A Pharmacokinetic and Tissue Residue Depletion Study after Intramuscular Injection of Leotrox (Sulfatroxazole (STX)/Trimethoprim (TMP)) Combination in Calves

J. Szancer, H.J. Edwards, A-M. Kissmeyer & J.M. Pott

Leo Pharmaceutical Products
55 Industriparken
DK-2750 Ballerup
Denmark

Introduction

The object of this study was to determine 1) lung and plasma pharmacokinetic profiles of STX and TMP, 2) tissue depletion and residues of STX and TMP.

Material & Methods

34 calves (approx. 20 days of age) were treated with Leotrox 24% inj. i.m. (each ml containing 200 mg STX and 40 mg TMP) in the neck at a dosage of 16 mg/kg/day for 5 consecutive days. The animals were slaughtered at 2, 4, 6, 24, 48 hours and at 10, 12, 16 and 17 days after the last Leotrox inj. Plasma, lung, liver, kidney, muscle, fat and urine samples, as well as samples of the last 3 injection sites were collected and assayed for STX and TMP. Samples collected at 10, 12, 16 and 17 days after the last injection were also assayed for N_4-acetyl-STX (main metabolite of STX).

The residues of STX, N_4-acetyl-STX and TMP in tissue from calves were assayed by reverse-phased HPLC with UV-detection.

Assay Procedure: Residues in tissue (approx. 100g) from calves were determined by mixing 2g of the homogenate with 0.33 0.22 N perchloric acid followed by extraction with chloroform at pH 5, 6.8, or 9 (N_4-acetyl-STX, STX or TMP, respectively).

Analysis conditions: Example for STX: Column: 250 mm, 4 mm i.d. packed with LiChrosorb RP18, 7µ (Merck). Eluent: CH_3CN-0.01 M KH_2PO_4 with 0.01 M tetramethylammonium perchlorate pH 3 (15:85). Flow: 2.5 ml/min. Detector wavelength: 270 nm. Range: 0.02 AUFS. Paperspeed: 0.5 cm/min. Injection volume: 100 µl.

Analysis conditions for TMP and N_4-acetyl-STX were similar to those of STX, with minor variations. The extraction and clean-up procedures for STX, N_4-acetyl-STX and TMP in blood and urine were similar to those used for tissues.

Validation of the HPLC assay: Blank tissues were spiked with different concentrations of STX, N_4-acetyl-STX or TMP over the range of concentrations (range for TMP: 0.05-0.8 µg/g). The standard curves were analysed by linear regression analysis to determine linearity of the detector response for peak height measurements over the concentration range, and the correlation coefficient r was greated than 0.999 in all tissues. The limit of detection was 0.05 ppm for TMP, 0.1 ppm for STX and N_4-acetyl-STX.

The recovery level was approximately 93% for STX, 98% for TMP and 99% for N_4-acetyl-STX.

The elimination rate constant for STX and TMP were calculated from the experimental data by least squares regression analysis.

Results

Tables 1 and 2 present the calculated elimination rate constants (β), the elimination half-life ($t^{1}/_{2}\beta$) and the calculated time required for tissue to reach detection limit.

Table 1

Elimination of trimethoprim (TMP) after intramuscular administration of 16 mg/kg/day Leotrox® vet. 24% injection

Tissue	Elimination rate constant β ($hour^{-1}$)	Half-life $t^{1}/_{2}\beta$ (hours)	Estimated time of TMP concentrations reaching detection limit 0.05 ppm (hours)	AUC∞
Plasma	-0.347	2	9	3 µg h/ml
Lung	-0.640	1	7.6	10 µg h/g
Liver	-0.358	2	11.6	
Kidney	-0.530	1.3	9.7	
Muscle	-0.424	1.6	7.9	
Fat	-0.540	1.3	6.6	

It appears from Table 1 that the concentrations of TMP would reach the detection limit of 0.05 ppm in all examined tissues within 24 hours and that STX would reach the detection limit of 0.1 ppm in all examined tissues after 10 days (Table 2). This is corroborated by the fact that

Table 2

Elimination of sulfatroxazole (STX) after intramuscular administration of 16 mg/kg/day Leotrox® vet. 24% injection

Tissue	Elimination rate constant β ($hour^{-1}$)	Half-life $t^{1}/_{2}\beta$ (hours)	Estimated time of STX concentrations reaching detection limit 0.1 ppm (days)	AUC∞
Plasma	-0.049	14	5.4	1870 µg h/ml
Lung	-0.029	24	6.6	340 µg h/g
Liver	-0.017	41	10.5	
Kidney	-0.024	29	8.6	
Muscle	-0.031	22	6	
Fat	-0.036	19	5	

concentrations of STX and TMP were below 0.1 ppm in all the samples collected at 10, 12, 16 and 17 days after the last Leotrox injection with the exception of the site of the intramuscular injection, where only STX was still detected 12 days post injection (p.i.) (Table 3). Concentrations of N_4-acetyl-STX in all tissue samples examined and

Table 3

Concentrations of sulphatroxazole (STX) in urine and at the site of injection of calves slaughtered 10, 12, 16, 17 days post last injection

Days post last injection	3rd inj. site µg/g	4th inj. site µg/g	5th inj. site µg/g	Urine µg/ml
10	<0.10	<0.10	<0.10	-
12	0.19	0.25	0.18	<0.1
16	<0.10	<0.10	<0.10	-
17	<0.10	<0.10	<0.10	-

- not determined

at the site of injection were below 0.1 ppm in calves slaughtered 10 and 12 days p.i. From day 10 p.i. N_4-acetyl-STX could only be found in the urine collected 12 days after dosing. According to the results of this study STX may be selected as a marker for residues of Leotrox in the tissue of calves.

Mean TMP and STX concentration in plasma and lung tissue after i.m. administration of Leotrox are plotted in Fig. 1 and 2. The plasma ratio of drug concentration of TMP to STX in the first 6 hours ranged from 1:90 to 1:200 and the lung ratio of drug concentration in the first 6 hours ranged from 1:5 to 1:50.

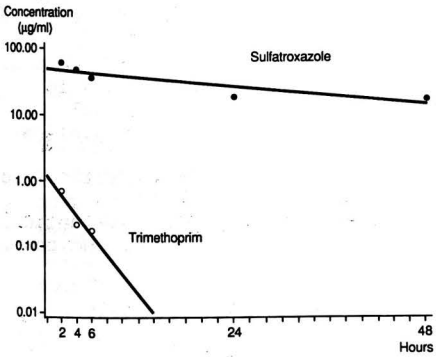

Fig. 1
Semi-logarithmic plot of the mean sulfatroxazole (STX) and trimethoprim (TMP) concentrations (µg/ml) in plasma vs time (h) after last administration of Leotrox 16 mg/kg in calves.

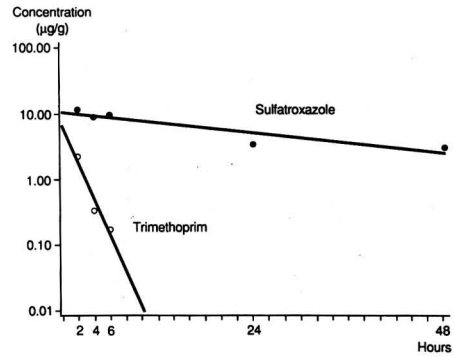

Fig. 2
Semi-logarithmic plot of the mean sulfatroxazole (STX) and trimethoprim (TMP) concentrations (µg/g) in lung tissue vs time (h) after last administration of Leotrox 16 mg/kg in calves.

It appears from the study that lung and plasma concentrations of STX and TMP were ≥ 5 and ≥ 0.05 µg/ml, respectively, for the first 8-10 hours after i.m. administration of Leotrox, and STX only was present in plasma (approx. 17 µg/ml) and lung tissue (approx. 3 µg/g) after 48 hours.

Discussion

The prolonged half-life of STX (20-day-old calves - $t^1/_2\beta$ 14 h) in this study is in agreement with previous findings by Nouws et al. (4). They found a diminished total body clearance of STX in 6-day-old calves ($t^1/_2\beta$ 18.8 h) as compared to the 10-week-old calves ($t^1/_2\beta$ 9.3 h) and cows of 4-5 years ($t^1/_2\beta$ 6.6 h).

The half-life for TMP in calves ($t^1/_2\beta$ 1-2 h) in all examined tissues was generally similar to the half-life in swine (ref. 1). The half-life for STX elimination was generally 2-3 times longer in calves than in swine (ref. 1). Elimination of STX from tissues proved approx. 1.5-3 times slower than that of plasma in calves (Table 2), but elimination of TMP is similar to that of plasma and tissue (Table 1). The high and persisting concentrations of STX in lung tissue indicates its suitability for the treatment of respiratory tract infections in combination with TMP.

The MIC of TMP/STX for *Pasteurella haemolytica* and *Pasteurella multocida* is <0.05/5 µg/ml. Based on this pharmacokinetic study, the concentrations in plasma and lung tissue of TMP/STX will be above MIC values for the first 12 hours after i.m. administration of 16 mg/kg. After approx. 12 hours, subinhibitory concentrations of TMP/STX will contribute to the post-antibiotic effect for a maximum of 2-6 hours.

This pharmacokinetic study indicates that 16 mg/kg bodyweight once daily is sufficient for the treatment of respiratory tract diseases.

Preliminary clinical investigations have confirmed the efficacy of the STX and TMP combination (Leotrox) in the treatment of bacterial pneumonia in calves and pigs at a dosage of 16 mg/kg/day (ref. 2 and 3).

References

1) J. Szancer, J.M. Pott, A-M. Kissmeyer & B. Skov: Study of residues and local tissue damage after intramuscular injection of Leotrox® (Sulfatroxazole/Trimethoprim combination) in pigs. Accepted for publication in the IPVS Proceedings, The Hague 1992.
2) J.M. Pott, A-M. Kissmeyer, C.J. Riley, H.J. Edwards, J. Jacobsen & J. Szancer: Distribution of trimethoprim/sulfatroxazole (Leotrox) in plasma and lung tissue. In vitro activity. Preliminary clinical studies in the treatment of pneumonia in pigs. Accepted for publication in the IPVS Proceedings, The Hague 1992.
3) J.M. Pott, H.J. Edwards & C.J. Riley: Internal Report (Leo Pharmaceutical Products 1991) being prepared for publication.
4) J.F.M. Nouws, T.B. Vree, D. Mevius & M. Degen: Pharmacokinetics, metabolism and renal clearance of sulphatroxazole in calves and cows. J.vet.Pharmacol.Therap. **12**, 50-57, 1989.