nick G. Coldham and Maurice J. Sauer (2001) Multi-Residue Analysis Of Macrolide Antibiotics In Bovine Kidney Tissue By Lc-Msn Can Vet J.Volume 25,418-422.
17. Chan, W., Gerhardt, G.C., and Salisbury, C.D.C., (1994). Determination of tylosin and tilmicosin residues in animal tissues by reversed-phase liquid chromatography. J. AOAC Int. 77: 331-333.
18. Delepine B., Hurtaud D., Sanders P. (1994). Identification of tylosin in bovine muscle at the maximum residue limit level by liquid chromatography-mass spectrometry, using a particle beam interface. Analyst 119, 2717-2721.
19. Cochrane, R.L., and Thomson, T.D., (1990). Toxicology and pharmacology of tilmicosin following administration of subcutaneous and intravenous injections to sheep. Research Report T5C768908, Sponsor Submitted
20. Susan, L. Helton-Groce, Tucker D. Thomson, Robin S. Readnour (1993). A study of tilmicosin residues in milk following subcutaneous administration to lactating dairy cows. Can Vet J Volume 34,619-621.
21.Delepine, B., Hurtaud-Pessel D., Sanders, P. (1996). Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography- mass spectrometry. Journal of AOAC International, 79, 397-404.
22. Naccari, F., Pellegrino, M., Calò,M., Licata, P., Giofré, F.,and Carli, S.(2001). Effectiveness and kinetic behaviour of tilmicosin in the treatment of respiratory infections in sheep. Veterinary Record 2148:773-776.
23. Clark, C.,M. Dowling, M., (2008). "Development and validation of a method for determination of tilmicosin residues in sheep plasma and tissues using HPLC. J. Liq. Chromatogr. Relat. Technol.175: 888-892.

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2011,(5), (1) :07-12

الحركية الدوائية للتليموكسين وطرحه في أنسجة وحليب الأغنام بعد أعطائه بجرعة واحدة

عروبة محمد سعيد إبراهيم

الخلاصة:

تناولت الدراسة أعطاء التليموكسين Tilmicosin إلى الأغنام عن طريق الحقن الوريدي وتحت الجلد وذلك لتحديد تركيزه في الدم ، الأنسجة والحليب والسلوك الحركي. بعد الحقن البطيء في الوريد وتحت الجلد، أظهر منحنى تركيز المصل للتليموكسين مع الوقت نموذج الحجرتين المفتوح، وسجل عمر نصف الإطراح (t1/2β) (4·36±0·04)ساعة. وبعد الحقن تحت الجلد سجل العقار أعلى تركيز له في البلازما (0.62 ± 22.54) ملغم / لتر (Cmax) في 0.75 ساعة (Tmax) حيث أعطى العقار سرعة امتصاص و بطء في الإطراح (0·08 ± 5·02 :t1/2β) ساعة وكان حجم الانتشار الظاهري للـ tilmicosin أكثر من (0.041 ± 1.89). متبقيات التليموكسين وجدت في الكبد والكلية والأنسجة بعد (14 و 28 يوم) من الإعطاء الوريدي وتحت الجل. وقد فرز Tilmicosin على نطاق واسع في الحليب، حيث تمكن الكشف عن متبقياته في الحليب لمدة (5 و 28 يوم) بعد إعطائه لجرعة واحدة عن طريق الحقن الوريدي وتحت الجلد على التوالي.