academicJournals

Vol. 7(15), pp. 801-808, 22 April, 2013
DOI 10.5897/AJPP2013.3521
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Effect of dose on disposition kinetics of isometamidium chloride/hydrochloride in trypanosomiasis induced calves

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Accepted 9 April, 2013

Effect of dose on disposition kinetics of isometamidium (ISMM) in Indian buffalo calves was studied in view of limited applications of the drug in India. Buffalo calves were pre grouped and trypanosomiasis was induced using *Trypanosoma evansi.* ISMM was administered intravenously at 0.25, 0.5 and 1 mg/kg (body weight) to the grouped animals. Blood samples were collected at 0.08, 0.16, 0.33, 0.66, 1, 2, 4, 6, 8, 12, 24 and 36 h post drug administration for disposition kinetics. ISMM chloride/hydrochloride in plasma was estimated by trial and error method using high performance liquid chromatography (HPLC). The time to reach the maximum plasma concentration (Tmax), was 0.08 h for all the doses. The maximum plasma concentration (Cmax) declined rapidly and the drug could not be detected in plasma samples collected beyond 8 h post dose of 0.25 mg/kg and 24 h post dose of the other two doses, that is, 0.5 and 1 mg/kg. The distribution rate constant (α) in groups I (0.25 mg/kg), II (0.5 mg/kg) and III (1 mg/kg) were 4.77 ± 1.54, 7.44 ± 0.55 and 6.91 ± 2.57 h^{-1}, respectively, while t½ β values were 2.01 ± 0.16, 3.09 ± 0.30 and 2.46 ± 0.16 h, respectively. The apparent volume of distribution like Vd_{area}, Vd_c and Vd_B were 0.50 ± 0.01, 0.69 ± 0.06 and 0.03 ± 0.005 L/ kg in group I; 0.35 ± 0.02, 1.98 ± 0.02 and 0.07 ± 0.005 L/kg in group II; 0.26 ± 0.01, 3.88 ± 0.49 and 0.035 ± 0.003 L/kg in group III, respectively. The value of K_{12} and K_{21} were 1.97 ± 0.83 and 0.67 ± 0.07 h^{-1} in group I; 4.92 ± 0.45 and 1.60 ± 0.07 h^{-1} in group II; 4.12 ± 0.21 and 0.89 ± 0.39 h^{-1} in group III, respectively. It was concluded that ISMM follows dose independent kinetics after intravenous administration in buffalo calves.

Key words: Disposition kinetics, isometamidium chloride/hydrochloride, trypanosomiasis, intravenous, calves.

INTRODUCTION

"Trypanosomiasis," is one of the most widely distributed pathogenic, mechanically transmitted vector borne haemoprotozoan (*Trypanosoma evansi)* disease of domestic and wild animals in India (Juyal, 2011). It leads to severe anaemia, oedema, immunosuppression and various neurological disorders causing huge economic losses to the farmers in terms of morbidity, mortality, abortion, infertility, reduced milk yield, etc (Juyal, 2011). The economic losses due to disease are underestimated in cattle and buffaloes mainly because of its sub-clinical nature. Chemotherapy and chemoprophylaxis has been the mainstay of control in domestic animals in India. Currently, drugs in practice, in India include diaminazene aceturate (berenil), quinapyramine sulphate and chloride (Triquin, Antrycide Prosalt) and quinapyramine sulphate (Triquin-S and Antrycide) for treatment and prophylactic use against trypanosomiasis in domestic animals (Juyal, 2011). However, this approach has also been associated

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with major problems such as high price of drugs, availability of few drugs and the development of drug resistance.

Isometamidium (ISMM) chloride/hydrochloride, synthesized by combining diazotized Para–Amino Benzamide moiety of diaminazene with homidium in the presence of sodium acetate (Kinabo et al., 1988) has been used for chemotherapy and chemoprophylaxis of disease in cattle, sheep, and goats under conditions of natural tsetse challenge for more than 35 years (Geerts and Holmes, 1998; Anene et al., 2001) in countries other than India. Mode of action of ISMM is not fully understood, but evidence is there that kinetoplastic topoisomerase type II of trypanosoma is selectively inhibited by the drug (Kaminsky et al., 1997; Boibessot et al., 2002; Mehlhorn, 2008). The claimed duration of protection afforded by ISMM is as long as 5 months (Whitelaw et al., 1991; Mehlhorn, 2008). Use of ISMM as remedial measure against trypanosomiasis has been cited (Magona et al., 2004; Delespaux and de Koning, 2010; Karaye, 2012) and disposition kinetics have been studied in cow (Dowler et al., 1989; Moloo and Kutuza, 1990), goats and sheep (Braide and Eghianruwa, 1980; Wesongah et al., 2000) and camel (Ali and Hassan, 1984). To determine the dosage regimen in buffalo and thereby its use against trypanosomiasis in buffalo, disposition kinetics of administered ISMM is essentially required. Keeping these points in view, the present study was undertaken to determine the disposition kinetics of intravenously administered ISMM in buffalo calves.

MATERIALS AND METHODS

Test drug and chemicals

ISMM chloride/hydrochloride, a trypanocidal drug (technical grade) was procured from M/S Alembic Ltd., Veterinary Division, Mumbai (India). The purity of the compound was >90%. All the other chemicals used in this experiment were obtained from E. Merck (India) and Sigma Chemicals Co. (USA).

Experimental animals

Clinically healthy calves (6 months age) weighing between 70 and 90 kg were used in this experiment. They were kept in animal room of clinical complex at Mohanpur campus. The animals were stall fed and were also allowed to graze. Water was provided *ad-libitum*. The composition of feed was 3 parts paddy straw, 1 part mustard cake and 1 part wheat husk. Before starting the experiment, the animals were dewormed with Fluzan® (suspension of Oxyclozanide 3% w/v and levamisole hydro-chloride, 1.5% w/v) at 10 mg/kg body weight. Animals were also dewormed against cestode with Cestophane® (Dichlorphan) at 0.5 g/2.5 kg body weight orally after 7 days of Fluzan® administration. After 21 days of deworming, the animals were acclimatized in experimental environment for 7 days.

Experimental grouping of animals

Eighteen calves of both sexes were grouped into 3, each containing 6 animals. ISMM was administered as a single intravenous dose at 0.25, 0.5 and 1 mg/kg (as 1% solution in normal saline) to animals of Groups I, II and III respectively.

Induction of infection (trypanosomiasis)

Strain of *Trypanosoma evansi* was brought from Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, U.P and the strain was serially passaged in mice in the Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Science. About 1 ml of heart blood was taken from mice and was mixed with equal volume of Alsevers solution. The mixed solution (2 ml) was administered in the calves subcutaneously.

Confirmation of trypanosomiasis

Blood (0.2 ml) from each infected calf was injected in mice intraperitoneally. Wet blood film of mice was examined at every 12 h interval under microscope and Trypanosoma was visible in the film after 48 h post induction.

Development of signs and symptoms

After 28 days of induction of infection, different signs and symptoms were observed in calves like posterior paresis, rise in temperature (103.6 to 104.8˚F), anorexia, dullness and depression, urticarial patches with hemorrhage in some calves and staggering gait.

Collection of blood samples

Blood samples were collected from jugular vein of calves in Groups I, II and III in test tubes containing ethylenediaminetetraacetic acid (EDTA; 99.5%) at 0.08, 0.16, 0.33, 0.66, 1, 2, 4, 6, 8, 12, 24 and 36 h. About 4 ml of blood was collected at the aforementioned time period. Plasma was then separated by centrifugation at 3000 rpm for 20 min. One milliliter of plasma was utilized for the analysis of ISMM concentration.

Analysis of ISMM in blood

Analysis of ISMM in blood method was developed by trial and error. About 3 ml of blood was collected from the jugular vein of the calf and was centrifuged at 3000 rpm for 20 min. Plasma (1 ml) was separated and 3 ml of acetonitrile was added to it and was well mixed. Then, the test tubes were allowed to centrifuge at 3500 rpm for 20 min. The supernatant was collected to another test tube. Acetonitrile (3 ml) was added in the test tubes containing sediment and was shaken well. Then, the test tubes were allowed to centrifuge at 3500 rpm for 20 min. The supernatant was collected and was added in the first supernatant. The aforementioned process was repeated for 5 times for complete extraction of the drug. The test tube containing supernatants were completely dried using vacuum evaporator at 40°C and the dried residue was dissolved in small quantity of high performance liquid chromatography (HPLC) grade water and final volume was made to 1 ml for subsequent analysis by HPLC.

Apparatus

SHIMADZU LC-20AT liquid chromatography coupled with diode-array detector (UV/V) attached with computer SPDMXA 10 software were used.

Condition of HPLC

Mobile phase: Phosphate buffer: Acetonitrile (77:23) (This mixture was subjected to membrane filtration); column: 5 µ Luna C_{18} (2), 125 × 4 mm LiChrospher 60 (RP); flow rate: 1 ml/min; wavelength: 320 nm; injection: (25 µl loop with Hamilton syringe). Standard and samples (20 µl) were injected into the injector part of liquid chromatography with the first and last being the standard.

Calibration

A stock solution of 100 µg/ml of technical grade of ISMM was prepared in 70 ml HPLC grade water and 30 ml HPLC grade acetonitrile.

Buffer

For buffer, 2.7 g of potassium dihydrogen phosphate was dissolved in 950 ml of distilled water, pH was adjusted to 2.5 with orthophosphoric acid and volume was made to 1000 ml with distilled water. Three peaks were observed with retention time (RT) of ISMM at 5.81, 7.09 and 8.22 min, respectively. The ISMM concentration in blood was calculated using following equation:

$$\text{Concentration of ISMM in blood (µg/ml)} = \frac{a_2 \times v_2 \times c}{a_1 \times v_1}$$

where a_1= area of standard chromatogram, a_2= area of sample chromatogram, v_2= final volume of sample (ml) v_1= volume of blood taken (ml), and c = concentration of standard (ppm).

Recovery of ISMM from plasma

Recovery of ISMM from calf plasma was carried out *in vitro* to ascertain the reliability of analytical method after fortifying with 5, 10, 20, 50 and 100 µg/ml of ISMM in plasma. The limit of detection for ISMM was below 0.1 ppm. The linearity for different concentrations of ISMM was plotted on graph paper and linearity was found to be maintained. The recovery was 85 to 92% and therefore, analytical method was considered ideal for estimation of ISMM in this experiment.

Pharmacokinetic parameters

Pharmacokinetic parameters of ISMM were determined from computerized curve fitting programme 'PHARMKIT' supplied by the Department of Pharmacology, JIPMER, Pondicherry, India as the following.

1) A, B: Zero time blood ISMM concentrations intercepts of biphasic intravenous disposition curve. The co-efficient A is the point of intercept of regression line of distribution phase and coefficient B is based on the terminal elimination phase. These are expressed in µg/ml.
2) C°p: The theoretical zero time plasma ISMM concentration: C°p (µg/ml) = A + B
3) α and β: The hybrid rate constants of disposition curve. Values of α and β are related to the slope of distribution and elimination curve. These rate constants are obtained from the terminal slope of semi-logarithmic plot of blood ISMM concentrations versus time and are expressed as h^{-1} $t_{½}$ α and $t_{1/2}$ β, the half-lives of the ISMM in distribution and elimination phase, respectively. They are expressed in hour (Baggot, 1977).

$$t_{1/2}\,\alpha = \frac{0.693}{\alpha}$$

$$t_{1/2}\,\beta = \frac{0.693}{\beta}$$

4) K_{12}: The first order rate constant for transfer of ISMM from central to peripheral compartment.
5) K_{21}: The first order rate constant for transfer of ISMM from peripheral to central compartment. Rate constants are expressed as per hour (h^{-1}).
6) K_{el}: First order rate constant for drug elimination from the central compartment.

$$K_{21} = \frac{A\beta + B\alpha}{A + B}$$

$$K_{12} = \alpha + \beta - K_{21} - Kel$$

$$Kel = \frac{\alpha\beta}{K_{21}}$$

7) Vd_c: The apparent volume of distribution of ISMM in central compartment is expressed as L/kg,
where D is the dose (mg/kg).

$$Vd_c = \frac{D}{A+B}$$

8) Vd_{area}: The apparent volume of ISMM distribution based on total area under blood concentration versus time area (area method) is expressed as L/kg where D is the dose (mg/kg).

$$Vd_{area} = \frac{D}{Area \times \beta}$$

$$= \frac{D}{(A/\alpha + B/\beta) \times \beta}$$

(Baggot, 1977)

$$= \frac{D}{AUC \times \beta}$$

9) Vd_B: The apparent volume of drug distribution obtained by avoiding the distribution phase of drug distribution and is expressed as L/kg.

$$Vd_B = \frac{D}{B}$$

10) V_{dss}: Apparent volume of distribution at steady state is expressed as L/kg.

$$Vdss = (K_{12} + K_{21}) \times \frac{Vd_c}{K_{21}}$$

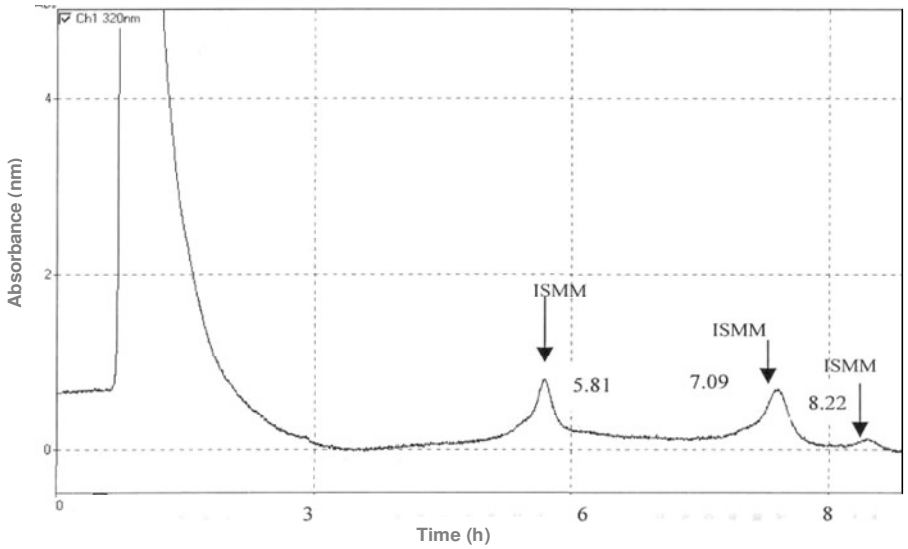

Figure 1. Chromatogram of ISMM chloride/hydrochloride, standard (100 ppm).

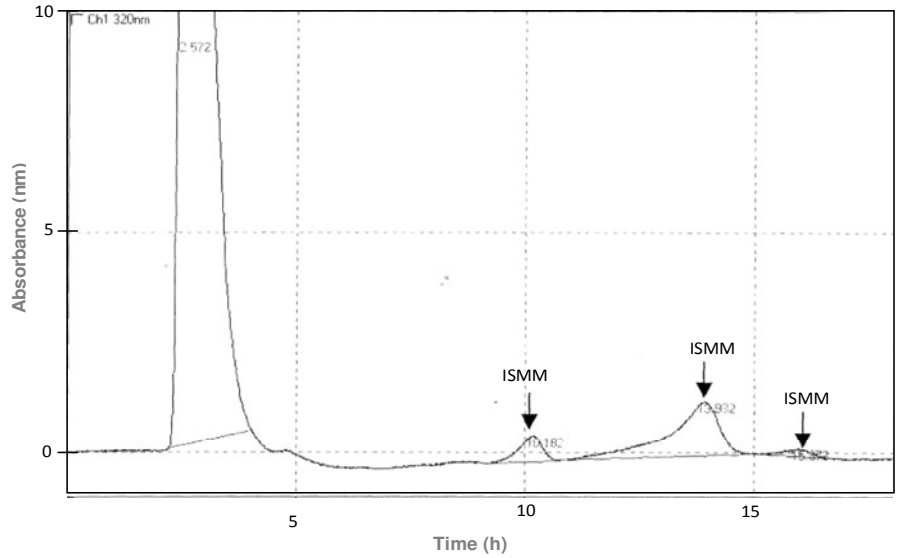

Figure 2. Chromatogram of Isometamidium chloride/hydrochloride recovered from plasma fortified with 100 ppm.

11) AUC: The total area under the blood ISMM concentration versus time curve from 't_0' to 't' after administration. The unit of measurement is µg/h/ml (for two compartment).

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

12) Cl_B: The total body clearance of ISMM representing the sum of all clearance process in the body and is expressed as $Lkg^{-1}h^{-1}$.

$$Cl_B = Vd_{area} \times \beta$$

Statistical analysis of data

Analysis of variance (ANOVA) using SPSS (10) was used for the analysis of data where applicable.

RESULTS

Recovery experiment

The chromatogram of HPLC, showed three peaks of ISMM and the retention time (RT) was found to be 5.81, 7.09 and 8.22 min under the operating conditions as described earlier (Figure 1). The recovery of ISMM from plasma varied from 85 to 92% (Figure 2). The sensitivity was found to be below 0.1 ppm.

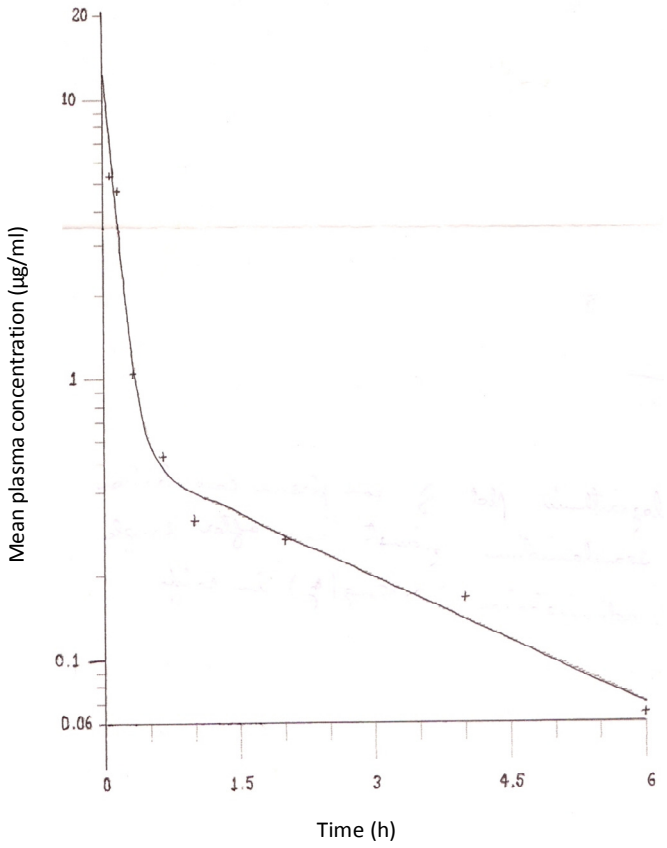

Figure 3. Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 0.25 mg/kg body weight in calf.

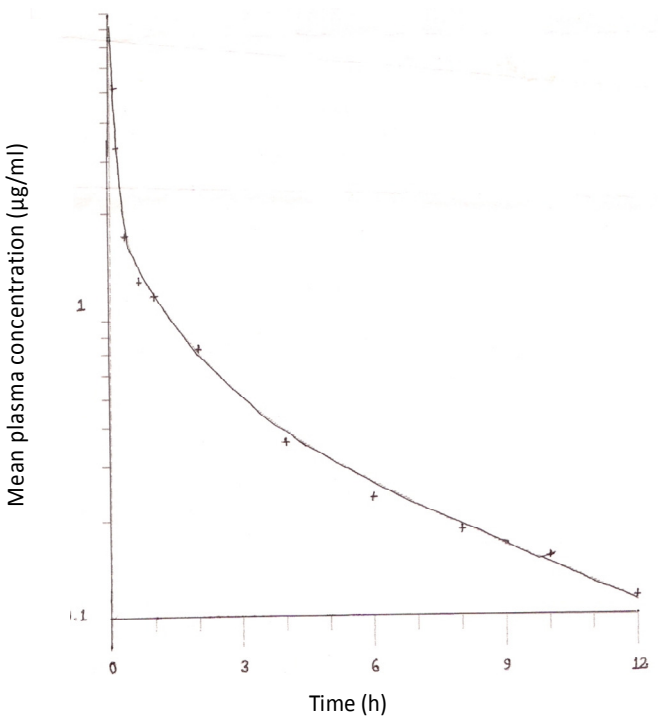

Figure 4. Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 0.5 mg/kg body weight calf.

Plasma level of ISMM chloride/hydrochloride

Mean values with standard error of plasma concentration of ISMM in calves at different time intervals after single dose intravenous administration at 0.25, 0.5 and 1 mg/kg are presented in Table 1 and Figures 3, 4 and 5. Maximum plasma concentration of ISMM at 0.25 mg/kg was 5.28 ± 0.14 µg/ml at 0.08 h and the minimum plasma concentration of 0.07 ± 0.008 µg/ml was recorded at 6 h post dosing (pd) (Figure 3). ISMM could not be detected in plasma collected beyond 8 h at the dose of 0.25 mg/kg. The highest plasma concentration of ISMM at 0.5 mg/kg dose level was 9.85 ± 0.15 µg/ml at 0.08 h and the lowest plasma concentration of 0.10 ± 0.01 µg/ml was found at 12 h pd (Figure 4). ISMM could not be detected in plasma collected at 24 h. Maximum plasma concentration of ISMM at 1 mg/kg was 19.92 ± 1.17 µg/ml at 0.08 h and minimum plasma concentration of 0.09 ± 0.01 µg/ml was at 12 h pd. No drug in plasma could be detected at 24 h (Figure 5).

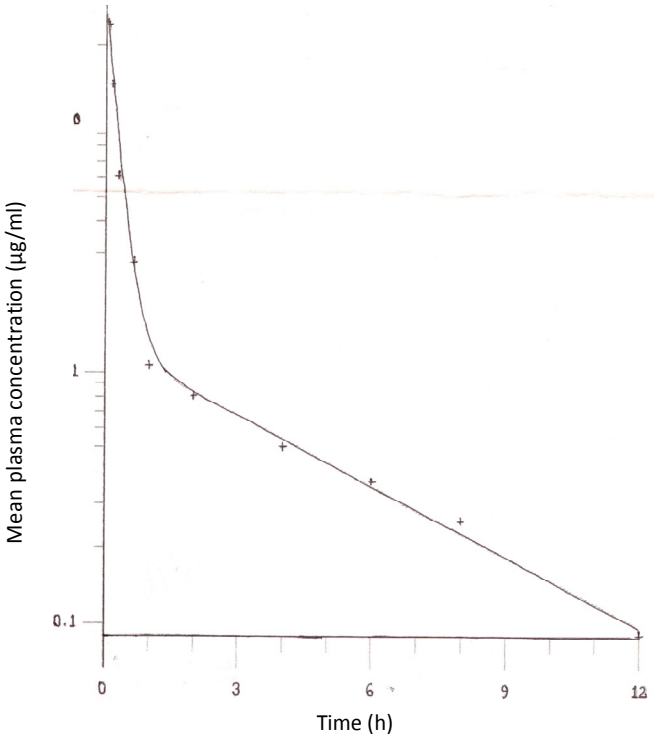

Figure 5. Semilogarithmic plot of mean plasma concentration of ISMM against time after single intravenous administration at the dose rate of 1 mg/kg body weight in calf.

Kinetic profile

Semilogarithmic plot of mean plasma level time profile of

Table 1. Plasma concentration of isometamidium (µg/ml) following single dose intravenous administration in calves at 3 dose levels (Mean of 6 replicates with SE).

Time (h)	Mean plasma concentration (µg/ml)		
	Group I	Group II	Group III
0.08	$5.28^{a} \pm 0.14$	$9.85^{a} \pm 0.15$	$19.92^{b} \pm 1.17$
0.16	$4.66^{a} \pm 0.16$	$5.36^{a} \pm 0.14$	$11.91^{b} \pm 0.95$
0.33	$2.21^{a} \pm 0.23$	$3.90^{a} \pm 0.09$	$6.41^{b} \pm 0.27$
0.66	$0.88^{a} \pm 0.23$	$1.30^{a} \pm 0.07$	$3.06^{b} \pm 0.14$
1	$0.40^{a} \pm 0.04$	$1.05^{b} \pm 0.06$	$1.94^{b} \pm 0.24$
2	$0.27^{a} \pm 0.01$	$0.82^{b} \pm 0.04$	$1.27^{b} \pm 0.17$
4	$0.18^{a} \pm 0.02$	$0.43^{b} \pm 0.02$	$0.72^{b} \pm 0.10$
6	$0.07^{a} \pm 0.008$	$0.27^{b} \pm 0.02$	$0.51^{b} \pm 0.09$
8	BDL	0.18 ± 0.01	0.26 ± 0.02
12	BDL	0.10 ± 0.01	0.09 ± 0.01
24	BDL	BDL	BDL

Mean value with dissimilar superscript in a row vary significantly ($P < 0.05$) and denoted as superscript a,b. BDL: Below detection limit; SE: standard error.

Table 2. Pharmacokinetic parameters of Isometamidium following single dose intravenous administration in calves at 3 dose levels (Mean of 6 replicates with SE).

Kinetic parameter	Group I	Group II	Group III
C°p (µg/ml)	$5.24^{a} \pm 0.83$	$7.27^{a} \pm 0.38$	$29.18^{b} \pm 2.97$
α (h^{-1})	4.77 ± 1.54	7.44 ± 0.55	6.91 ± 2.57
β (h^{-1})	0.34 ± 0.01	0.23 ± 0.02	0.28 ± 0.01
t ½ α (h)	0.35 ± 0.15	0.11 ± 0.01	0.11 ± 0.02
t ½ β (h)	2.01 ± 0.16	3.09 ± 0.30	2.46 ± 0.16
AUC (µg/h/ml)	$3.30^{a} \pm 0.16$	$6.16^{b} \pm 0.3$	$13.89^{c} \pm 0.84$
Cl_B (L/kg/h)	$14 \times 10^{-4} \pm 1 \times 10^{-4}$	$15 \times 10^{-4} \pm 1 \times 10^{-4}$	$14 \times 10^{-4} \pm 1 \times 10^{-4}$
Vd area (L/kg)	0.5 ± 0.01	0.46 ± 0.02	0.35 ± 0.01
Vd_B (L/kg)	$0.69^{a} \pm 0.06$	$1.98^{b} \pm 0.02$	$3.88^{c} \pm 0.49$
Vd_C (L/kg)	0.03 ± 0.005	0.07 ± 0.005	0.04 ± 0.003
Kel (h^{-1})	2.52 ± 0.69	1.17 ± 0.03	2.17 ± 0.19
K_{12} (h^{-1})	$1.97^{a} \pm 0.83$	$4.92^{b} \pm 0.45$	$4.12^{b} \pm 2.11$
K_{21} (h^{-1})	$0.67^{a} \pm 0.07$	$1.60^{b} \pm 0.07$	$0.89^{a} \pm 0.39$
C max (cal) (µg/ml)	$6.97^{a} \pm 0.82$	$7.28^{a} \pm 0.54$	$29.18^{b} \pm 2.97$
f_C	$0.18 \pm .04$	0.21 ± 0.01	0.12 ± 0.02
T~P	$5.88^{a} \pm 0.24$	$3.64^{b} \pm 0.15$	$6.44^{a} \pm 0.69$

Mean value with dissimilar superscript in a row vary significantly ($P < 0.05$) and denoted as superscript a,b, c. Group I – at dose rate of 0.25 mg/kg body weight , Group II – at dose rate of 0.5 mg/kg body weight, Group III – at dose rate of 1 mg/kg body weight.

ISMM obtained from computerized pharmacokinetic programme "PHARMKIT" and disposition kinetic parameters of ISMM in calves following single dose intravenous administration at 0.25, 0.5 and 1 mg/kg have been depicted in Table 2. It was observed that plasma concentration of ISMM was maximum at 0.08 h which rapidly declined till 1 h and then gradually decreased in concentration till 6 h (0.25 mg/kg) and 12 h (0.5 and 1 mg/kg).

Mean value with standard error of C°p (The theoretical zero time plasma ISMM concentration), t½ α (half life of ISMM in distribution phase), t½ β(half life of ISMM in elimination phase), β (hybrid rate constants of elimination phase), α (hybrid rate constant of disposition phase), AUC (total area under the blood ISMM concentration versus time curve), Cl_B (total body clearance of plasma), Vd_{area} (apparent volume of ISMM distribution based on total area under blood concentration versus time area), C_{max} cal, Vd_B (apparent volume of drug distribution obtained by avoiding the distribution phase of drug distribution), Vd_c (apparent volume of distribution of ISMM

in central compartment), K_{el} (first order rate constant for drug elimination from the central compartment), K_{12} (first order rate constant for transfer of ISMM from central to peripheral compartment), K_{21} (first order rate constant for transfer of ISMM from peripheral to central compartment), f_c (fraction of drug in the body that is contained in the central compartment), T~P (tissue to plasma ratio) at 0.25, 0.5 and 1 mg/kg dose level are presented in Table 2.

The data presented in Table 2 showed that the mean values of C°p, Cmax, AUC and Vd_B were significantly ($P < 0.05$) greater in Group III animals as compared to animals of Groups I and II. The values for Vd_{area} and Vd_C at three dose levels were comparable among themselves, whereas V_{dB} values increased significantly with increase in dose level. There was insignificant difference in Kel values at three dose levels. The value for K_{12} and K_{21} were the highest at 0.5 mg/kg body weight, significantly higher than the values at 0.25 mg/kg body weight, but comparable to the values at 1 mg/kg body weight, Cmax was significantly higher at 1 mg/kg body weight than the other two which were comparable between themselves. T~P value was lower at 0.5 mg/kg body weight than the other two which shares a comparable values in between them.

DISCUSSION

There have been much studies on the distribution and pharmacokinetics of ISMM in several animal species, including rats, mice, dogs, monkeys, goats, pigs, camels and cattle (Hill and McFadzean, 1963; Philips et al., 1967; Braide and Eghianruwa, 1980; Kinabo and Bogan, 1988a, b; Kinabo et al., 1991; Eisler, 1996; Murilla et al., 1996; Wesongaha et al., 2004). In our recovery experiment study, the retention time showed a linear trend with dose and recover value ranged between 85 and 925 much higher than usual standard of 80% indicative of standard operating conditions and satisfactory recovery level. Mean plasma concentration of ISMM in animals of Group III at 0.08, 0.16, 0.33 and 0.66 h were significantly ($P < 0.05$) higher in comparison to Groups I and II, whereas at 1, 2, 4 and 6 h, mean values of ISMM in Groups II and III were higher significantly ($P < 0.05$) as compared to Group I. Plasma concentration of ISMM was increased in all the three groups with enhanced doses at different times except at 0.08, 0.16 and 0.33 h in animals of Group II suggesting dose dependent increase in drug concentration in calves. Similar dose dependent increase in concentration was also reported by Ardelli and Woo (2001) and Eze et al. (2012) in aquatic animals. The plasma concentration of ISMM falls to below detection level within 24 h at all the dose level, indicates rapid distribution of drug in the tissues. Similar findings for rapid distribution were reported by Boibessot et al. (2006).

Dose dependent significant increase in the value of AUC was obvious and in consistence with total systemic clearance of the drug. Vd_{area} value at three dose levels indicates moderate distribution of ISMM in the body. But Vd_C value was very low (0.03 ± 0.005 to 0.07 ± 0.005 L/kg) suggesting persistence of the drug in the peripheral compartment/tissue compartment. This suggests that disposition kinetics of ISMM in buffalo calves were best fitted to a two-compartment open model at all three dose levels. Higher K_{12} values and lower K_{21} values along with higher T~P ratio of ISMM in all three groups might have cause longer persistence of the drug in tissues leading to lower Cl_B value. Murilla et al. (1995) reported that 80% of ISMM was excreted within 21 days out of which only 18% was through urine and remaining through faeces when administered at 1 mg/kg intravenously in cattle. Kinabo et al. (1990) found that the elimination of half life of the drug was 3.2 h, and the mean residence time was 2.4 h following intravenous (I/V) administration at 0.5 mg/kg in lactating goats. The apparent volume of distribution averaged 1.52 L/kg and the mean total body clearance was 0.31 L/kg/h. Uptake of ISMM chloride demonstrated Michaelis-Menten-type kinetics. The difference in parameters with the present findings might be due to species variation. An exceptionally higher value for C_{max} at 1 mg/kg body weight than the other two dosages suggested for its accumulation as depots in tissues at higher dose level. The reports of prophylactic use of ISMM against trypanosomiasis also substantiate the same (Awa and Ndamkou, 2006). The values of t½β, Cl_B, Vd_{area} and Vd_c showed mild alteration at three dose level which may suggest that ISMM does not show dose-dependent kinetics.

It is concluded that selection of the optimal dose of ISMM in buffalo intravenously, should be based on the information regarding its disposition kinetics, efficacy and safety. The results of the present study have established that ISMM does not show dose-dependent kinetics while administered intravenously, and the same would be helpful in designing appropriate drug regimens in the strategic use of ISMM chloride in buffalo.

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