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# Comparative Pharmacokinetics of Diminazene in Lactating Goats and Sheep

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*With 2 figures and 3 tables*

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## Summary

The pharmacokinetic aspects of diminazene aceturate were studied in lactating goats and sheep after single intravenous and intramuscular administrations of 3.5 mg/kg b.wt. Plasma and milk concentrations were determined by use of reversed phase high-performance liquid chromatography (HPLC) after ion-pair extraction. Following intravenous injection, the disposition of diminazene in goats and sheep conformed to a two-compartment model with rapid distribution and slower elimination phases. Values of ($t_{1/2\beta}$) were obtained indicating a slower final disappearance of the drug from plasma of sheep (21.17 h) than in goats (16.39 h). Diminazene concentrations were maintained for more than 4 days in the plasma of goats and sheep. In both species of animals, diminazene was rapidly absorbed following intramuscular administration of 3.5 mg/kg b.wt. The peak plasma concentrations ($C_{max}$) were 7.00 and 8.11 $\mu$g/ml and were attained at ($T_{max}$) 0.92 and 1.12 hours in goats and sheep, respectively. The elimination half-life (t1/2el) of diminazene after intramuscular administration was shorter in goats (16.54 h) than in sheep (18.80 h). Systemic bioavailabilities (F%) of diminazene after intramuscular administration were 94.94 % and 82.64 % in goats and sheep, respectively. Diminazene could be detected in milk of goats and sheep within 10 min post-injection. Milk concentrations of the drug were lower in goats than in sheep and were detected for 5 and 6 days following both routes of administration, respectively.

## Introduction

The treatment of livestock diseases caused by protozoans constitutes a major problem in tropical and subtropical regions. African trypanosomiasis, as sleeping fever in man and ngana in cattle, is a parasitic disease of considerable economic and epidemiological importance (WHO, 1979). Numerous clinical investigators have confirmed that diminazene aceturate is highly effective against both trypanosomiasis and babesiasis (KLATT and HAJDU, 1976). Efficacy of diminazene at the recommended single intramuscular dose of 3.5 mg/kg of body weight is widely acknowledged (LEACH and ROBERTS, 1981). Furthermore, diminazene has been proved to be effective in prophylaxis of trypanosomiasis (RAETHER et al., 1972). Recent studies have been documented that *Trypanosoma congolense* infected goats were cured when treated with diminazene one day post-infection (SILAYO et al., 1992; MAMMAN et al., 1993). Resistance to diminazene has been reported after administration of subcurative doses (CLAUSEN et al., 1992; OSMAN et al., 1992). GOODWIN and TIERNEY (1977) suggested that some activity of diminazene is retained for two or three weeks following its intramuscular administration. Little literature is available concerning the pharmacokinetics of diminazene in different animals, (rabbits, GILBERT 1983; cattle, ALIU et al., 1993; goats, MAMMAN et al., 1996). The objective of this study was to determine the plasma and milk concentrations of diminazene in lactating

goats and sheep using reversed-phase HPLC technique. Moreover, to investigate the pharmacokinetic variance and bioavailability of diminazene between both species.

## Materials and Methods

### *Drug*

Diminazene aceturate was generously supplied by Hoechst AG (Frankfurt, Germany).

### *Animals*

*Goats.* Five clinically healthy, non-pregnant Baladi spp. lactating goats; weighing 27–32 kg and aged 2 to 3 years, were used.

*Sheep.* Five clinically healthy, non-pregnant Barki spp. lactating ewes; weighing 30–35 kg and aged 3 to 5 years, were used.

During acclimatization for three weeks and the subsequent treatment period, all animals were fed on a concentrate ration with drinking water freely available.

### *Drug administration and samples collection*

A freshly prepared 7.0 % w/v solution of diminazene aceturate in sterile distilled water was used with each animal, at the recommended dosage of 3.5 mg/kg of body weight. Diminazene aceturate was injected intravenously in each animal of both species through the left jugular vein. One month after the intravenous study, the animals were administered diminazene at the same dose intramuscularly into the left gluteal muscles.

Blood samples were collected from the right jugular vein in heparinized tubes just prior to injection and at 10, 20, 30 min and 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144 and 168 h after intravenous and intramuscular injections. The blood was centrifuged at 1000 ***g*** for 15 min to obtain clear plasma which was kept at −20 °C until assayed.

Milk samples were collected at the same time as the blood samples by hand milking. Complete evacuation of the udder was done after each sampling.

### *Drug analysis*

Concentrations of diminazene in plasma and milk samples were determined by use of reversed phase high-performance liquid chromatography (HPLC) after ion-pair extraction according to GUMMOW et al., 1995.

### *HPLC instrumentation*

A Shimadzu modular HPLC system (Japan) was used. It consisted of a constant-flow solvent delivery pump (Model LC-10 AS) and an injector (Model Rheodyne7161) equipped with 20 $\mu$l loop. Separation of injected compound was achieved by a Nova Pak $C_{18}$ column (10 cm × 5 mm I.D., 10 $\mu$m particle size) fitted with a $C_{18}$ guard column. The column effluent was monitored with a 355 nm UV detector (Model SPD-10 A) operated at $2 \times 10^{-2}$ absorbance units full scale (a.u.f.s.). Area and concentrations of peaks were determined by an on-line integrator (Model C-R6A Chromatopac). Mobile phase was pumped at a rate of 0.8 ml/min. The cartridge (Sep-Pak $C_{18}$) which was used to extract diminazene from plasma and milk was supplied by Alltech Associates Inc.

### *HPLC mobile phase*

The mobile phase used for isocratic elution of diminazene consisted of acetonitrile/0.005 M Na-octane-sulphonate and 0.1 % of triethylamine. The pH was adjusted to 3.2 with acetic acid. All chemicals used were HPLC grade.

### *Sample preparation*

To 1 ml of plasma or milk, 1 ml of methanol was added and mixed on a Vortex mixer. The sample was passed through the Sep-Pak $C_{18}$ cartridge which was pre-washed with 2 ml of methanol and 5 ml of distilled water. After washing, 1 ml of acetonitrile/0.005 M Na-octane-sulphonate and 2 % acetic acid was used to elute diminazene. The solvent was allowed to drip through the cartridge from the syringe without applying pressure, except the last few drops, which were forced through by air applied from the syringe. The effluent was vortexed and 20 $\mu$l were injected into the HPLC system.

*Statistical validation of the method*

The limit of quantitation by this method was 15 ng/ml in plasma and milk. The response of diminazene was linear over the range of concentration between 0.015–5 $\mu$g/ml and the mean correlation coefficient ($r^2$) of the standard curves was 0.998. The recovery from plasma and milk was $93.5 \pm 4.7$ % with a mean variation coefficient of 10 %.

*Pharmacokinetic analysis*

A computerized curve-stripping program (Rstrip, Micromath Scientific Software, Salt Lake City, UT, and USA) was used for data analysis for each animal after administration of diminazene. Following intravenous injection the disposition curve of diminazene which expresses the decline in plasma drug concentration as a function of time was described by a biexponential expression:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

Cp = The concentration of drug in plasma at time t
A = Intercept of the distribution line with the concentration axis expressed in ($\mu$g/ml)
B = Intercept of the elimination line with the concentration axis expressed in ($\mu$g/ml)
$\alpha$ = Distribution rate constant expressed in units of reciprocal time ($h^{-1}$)
$\beta$ = Elimination rate constant expressed in units of reciprocal time ($h^{-1}$)
e = Base of natural logarithm.

Following intramuscular administrations, each individual curve of diminazene vs time was analyzed to determine peak concentration ($C_{max}$), time to peak concentration ($T_{max}$). This program also calculated compartmental analysis by statistical moment theory. Values calculated included elimination half-life ($T_{1/2el}$), area under the curve (AUC) from zero to infinity by the trapezoidal integral, area under the first moment curve (AUMC) and mean residence time (MRT) in plasma. The systemic bioavailability (F), is the fraction of intramuscular dose absorbed and was calculated as $AUC_{i.m.}/AUC_{i.v.} \times 100$.

The results obtained were statistically analyzed by using Student's '*t*' test, according to (SNEDECOR and COCHRAN, 1976).

## Results

Mean plasma diminazene concentrations following single intravenous and intramuscular administrations of 3.5 mg/kg b.wt. are presented in Figs 1 and 2. Values of pharmacokinetic constants for diminazene in lactating goats and sheep are shown in Tables 1 and 2. After intravenous injection, disposition of diminazene in both species conformed to a two-compartment model (Fig. 1). A rapid distribution and slower elimination phases were observed after intravenous injection of diminazene. The mean distribution half-lives ($t_{1/2\alpha}$) of diminazene were 16.20 and 15.57 min in goats and sheep, respectively. Values of ($t_{1/2\beta}$) obtained indicating a slow final disappearance of the drug from plasma of sheep (21.17 h) than in goats 16.39 h). Also, the MRT values after intravenous injection were 23.30 and 29.66 h in goats and sheep, respectively. The volume of the central compartment ($V_c$) was significantly higher in goats (0.191 L/kg) than in sheep (0.132 L/kg). Diminazene concentrations were maintained for more than 4 days in plasma of goats and sheep.

Following intramuscular administration of 3.5 mg/kg b.wt., diminazene was rapidly absorbed. The mean absorption half-life ($t_{1/2ab}$) was slightly shorter in goats (0.13 h) compared to sheep (0.16 h). The peak plasma concentrations ($C_{max}$) were 7.00 and 8.11 $\mu$g/ml and were attained at ($T_{max}$) 0.92 and 1.12 h in goats and sheep, respectively (Fig. 2). The elimination half-life ($t_{1/2el}$) of diminazene after intramuscular administration was shorter in goats (16.54 h) than in sheep (18.80 h). Also, the MRT values were 24.04 and 27.27 h, respectively. Systemic bioavailabilities (F%) of diminazene after intramuscular administration were 94.94 % and 82.64 % in goats and sheep, respectively.

Milk concentrations of diminazene after intravenous and intramuscular injections of 3.5 mg/kg b.wt. in goats and sheep are shown in Table 3. Diminazene was detected in milk of both species within 10 min after injections. Milk concentrations were higher in sheep than those of goats. The mean peak concentration of diminazene in milk was obtained one-hour

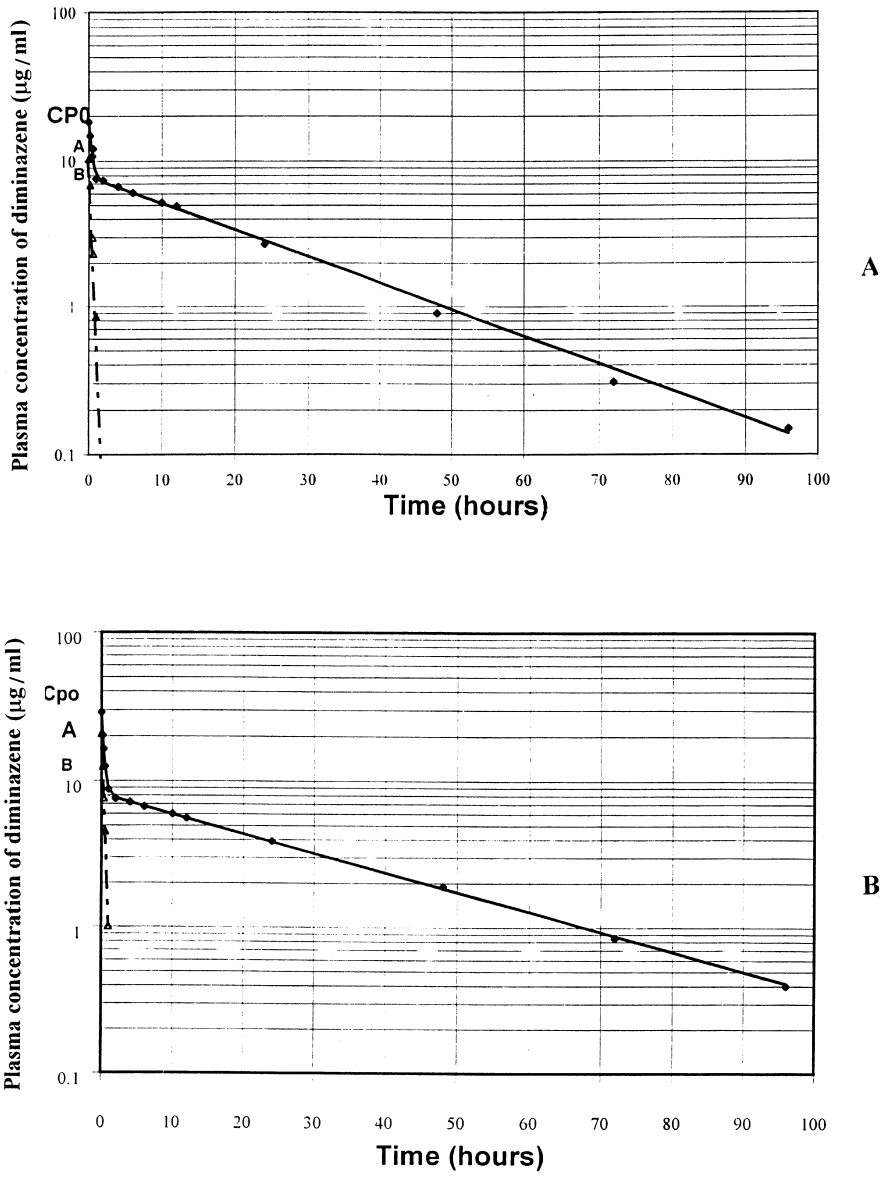


Fig. 1. Semilogarithmic graph depicting the time plasma concentration curve of diminazene following intravenous injection of 3.5 mg/kg b.wt. (n = 5) in (A) Goats and (B) Sheep.

post intravenous and intramuscular injections. The drug was slowly eliminated from milk as it was detected in milk for 5 and 6 days after single injections in goats and sheep, respectively.

## Discussion

This study used the reversed-phase HPLC technique to determine diminazene concentrations in plasma and milk of goats and sheep. This method is specific for the intact

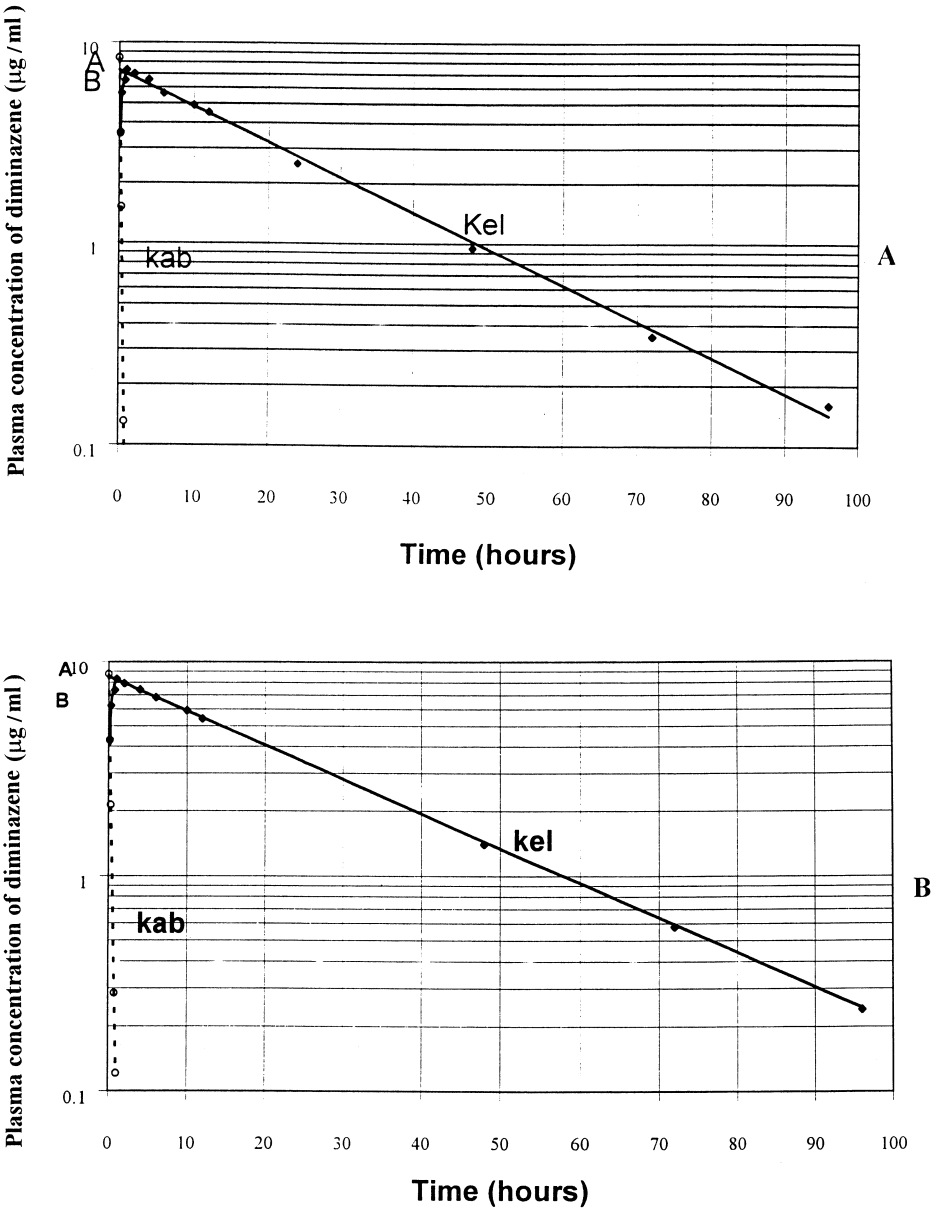


Fig. 2. Semilogarithmic graph depicting the time plasma concentration curve of diminazene following intramuscular injection of 3.5 mg/kg b.wt. (n = 5) in (A) Goats and (B) Sheep.

diminazene molecule and is suitable for its pharmacokinetics evaluation because in animals treated with diminazene there may be closely related metabolites which are interfering with the estimation of the parent compound (ALIU and ODEGAARD, 1983).

The disposition kinetics of intravenous administered diminazene in goats and sheep are very similar to those described in rabbits (GILBERT, 1983), cattle (MAMMAN et al., 1993) and goats (ALIU et al. 1984). A two-compartment open model best described the intravenous data

Table 1. Kinetic parameters of diminazene following intravenous administration of 3.5 mg/kg b.wt. to goats and sheep. (n = 5)

| Parameter | Unit | Goats | Sheep |
|---|---|---|---|
| A | $\mu$g/ml | 10.41 ± 1.05 | 17.56 ± 1.32** |
| $\alpha$ | h | 2.50 ± 0.21 | 2.62 ± 0.17 |
| $T_{1/2\alpha}$ | min | 16.20 ± 0.81 | 15.57 ± 0.92 |
| B | $\mu$g/ml | 7.83 ± 0.57 | 8.93 ± 0.71 |
| $\beta$ | $h^{-1}$ | 0.042 ± 0.003 | 0.033 ± 0.001* |
| $T_{1/2\beta}$ | h | 16.39 ± 1.14 | 21.17 ± 1.66* |
| MRT | h | 23.30 ± 1.69 | 29.66 ± 2.11* |
| $Cp^0$ | $\mu$g/ml | 18.24 ± 1.42 | 26.49 ± 2.87* |
| $V_c$ | L/kg | 0.191 ± 0.011 | 0.132 ± 0.010** |
| $K_{21}$ | $h^{-1}$ | 1.08 ± 0.014 | 0.89 ± 0.017*** |
| $K_{el}$ | $h^{-1}$ | 0.092 ± 0.007 | 0.095 ± 0.001 |
| $K_{12}$ | $h^{-1}$ | 1.37 ± 0.070 | 1.67 ± 0.10* |
| $V_{d(area)}$ | L/kg | 0.42 ± 0.022 | 0.38 ± 0.021 |
| $V_{d(SS)}$ | L/kg | 0.43 ± 0.023 | 0.36 ± 0.023 |
| $CI_B$ | L/h/kg | 0.017 ± 0.001 | 0.012 ± 0.001 |
| AUC | $\mu$g/ml/h | 190.54 ± 8.74 | 276.50 ± 10.57*** |
| AUMC | $\mu$g/ml/h | 4440.44 ± 251.87 | 8202.74 ± 326.39*** |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

Table 2. Kinetic parameters of diminazene following intramuscular administration of 3.5 mg/kg b.wt. to goats and sheep. (n = 5)

| Parameter | Unit | Goats | Sheep |
|---|---|---|---|
| A | $\mu$g/ml | 8.41 ± 0.72 | 8.74 ± 0.65 |
| $K_{ab}$ | $h^{-1}$ | 5.20 ± 0.27 | 4.28 ± 0.21* |
| $T_{1/2ab}$ | h | 0.13 ± 0.007 | 0.16 ± 0.011* |
| B | $\mu$g/ml | 7.31 ± 0.62 | 8.53 ± 0.81 |
| $K_{el}$ | $h^{-1}$ | 0.041 ± 0.001 | 0.037 ± 0.001* |
| $T_{1/2el}$ | h | 16.54 ± 1.12 | 18.80 ± 0.95 |
| MRT | h | 24.04 ± 2.68 | 27.27 ± 3.41 |
| $C_{max}$ | $\mu$g/ml | 7.00 ± 0.55 | 8.11 ± 0.74 |
| $T_{max}$ | h | 0.92 ± 0.081 | 1.12 ± 0.101 |
| AUC | $\mu$g/ml/h | 80.90 ± 11.25 | 28.50 ± 15.57* |
| AUMC | $\mu$g/ml/h | 4348.91 ± 192.46 | 6231.30 ± 155.28*** |
| F | % | 94.94 ± 4.04 | 82.64 ± 3.45* |

* Significant at $P \leqslant 0.05$.
*** Significant at $P \leqslant 0.001$.

in those studies. The mean distribution half-lives ($t_{1/2\alpha}$) of diminazene were 16.20 and 15.57 min in goats and sheep, respectively. Values of ($t_{1/2\beta}$) obtained indicating a slow final disappearance of the drug from plasma of sheep (21.17 h) than in goats (16.39 h). The volume of the central compartment ($V_c$) was significantly higher in goats (0.191 L/kg) than in sheep (0.132 L/kg). These values were lower than (0.57 L/kg) reported by MAMMAN et al. (1996) in goats. The difference found may be due mainly to the use of lactating animals in our study. The volume of distribution at steady state of a drug $V_{d(ss)}$ is an indication of its diffusion in body tissues

Table 3. Mean ± SEM (μg/ml) of diminazene in milk of lactating goats and sheep after single intravenous and intramuscular injections of 3.5 mg/kg b.wt. (n = 5)

| | Concentration of diminazene (μg/ml) in milk | | | |
|---|---|---|---|---|
| | Goats | | Sheep | |
| Time | i.v. | i.m. | i.v. | i.m. |
| 10 min | 0.640 ± 0.015 | 0.470 ± 0.016 | 1.050 ± 0.021 | 1.200 ± 0.017 |
| 20 min | 0.780 ± 0.017 | 0.620 ± 0.021 | 1.770 ± 0.044 | 1.690 ± 0.032 |
| 30 min | 2.400 ± 0.510 | 1.210 ± 0.320 | 3.250 ± 0.310 | 2.480 ± 0.290 |
| 1 h | 3.800 ± 0.320 | 2.070 ± 0.220 | 4.880 ± 0.241 | 4.140 ± 0.263 |
| 2 h | 3.500 ± 0.370 | 1.950 ± 0.270 | 4.505 ± 0.167 | 3.880 ± 0.251 |
| 4 h | 3.250 ± 0.610 | 1.850 ± 0.410 | 4.113 ± 0.304 | 3.640 ± 0.149 |
| 6 h | 2.990 ± 0.410 | 1.720 ± 0.300 | 3.820 ± 0.243 | 3.350 ± 0.441 |
| 8 h | 2.750 ± 0.290 | 1.550 ± 0.250 | 3.500 ± 0.211 | 3.050 ± 0.317 |
| 10 h | 2.500 ± 0.340 | 1.450 ± 0.310 | 3.100 ± 0.243 | 2.800 ± 0.211 |
| 12 h | 2.200 ± 0.210 | 0.860 ± 0.140 | 2.500 ± 0.261 | 2.100 ± 0.234 |
| 24 h | 1.350 ± 0.350 | 0.480 ± 0.120 | 1.950 ± 0.111 | 1.750 ± 0.129 |
| 48 h | 0.480 ± 0.090 | 0.210 ± 0.100 | 0.684 ± 0.053 | 0.580 ± 0.043 |
| 72 h | 0.160 ± 0.013 | 0.140 ± 0.011 | 0.260 ± 0.028 | 0.220 ± 0.020 |
| 96 h | 0.050 ± 0.001 | 0.035 ± 0.002 | 0.150 ± 0.010 | 0.130 ± 0.009 |
| 120 h | 0.025 ± 0.001 | 0.015 ± 0.001 | 0.075 ± 0.002 | 0.060 ± 0.002 |
| 144 h | – | – | 0.015 ± 0.001 | 0.015 ± 0.001 |
| 168 h | – | – | – | – |

– Not detected.

(GILMAN et al., 1980). Diminazene showed relatively low volume of distribution $V_{d(ss)}$ (0.43 and 0.36 L/kg) in goats and sheep, respectively, indicating that the drug is less extensively distributed in extravascular tissues (BAGGOT, 1983). The clearance rate was faster in goats (0.017 L/h/kg) than in sheep (0.012 L/h/kg). Extensive binding of diminazene to plasma proteins may have caused the relatively slow $Cl_B$ and low volume of distribution in both species. Similarly, suramin (antitrypanosomal drug) has limited extravascular distribution because of extensive binding to plasma proteins, which accounts for its persistence for at least 3 months after a single dose (GILMAN et al., 1980). Diminazene plasma concentrations were maintained for more than 4 days after both intravenous and intramuscular injections in both species of animals. In this respect, NEWTON and GILBERT (1982) found that diminazene aceturate at 3.5 mg/kg b.wt. reached a peak concentration within 30 min, then fell rapidly over the next 5 h and remained at a significant level for 4–6 days. The concentration of diminazene required to trypanocidal activity in body fluids is unknown (GILBERT, 1983). But measurements by GOODWIN and TIERNEY (1977) of the dilution of a known concentration of the drug required to kill *Trypanosoma brucei* in culture medium at 35°C indicated that 2 ng/ml of diminazene was trypanocidal in 24 h incubation. Primary concentrations detected throughout the current work were considerably higher than this value in blood of goats and sheep.

After intramuscular administration, diminazene was rapidly absorbed in goats ($t_{1/2ab}$; 0.13 h) than in sheep ($t_{1/2ab}$; 0.16 h). Peak plasma concentration was reached rapidly when diminazene was administered intramuscularly at a dose of 3.5 mg/kg with ($C_{max}$) of 7.00 and 8.11 μg/ml in goats and sheep, respectively. These values were higher than these observed in cattle; 4.76 μg/ml (MAMMAN et al., 1993). In this study, the $T_{max}$ (0.92 and 1.12 h in goats and sheep, respectively) were occurred longer than those in rabbits; (15 min; GILBERT, 1983) and in (cattle 36 min; MAMMAN et al., 1993). The elimination half-life ($t_{1/2el}$) of diminazene after intramuscular administration was shorter in goats (16.54 h) than in sheep (18.80 h). Systemic bioavailabilities (F%) of diminazene after intramuscular administration were 94.94 % and 82.64 % in goats and

sheep, respectively indicating that the drug is well absorbed from this route of administration in goats more than sheep. The detection of diminazene in milk within 10 min after intravenous and intramuscular injections was not surprising since earlier work with tylosin, an organic base, suggested that tylosin passes readily into milk by nonionic passive diffusion (GINGERICH et al., 1977). Diminazene (organic base) is non-ionized at plasma of pH 7.4 and ionized at the ovine milk of pH 6.5. Since the non-ionized fraction is diffusible; passive diffusion from plasma to milk is favoured over diffusion from milk to plasma. A similar mechanism was proposed for the persistence of spiramycin and tilmicosin other macrolide antibiotics in milk (NOUWS and ZIV, 1980) and dry udder secretion (ZIV et al., 1995). Diminazene milk concentrations were higher in sheep than those of goats. The drug was slowly eliminated from milk as it was detected in milk for 5 and 6 days after single injection in goats and sheep, respectively. So, the milk from diminazene treated animals should be discarded for at least one week post-treatment to ensure that the drug is completely eliminated.

In conclusion, there is a great similarity between the pharmacokinetic behaviour of diminazene in lactating goats and sheep.

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