Disposition Kinetics of lincomycin following intravenous administration in hypothyroid goats

Meemansha Sharma*, Vinod Kumar Dumka, Saloni Singla, Rajdeep Kaur and Raushan Kumar Singh

Department of Veterinary Pharmacology and Toxicology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

ABSTRACT

Hypothyroidism is a common disorder of small ruminants and is expected to alter the pharmacokinetics of drugs. Hypothyroidism was induced by feeding thiourea at the dose rate 50 mg.kg⁻¹ daily for 28 days to goats. Disposition of lincomycin, after intravenous administration at dose rate 10 mg/kg, was investigated in hypothyroid goats to determine the potential dosage regimen against susceptible microorganisms. Blood samples were collected from 1 min to 24 h of drug administration. The drug was detected in plasma up to 8 h and lincomycin was rapidly distributed from blood to the tissue, as evidenced by the high value of the distribution coefficient (mean ± SEM) 12.3±1.09 h⁻¹. The large Vd (1.78±0.18 L/kg) indicated vast tissue distribution of lincomycin in goats. The elimination half life, AUC and total body clearance were 3.99± 0.25 h, 33.2±1.71 ìg.h/mL and 0.31±0.02 L/h/kg, respectively. Based on results, lincomycin in hypothyroid goats is suggested to be repeated at 12 h interval for organisms sensitive to lincomycin having MIC up to 0.1 µg.ml⁻¹.

Key words: Disposition, Goats, Hypothyroid, Lincomycin.

INTRODUCTION

In large animal practice, clinical cases involving deep-seated infections affecting bones, joints, meninges and the larynx are particularly difficult to treat. The antibiotic lincomycin has the ability to penetrate tissue of poor vascularity and is also effective in the presence of pus. Treatment with lincomycin has been shown to be effective in cattle, sheep and horses at various doses (Plenderleith 1988). Successful treatment of arthritis and pedal osteomyelitis usually associated with Trueperella pyogens (Arcanobacterium pyogens) has been reported with lincomycin in sheep (Giguere 2013). Lincomycin disposition has been studied in calves (Burrows et al. 1983; 1986), cattle (Weber et al. 1981), buffalo calves (Gouri et al. 2014), pigs (Chaleva and Nquyen 1987), cats (Albarellos et al. 2012) and chickens (El-Sayed et al. 2015). The disposition of antimicrobials has been reported to be altered by disease conditions (Burrows 1986) and hypothyroidism is a common type of disorder encountered in the small ruminants (Gupta et al. 1999). When thyroid function was altered a series of physiological changes were recorded which are likely to affect drug absorption, metabolism or excretion, and altered pharmacokinetics of variety of drugs in both hypothyroidism and hyperthyroidism (Shenfield 1981). There is lack of data on influence of hypothyroid state on pharmacokinetics of lincomycin in goats. Therefore, the present study was undertaken to determine the disposition of lincomycin in hypothyroid goats.

MATERIALS AND METHODS

Animals: The experiments were performed on six healthy female goats of 16-24 months age and weighing between 35-50 kg, procured from University dairy farm. Animals were kept under loose housing system in clean and hygienic experimental sheds with brick flooring, asbestos roofing and sufficient space for the free movement of the animals. During this period, all animals were subjected to regular clinical examination and treated with anthelmintics for deworming. During the experimental period, the animals were maintained on concentrate and free grazing. The animals were maintained on green fodder and wheat straw and water was provided ad libitum. The study was approved by the Institutional Animal Ethics Committee (Vide Ref No. VMC/2014/IAEC/1046-73 dated 07-04-2014).

Induction of hypothyroidism: Thiourea was used to induce hypothyroidism at the dose rate 50 mg.kg⁻¹ daily drenched to goats for 28 days. The blood samples were analysed weekly up to 42 days for thyroid hormones, TSH, cholesterol and total protein. Significant reduction in plasma T₃ and T₄ levels and increase in level of TSH, cholesterol and total proteins to the extent beyond 50% were taken as indicators of hypothyroid state in goats.

Drug administration and blood sampling: On induction of hypothyroidism, lincomycin was administered as single IV injection into jugular vein of all animals at the dose...
rate 10 mg/kg body weight. Blood samples (3-5 ml) were drawn by venepuncture from the contralateral jugular vein at 0, 1, 2.5, 5, 10, 15, 30 min and 1, 2, 4, 8, 12 and 24 h into heparinized test tubes and plasma from the samples were separated by centrifugation at 2500 rpm for 15 min for drug estimation.

Sample processing: Two ml plasma sample was added to 2.3 ml of acetonitrile in centrifuge tube, mixed for 10 seconds and centrifuged at 2500 rpm for 10 min. After centrifugation, 3.6 ml of clear supernatant was pipetted into a fresh test tube and kept for evaporation. The evaporated sample was reconstituted in 0.4 ml water and mixed for 10 s. The mixed clear supernatant (0.2 ml) was pipetted into the autosampler vial.

Drug Assay: Lincomycin was estimated in plasma by reverse-phase chromatography using HPLC (Perkin Elmer, USA, series 200) by the method of Nielsen and Gyrd-Hansen (1998) with analytical C18 column (particle size 5µ, 4.6×250mm, Waters, USA), acetonitrile as mobile phase A (25%), phosphate buffer as mobile phase B(75%), flow rate of 1 ml/ min, UV/VIS detector set 210 nm. The data were analyzed using Total Chrome software (version 6.1). The limits of detection and quantification were 0.1 and 0.5 µg/ mL., respectively. Retention time of lincomycin was 7 min and calibration curve was linear between 0.5 and 100 µg/mL (r = 0.998). Accuracy and precision were evaluated with quality control samples at concentrations of 0.5, 5 and 25 µg/mL.

Pharmacokinetic analysis: Appropriate pharmacokinetic model was determined by application of Akaike’s Information Criterion (AIC). The mean pharmacokinetic variables were obtained by averaging the variables for drug disposition from individual animals. The pharmacokinetic parameters were calculated according to classical equations (Gibaldi and Perrier 1982). The mean pharmacokinetic variables were obtained by averaging the variables calculated for drug disposition after IV drug administration to each animal. The time for which the plasma drug levels remain above or equal to minimal inhibitory concentration (MIC) value is calculated using the formula:

\[
\% T>MIC = \ln \left( \frac{D}{Vd(area) \times MIC} \right) \times \frac{t_{1/2}}{ln(2)} \times \left[ \frac{100}{DI} \right]
\]

Where T>MIC is the time interval (in percent) during which the plasma concentration is above or equal to the MIC values, ln is natural logarithm, D is the proposed dose, Vd(area) is the volume of distribution, t_{1/2} is the terminal elimination half-life, and DI is the dose interval (Turnidge 1998).

RESULTS AND DISCUSSION

Effect of thiourea feeding on plasma levels of triiodothyronine (T₃), thyroxine (T₄) and TSH hormones are presented in Table 1. Alteration in plasma cholesterol and total proteins are depicted in Table 2. Significant reduction in levels of plasma T₃, T₄ and increase in level of TSH were recorded (61%, 82% and 79%, respectively) on 28th day of thiourea feeding as compared to zero day value (normal value). Similarly, plasma levels of cholesterol and total proteins were significantly increased on 28th day (82% and 65%, respectively) of thiourea feeding as compared to zero day value (normal value). Pharmacokinetic determinants of lincomycin after its intravenous injection in hypothyroid goats are presented in Table 3. The peak plasma concentration of lincomycin was 49.1± 9.68 µg/mL at one minute and the drug was detected above MIC level up to 8 h (2.39± 1.01 µg/mL). The minimum inhibitory concentration (MIC₉₀) of lincomycin have been reported as 0.06-2.0 µg/ml against Streptococcus, Mycoplasma, and Staphylococcus spp. (Nielsen and Gyrd-Hansen 1998; Petinaki et al. 2008; Albarellos et al. 2012; Giguere 2013). The T>MIC has been calculated for MIC₉₀ of 0.06, 0.1, 0.6 and 1µg/mL. Table 4 shows the calculated % T>MIC for lincomycin based on the estimated pharmacokinetic parameters obtained following IV injection in hypothyroid goats for 8, 12 and 24 h dosing interval.

The disposition curve of lincomycin in hypothyroid animals revealed that pharmacokinetics of lincomycin followed two-compartment open model (Fig 1). In agreement of the present findings, elimination pattern the plasma disposition of lincomycin has been reported to fit two compartment model in buffalo calves and broiler chickens. (Gouri et al. 2014; El-Sayed et al. 2015) and lincomycin
was detected for up to 12 h in pigs (Nielsen and Gyrd-Hansen 1998) after intravenous administration. Since there are no reports on the pharmacokinetic data of lincomycin in hypothyroid condition in any animal species, the results of the present study are compared with the pharmacokinetic data of other animal species reported under healthy condition. The large $V_d_{area}$ indicated good distribution of lincomycin in various body fluids and tissues of hypothyroid goats which was higher than the $V_d_{area}$ of 1.15 L.kg$^{-1}$ observed for lincomycin in buffalo calves (Gouri et al. 2014) and healthy and pneumonic calves (1.1-1.30 L.kg$^{-1}$) following IV administration (Burrows et al. 1983, 1986). The lipophilic nature and high pK$\alpha$ values of 7.6 of this compound might be the major reasons for the good distribution of lincomycin to the tissues. The values of AUC of lincomycin in hypothyroid goats were 33.2±1.71 µg.ml$^{-1}$.h indicating good antibacterial activity of the drug. The high value of AUC obtained in the present study was consistent to the high value reported for AUC of lincomycin as 41.6 µg.ml$^{-1}$.h in buffalo calves after single IV administration (Gouri et al. 2014) and in cats 39.76 µg.ml$^{-1}$.h. (Alberallos et al. 2012). Lincomycin was rapidly transferred from central to peripheral compartment in hypothyroid goats as is evident from the high value of distribution rate constant and short distribution half-life which are comparable with the high distribution rate constant (11.2 h$^{-1}$) and short distribution half-life (0.06 h) reported after intravenous injection of lincomycin in buffalo calves (Gouri et al. 2014). Rapid transfer of lincomycin from central to peripheral compartment was also evident by the high value of $K_{12}/K_{21}$ ratio which was higher than the $K_{12}/K_{21}$.

Table 3: Disposition of lincomycin following its single intravenous injection (10 mg.kg$^{-1}$) in hypothyroid induced goats $n=6$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d_{area}$</td>
<td>L.kg$^{-1}$</td>
<td>1.78±0.18</td>
</tr>
<tr>
<td>$V_d_{ss}$</td>
<td>L.kg$^{-1}$</td>
<td>1.53±0.15</td>
</tr>
<tr>
<td>AUC</td>
<td>µg.ml$^{-1}$.h</td>
<td>33.2±1.71</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg.ml$^{-1}$.h$^2$</td>
<td>163.2±9.43</td>
</tr>
<tr>
<td>$C_p^0$</td>
<td>µg.ml$^{-1}$</td>
<td>58.4±3.96</td>
</tr>
<tr>
<td>A</td>
<td>µg.ml$^{-1}$</td>
<td>53.3±3.90</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>h</td>
<td>12.3±1.09</td>
</tr>
<tr>
<td>$t_{1/2_{SS}}$</td>
<td>h</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>$K_{12}/K_{21}$</td>
<td>Ratio</td>
<td>7.79±0.79</td>
</tr>
<tr>
<td>B</td>
<td>µg.ml$^{-1}$</td>
<td>5.13±0.58</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>h</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>P/C</td>
<td>Ratio</td>
<td>9.28±1.10</td>
</tr>
<tr>
<td>$Cl_{h}$</td>
<td>L.kg$^{-1}$.h-1</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>$t_%{el}^0$</td>
<td>h</td>
<td>3.99±0.25</td>
</tr>
<tr>
<td>$t_{1/2_{el}}$</td>
<td>h</td>
<td>0.40±0.03</td>
</tr>
<tr>
<td>$V_C$</td>
<td>L.kg$^{-1}$</td>
<td>0.175±0.01</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>4.96±0.31</td>
</tr>
</tbody>
</table>

$V_{d_{area}}$, volume of distribution; $V_{d_{ss}}$, volume of distribution at steady state; AUC, area under concentration – time curve; AUMC, area under the first moment of the plasma concentration-time-curve; $C_p^0$, plasma drug concentration at zero time; A, zero time intercept of distribution phase; $t_{1/2}$, distribution rate constant; $t_{1/2_{SS}}$, distribution half life; $K_{12}/K_{21}$, ratio of rate constant for central to peripheral compartment and peripheral to central compartment; B, zero time intercept of elimination phase; $t_{1/2}$, elimination rate constant; P/C, ratio of peripheral to central compartment; $Cl_{h}$, total body clearance; $t_{1/2}^0$, elimination half life; $t_{1/2_{el}}$, elimination half life from central compartment; $V_C$, apparent volume of central compartment; MRT, mean residence time of the drug in body.

Fig 1: Plasma concentration time profile of lincomycin in hypothyroid goats following single IV dose of 10 mg.kg$^{-1}$. Distribution (— -0%—) and elimination phases ( ——— ) are represented by least square regression lines. Plasma concentrations at different time intervals are represented by dots (●).
K_{12}, ratio of 4.4 for lincomycin in buffalo calves after single IV administration (Gouri et al. 2014).

Total body clearance of lincomycin, which represents the sum of metabolic and excretory process in hypothyroid goats was 0.31 ± 0.02 L.kg^{-1}.h^{-1} which was higher than the value of Cl_{12} reported for lincomycin in buffalo calves (0.24 L.kg^{-1}.h^{-1}) and in cats (0.28 L.kg^{-1}.h^{-1}) but less than the corresponding value in pigs (0.46 L.kg^{-1}.h^{-1}) after IV administration (Gouri et al. 2014; Albarellos et al. 2012; Huimin et al. 2012).

The elimination half-life of lincomycin in the present study was 3.99 ±0.25 h indicating rapid elimination of lincomycin following IV administration in hypothyroid goats. This finding was comparable with the t_{1/2} of 3.30 h in buffalo calves, 3.38 h in pigs, and 2-3 h in calves reported after IV injection of lincomycin (Gouri et al. 2014; Burrows et al. 1983, 1986; Huimin et al. 2012).

It has been recommended that T>MIC, which is a crucial pharmacodynamic/pharmacokinetic parameter for time-dependent antibacterial drugs reflecting the length of time during which drug remains above the MIC value, should be at least 50% of the dosage interval for optimal antibacterial effect (Toutain and lees 2004). The purpose of present study was to calculate T>MIC of lincomycin, a time-dependent antibacterial drug and to modify its dosage regimen in hypothyroid goats. The results obtained from the present investigation suggest that lincomycin at dose rate 10 mg/kg body weight IV should be repeated at 12 h interval for that organism which are sensitive to lincomycin having MIC up to 0.1 µg/mL and at 8 h interval for bacteria having MIC up to 0.6 and 1µg/mL in hypothyroid goats.

CONCLUSION
The dosage regimen suggested in present study may be considered for clinical use in goats after establishing PD studies and potential clinical testing of lincomycin in this species. Since only six animals were used in the present study, the PK parameters need to be verified in a larger population of goats and variations in dosage regimens may be required.

Conflict of interest statement
The authors of this study do not have any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGEMENT
The junior research fellowship awarded to the senior author by Indian Council of Agricultural Research to conduct this study is thankfully acknowledged.

REFERENCES