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

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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 ($t_{1/2\beta}$) of (4.36 ± 0.04) hours. After a subcutaneous injection the peak plasma drug concentration was (22.54 ± 0.62) mg/ml (C_{max}) at 0.5h (T_{max}) and the drug was rapidly absorbed and slowly eliminated ($t_{1/2\beta}$: 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.

Key words : Pharmacokinetics ,tissue , milk disposition , Tilmicosin , sheep .

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 pleuropneumoniae*, 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.

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 evaluate 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 -20°C and assayed within 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 samples 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 *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.

$$Vd_{(area)} = \text{Dose (mg/kg)} / \beta \times (\text{AUC})$$
- d) $Vd_{(ss)}$, the apparent volume of distribution of drug at steady state.

$$Vd_{(ss)} = \text{Dose} \times \text{AUMC} / (\text{AUC})^2$$
- e) Cl_B , the total body clearance of drug.

$$Cl_B = \beta \times Vd_{(ss)} \times 1000$$
- f) MRT, the mean residence time.

$$\text{MRT} = \text{AUMC} / \text{AUC}$$

- g) F, the fraction of drug absorbed after non-vascular administration.

$$F = t_{1/2\beta} (\text{I.V.}) \times \text{AUC (S.C.)} / t_{1/2\beta} (\text{S.C.}) \times \text{AUC (I.V.)}$$

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 ($t_{1/2\alpha} : 0.14 \pm 0.01$ h, the apparent volume of distribution $Vd(\text{area})$ of tilmicosin was large volume (2.12 ± 0.036 L/kg). and elimination half-life ($t_{1/2\beta} : 4.36 \pm 0.04$ 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 (C_{max}) at 0.5h (T_{max}) and the drug was detected up to 24 h. The drug was rapidly absorbed $t_{1/2} k(a) : (0.08 \pm 0.02)$, widely distributed ($Vd(\text{area}) : 1.89 \pm 0.04$ l/kg) and slowly eliminated ($t_{1/2\beta} : 5.02 \pm 0.08$ h; $Cl_B : 2.98 \pm 0.44$ ml/min/kg) following subcutaneous injection. The bioavailability of tilmicosin was $68.0 \pm 6.0\%$ 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 *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

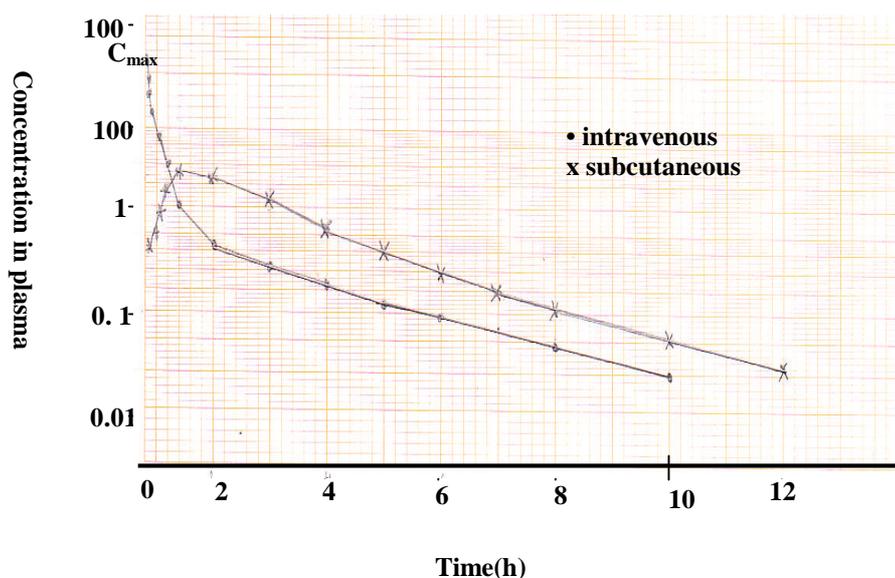


Fig1: Semilogarithmic plot of Tilmicosin in plasma versus time following single dose intravenous and subcutaneous administration at the dose of rate 10mg/kg of body weight in sheep. Each point represents mean ± S.E. of six animals

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 (C_{max}) 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 ($\alpha : 5.77 \pm 2.00$ h⁻¹) and slow elimination ($\beta : 0.46 \pm 0.02$ 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 ± 0.12 h.) , 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 calves , 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 C_{max}/MIC (19). The effective use of the antibacterial drugs against clinically important pathogens depends on designing dosages that attain a C_{max}/MIC ratio $> 8-10$ and an AUC/MIC ratio $> 100-125$ (Lode et al, 1998). Thus values of AUC/MIC and C_{max}/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 C_{max}/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).

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

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الخلاصة: تناولت الدراسة إعطاء 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 منحني تركيز المصل للتليموكسين مع الوقت نم وذج الحجرتين اللمفتوح، وسجل عمر نصف الإطراح $(t_{1/2\beta})$ (0.04 ± 4.36) ساعة. وبعد الحقن تحت الجلد سجل العقار أعلى تركيز له في البلازما (22.54 ± 0.62) ملغم / لتر (Cmax) في ٠.٧٥ ساعة (Tmax) حيث أعطى العقار سرعة امتصاص و بطء في الإطراح $(t_{1/2\beta}: 5.02 \pm 0.08)$ ساعة وكان حجم الانتشار الظاهري للـ tilmicosin أكثر من (1.89 ± 0.041) . متبقيات التليموكسين وجدت في الكبد والكلية والأنسجة بعد (١٤ و ٢٨ يوم) من الإعطاء الوريدي وتحت الجلد. وقد فرز Tilmicosin على نطاق واسع في الحليب، حيث تمكن الكشف عن متبقياته في الحليب لمدة (٥ و ٢٨ يوم) بعد إعطائه لجرعة واحدة عن طريق الحقن الوريدي وتحت الجلد على التوالي.