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Pharmacokinetics of sulphadimidine in Indian cattle calves

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Abstract
Sulphadimidine is commonly used in field condition to treat enteritis of bacterial origin in veterinary clinical practice. The ultimate success of any therapy depends upon the attainment of effective drug concentration *in vivo* which requires the selection of appropriate dosage for the drug. Pharmacokinetic studies provide the data necessary for calculating the dose and dosage regimen. In view of this, a pharmacokinetic study of sulphadimidine in cattle calves following i.v was undertaken to study the disposition kinetics. The concentration of sulphadimidine in plasma of cattle calves was analysed by High Performance Liquid Chromatography. A dose of 100 mg.kg^{-1} was used for i.v. Studies. The initial mean plasma concentration of 338.17µg.ml-1 sulphadimidine was found in plasma of male cattle calves, which declined to the minimum concentration of 0.21 µg.ml^{-1} (72 h), in these animals. The $t_{1/2\ \beta}$, Vd(area), Cl_B and AUC of sulphadimidine in male cattle calves were observed to be 10.43±0.53h, 0.68±0.02 L.kg^{-1}, 43.94±0.71 mL.kg^{-1}.h^{-1}, 2301.66±53.43 µg.mL^{-1}.h^{-1}. On the basis of present pharmacokinetic study, the i.v. dosage regimen of 80 mg.kg^{-1} was calculated as priming dose and 45 mg.kg^{-1} as maintenance dose for cattle calves at dosing intervals of 12h.

Keywords: Sulphadimidine, pharmacokinetics, dosage regimen, cattle calves

Introduction
Sulphadimidine or sulphamethazine is the widely used antibacterial agent to treat enteritis and also toxoplasmosis and coccidiosis in cattle. Sulphonamides are effective only in cells that must produce their own folic acid; mammalian cells do not synthesize folic acid, but get it from outside sources [1].

Pharmacokinetic studies of any compound are important to determine its dose and frequency of administration in order to maintain its effective therapeutic concentration in tissues and body fluids. Pharmacokinetic principles relate specifically to the variation of drug concentration with time, particularly in blood plasma or serum which may be interpreted in terms of drug effect by its extrapolation. The knowledge and application of pharmacokinetic principles leads to accelerated drug development, cost effective drug use and a reduced frequency of adverse effects and drug interactions [2].

The pharmacokinetic behavior of sulphadimidine in adult ruminant species is characterized by a high bioavailability, a low volume of distribution (0.24–0.50 l/kg) and a relatively short elimination half-life, compared to other sulphonamides, which varies between 3 and 10 h [1,3,4].

It has been observed that pharmacokinetic parameters of a drug may vary to a great extent from species to species. Thus it has become important for a clinician to know pharmacokinetic patterns occurring in different species of animals more specifically for a veterinarian who has to deal with the treatment of a variety of species of animals. In view of the above mentioned facts, a detailed pharmacokinetic study of sulphadimidine is beneficial for salient use of the drug in domestic animals.

Materials and Methods
The study was conducted in six cross-bred male cattle calves (1.0-1.5 yrs in age, weighing 120.11 ± 5.11 kg). Cross-bred male cattle calves for this study were procured from instructional dairy farm (IDF), Nagla of the university. All these animals were housed in animal house of department of Veterinary Pharmacology and Toxicology and kept on pre-experimental period of one month before the commencement of experiment to acclimatize them to the new environment. During this pre-experimental period all the animals were dewormed with ivermectin (Ivectin, 1% ivermectin injection, M/S Indian Immunologicals Ltd., New Delhi) @ 0.2 mg.kg^{-1} b.w. and albendazole

(Vetalben, albendazole 10% suspension, M/S Indian Immunologicals Ltd., New Delhi) @ 10mg/kg body weight one month prior to the experiment. Physical and clinical examination was done before the start of experiment. The animals were reared under uniform management and husbandry conditions, maintained on standard ration and water provided *ad libitum*. The animals were kept under constant observation before the commencement of the experiment. The study was approved by Institutional Animal Ethics committee. Principles were followed strictly throughout the course of this study. Animals were handled gently and carefully.

Drugs and chemicals used

Sulfamin® (Sulphadimidine injection I.P. (Vet), M/S Indian Immunologicals Ltd., Hyderabad). Chemicals for HPLC analysis such as acetonitrile and methanol were obtained from Merck Specialities Pvt. Ltd., Worli (Mumbai) whereas, the HPLC grade water was prepared in the laboratory using Millipore water purification assembly (Milli-Q®).

Instrument used

Drug estimation in plasma was done by high performance liquid chromatography. The HPLC system (Shimadzu corporation, Kyoto, Japan, model SPD 10 A LC 10 AT) comprised of double plunger pump, rheodyne manual loop injector with a 20µl loop and UV-VIS detector. Separation was achieved using C_{18} reverse phase column, particle size 5 µm (4x 150 mm, LiChroCART®, Merck) as a stationary phase. The flow rate was kept at 1 ml.min^{-1} and the elution was monitored at 20 ± 2 ^{0} C with UV detection at 272 nm. The chromatogram was analyzed by 'Chromatopak'. Sulphadimidine was quantified from their respective peaks.

Estimation of sulphadimidine

For intravenous pharmacokinetic study, sulphadimidine was injected as a single dose (100 mg kg^{-1}) intravenous (i.v.) bolus in jugular vein of animal. The blood samples were collected from contralateral jugular vein of each animal in marked heparinized microcentrifuge tubes (eppendorf®) by disposable plastic syringes at time interval of 0, 0.04, 0.08, 0.17, 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 h. The blood samples collected in heparinized tubes following administration of sulphadimidine were centrifuged at 3000 rpm (20 min) for separation of plasma. The plasma thus obtained was collected in clean microcentrifuge tubes and stored at -20 °C till further analysis.

Extraction of sulphadimdine from plasma

Drug extraction from plasma sample was carried out as per the method described [9] with slight modification. Deproteinization of plasma sample was carried out by adding 1 ml of 0.66 M Perchloric acid to 1 ml of separated plasma followed by vortex mixing at high speed for 1 min and subsequent centrifugation at 3000 rpm for 10 min. The clear supernatant was collected into a clean microcentifuge tube and pH adjusted with 40 % potassium hydroxide. The mixture was then filtered through millipore 0.22 µm filter and an aliquot of 20 µL of the sample thus obtained was injected into HPLC system for analysis.

The analysis of plasma samples for sulphadimidine was done as described [9] with an isocratic mobile phase consisting of 0.02 M Sodium acetate, 0.2 M acetic acid, methanol, acetonitrile and water (HPLC grade) in the ratio of 50:3.6:15:4:27.4. The pH of mobile phase was 4.9.

Preparation of standard curve

The standards for sulphadimidine were made by dissolving 1 mg of pure sulphadimdine in 1 ml of mobile phase from which the concentrations of 10, 5, 2.5, 1, 0.5, 0.25, 0.125, 0.1,0.05 and 0.025 µg.ml^{-1} were made in mobile phase. 20 µl of these concentrations was injected into HPLC under the HPLC conditions mentioned above. The standard calibration curve for sulphadimidine was obtained by plotting concentrations *verses* mean of the peak areas obtained for their respective standards. The limit of quantification (LOQ) for sulphadimdine was 0.025µg.ml^{-1}. The method for sulphadimidine was found to be linear and reproducible in the concentrations ranging 10 to 0.025 µg.ml^{-1}. A retention time of 9.49 min for sulphadimidine was observed.

The concentrations of the standard were made in drug free plasma as 10, 5, 2.5, 1, 0.5, 0.25, 0.125, 0.1, and 0.025 µg.ml^{-1} applying serial ten times dilution (100 µl standard + 900 µl drug free plasma) of 100, 50, 25, 10, 5, 2.5, 1.25, 1.0, and 0.25 µg.ml^{-1} of standard in mobile phase, in equal volumes of drug free plasma, each time. The extraction from plasma was done by the same procedure as mentioned earlier. The areas obtained by chromatography were plotted against concentration in order to get a standard calibration curve.

Recovery of the drug was done by deprotenizing the drug free plasma and then adding the above mentioned drug concentrations to the supernatant. The estimation of sulphadimidine was done as per the procedure outlined above. The peak areas of various supernatant drug concentrations were recorded and recovery of sulphadimidine from plasma was 67 percent.

Pharmacokinetic analysis

The pharmacokinetic analysis of the plasma concentration obtained following i.v. administration in this study was done by pharmacokinetic software 'Pharmkit'.

Results

The plasma concentration-time profile following single dose (100mg.kg^{-1}) i.v. administration of sulphadimidine in male cattle calves is depicted in Table.1 and Fig.1. The mean sulphadimidine concentration at 0.03 h post administration was 338.17 ± 5.94 µg.ml^{-1} which declined to 61.23 ± 0.70 µg.ml^{-1} at 12 h and then decreased slowly to a minimum of 6.37 ± 0.15 µg.ml^{-1} in 12 h. The therapeutic concentration (50µg.ml^{-1}) was maintained upto 12 h. The pharmacokinetic parameters describing the disposition kinetics of sulphadimidine following single dose (100mg.kg^{-1}) intravenous administration are presented in Table.2. A two-compartment model adequately described plasma concentration-time profile of sulphadimidine in male cattle calves following single dose i.v. administration.

The mean values of zero time intercept of distribution phase (A) and elimination phase (B) in the present study were calculated to be 271.51 ± 32.24 µg.ml^{-1} and 141.84 ± 1.89 µg.ml^{-1}, respectively. The elimination rate constant (β) was 0.0668 ± 0.002 h^{-1} with an elimination half-life ($t_{1/2\,\beta}$) of 10.43 ± 0.53 h. The $Vd_{(area)}$ and Cl_B of sulphadimidine were determined as 0.68 ± 0.02 L.kg^{-1} and 43.94 ± 0.71 ml.kg^{-1}.h^{-1}, respectively. The rate constant of distribution phase (α) was 12.88 ± 1.24 h^{-1} with distribution half-life ($t_{1/2\alpha}$) of 0.06 ± 0.01 h. The mean area under curve (AUC) and MRT were 2301.66 ± 53.43 µg.ml^{-1} h and 14.89 ± 0.19, respectively. The volume of distribution at steady state concentration (Vd_{ss}) was calculated to be 0.62 ± 0.02 L.kg^{-1}.

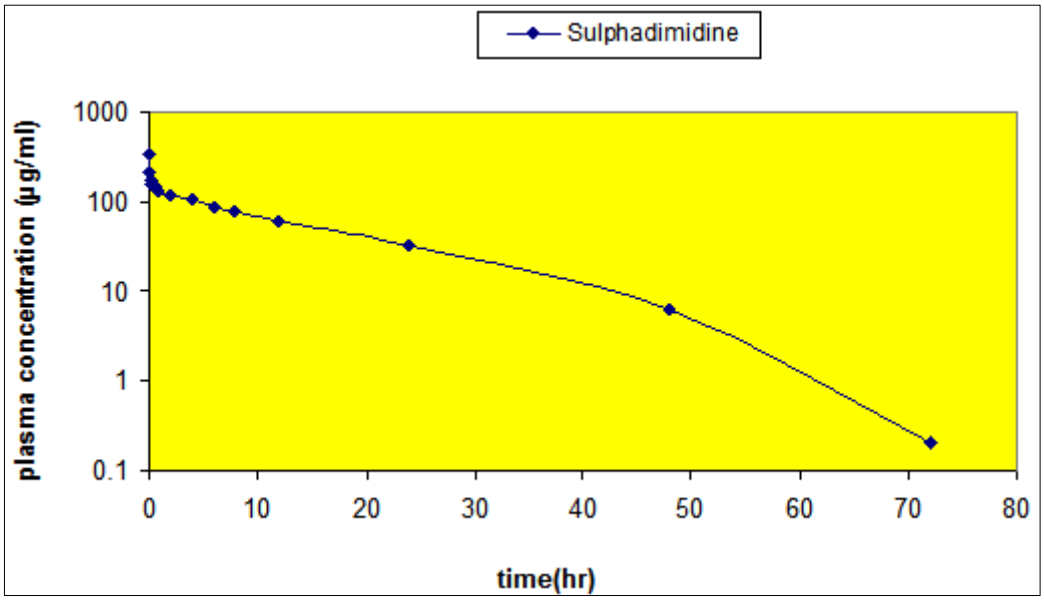

Fig 1: Semilogarithmic plot of sulphadimidine concentrations (mean± SE) in plasma versus time following i.v. administration of sulphadimidine (100mg.kg-1) in male cattle calves (n=6).

Table 1: Plasma concentration (µg.ml^{-1}) time profile of sulphadimidine following single dose (100mg.kg^{-1}) i.v. administration in male cattle calves (n=6)

Time (h)	Animal No.						Mean ± S.E.
	I	II	III	IV	V	VI	
0.03	360.12	350.24	339.62	326.34	324.19	328.49	338.17±5.94
0.08	240.20	230.15	200.09	209.93	205.27	210.32	215.99±6.39
0.17	181.21	174.21	174.64	166.75	164.46	172.57	172.31±2.46
0.25	160.52	158.42	164.78	156.85	155.70	155.55	158.64±1.45
0.5	149.69	151.36	145.61	151.37	150.65	140.28	148.16±1.80
0.75	142.12	144.40	138.15	138.39	135.36	131.09	138.25±1.94
1	136.57	135.79	136.57	132.48	128.19	125.17	132.46±1.97
2	119.23	118.35	115.84	113.83	111.26	111.22	114.95±1.41
4	108.22	104.23	112.60	106.12	102.13	101.24	105.76±1.72
6	87.51	85.37	87.86	83.28	80.24	82.37	84.44±1.23
8	76.97	79.59	81.80	78.65	77.53	78.25	78.80±0.71
12	61.11	63.24	62.17	60.88	58.19	61.78	61.23±0.70
24	35.21	31.51	34.78	34.48	29.46	30.29	32.62±1.02
48	6.01	5.92	6.66	6.87	6.21	6.54	6.37±0.15
72	0.32	0.21	0.19	0.20	0.17	0.19	0.21±0.02

Table 2: Pharmacokinetic parameters of sulphadimidine in plasma following single dose (100mg.kg-1) i.v. administration in male cattle calves (n=6)

Parameters	Unit	Animal No.						Mean ± S.E.
		I	II	III	IV	V	VI	
A	µg.ml-1	379.30	341.93	180.74	203.36	289.65	234.08	271.51±32.24
B	µg.ml-1	144.34	143.99	140.40	136.90	136.85	148.56	141.84±1.89
α	h-1	15.79	15.18	8.56	9.77	14.88	13.10	12.88±1.24
β	h-1	0.0615	0.0666	0.0629	0.0620	0.0651	0.0791	0.0668±0.002
t 1/2 α	h	0.04	0.05	0.08	0.07	0.05	0.05	0.06±0.01
t 1/2 β	h	10.57	10.40	11.01	11.17	10.64	8.76	10.43±0.53
AUC0-∞	µg.h.ml-1	2327.32	2266.79	2360.37	2514.76	2155.77	2184.96	2301.66±53.43
AUMC	µg.h2.ml-1	34596.62	32973.55	30995.28	36009.45	32039.36	31155.88	32961.69±815.28
MRT	h	14.86	14.54	15.25	15.56	14.86	14.25	14.89±0.19
Vd (area)	L.kg-1	0.65	0.67	0.75	0.70	0.71	0.58	0.68±0.02
Vd ss	L.kg-1	0.64	0.64	0.56	0.57	0.69	0.65	0.62±0.02
Cl $_B$	L. h-1.kg-1	42.09	44.08	42.34	43.15	46.30	45.70	43.94±0.71

Discussion

The present study was undertaken to investigate the disposition kinetics of a single i.v. dose (100 mg.kg^{-1}) of sulphadimidine in male cattle calves. A two-compartmental model adequately described plasma concentration time profile of sulphadimidine in male cattle calves following single dose i.v. administration as the drug distributed rapidly into the central compartment and comparatively slowly into the peripheral compartment.

Following i.v. administration, the mean sulphadimidine concentration at 0.03 h post administration was 338.17 ± 5.94 µg.ml^{-1} which declined to 61.23 ± 0.70 µg.ml^{-1} at 12 h and

then decreased slowly to a minimum of 0.46 ± 0.09 µg.ml^{-1} in 72 h. The initial plasma concentration of sulphadimidine in present study ranged from 324.19 µg.ml^{-1} to 360.12 µg.ml^{-1} among the animals under study. Some authors observed higher plasma concentration (407.80±41.58 µg/ml and 443±4.75 µg/ml, respectively) at 0.03 h and (70.46±3.80 µg/ml and 63.5 ±4.85 µg/ml, respectively) at 12 h post administration of sulphadimidine in calves [7, 8]. The present findings suggest that sulphadimidine acted as an intermediate acting sulfonamides which is in accordance with others who reported similar observation based on plasma concentration-time profile [3].

Following i.v. administration in cattle calves, the earliest plasma therapeutic concentration and the peak level appeared at 0.03 h and persisted upto 12 h in the present study. In other ruminant species, a higher initial plasma concentration (339.70±10.94 at 15 min) of sulphadimidine has been reported in sheep [11]. In another study by Bourne [5] who observed that sulfamethazine following i.v. dose of 107.25 mg/kg in lambs persisted at a therapeutic concentration (50µg/ml) for a longer time (18 h) as compared to cattle calves (12h) following i.v. route. The difference could be attributed to species variation in attainment of therapeutic plasma concentration and their persistence. Some authors also reported the species variation in attainment of plasma levels [10].

The mean elimination rate constant of 0.07 h^{-1} was observed in cattle calves in the present study. In another study mean elimination rate constant of 0.0858±0.02 h^{-1} was reported [6] and lower elimination rate (0.0482±0.0025 h^{-1}) was observed in cross-bred calves [8].

In the present study mean distribution rate constant (α), absorption half-life ($t_{½\alpha}$), volume of distribution (Vd_{area}) and volume of distribution at steady state (Vd_{ss}) in cattle calves following i.v. administration were found to be 12.88 h^{-1}, 0.06 h, 0.68 L.kg^{-1} and 0.62 L.kg^{-1}, respectively. The volume of distribution (Vd_{area}) is a measure of extra-vascular distribution of a drug and higher value is always advantageous for clinical purposes. It is an important parameter to decide upon the extent to which the drug gets distributed in body fluids and tissues. Volume of distribution at steady state (Vd_{ss}) is an accurate indicator of diffusion of drug into deep body tissues.

A low distribution rate constant (7.73±1.97 h^{-1}), slightly higher absorption half-life (0.10± 0.02 h) and volume of distribution of 0.52± 0.07 were observed in calves [7]. In another study they found distribution rate constant of 4.66±0.61 h^{-1}, absorption half life of 0.158±0.024 h and volume of distribution of 0.858±0.025 in cross bred-calves [8].

The half-life indicates the rate of elimination of drug and total plasma clearance of the drug is the volume of the blood or plasma cleared of drug by metabolism and excretion per unit of time. It is better index of efficiency of drug elimination than half-life as it gives the clearance of drug from blood per unit of time. The elimination half-life and total plasma clearance in present study were 10.43 ± 0.53 h and 43.94 ± 0.71 mL.h^{-1}.kg^{-1} following single dose i.v. administration of sulphadimidine in cattle calves which was comparable to that in calves [7] who observed elimination half-life of 9.67 h and clearance of 41.11 ml kg^{-1} h^{-1}. However, higher elimination half-life of 14.50 h and comparable plasma clearance of 41.53mL.kg^{-1}.h^{-1} were observed by in cross bred-calves [8].

Mean residence time (MRT) provides estimates regarding the persistence time of drug in the body. In the present study MRT of sulphadimidine was 14.89 ± 0.19 h in cattle calves. In another study, the mean residence time of 9.63 h and 10.48 h in buffalo calves and bovine, respectively was observed [3,4].

The area under curve (AUC) is the parameter that integrates both time and intensity of drug concentration. The AUC characterizes the relative availability of the drug in the body [6]. It is used for calculating drug clearance and other non-compartmental kinetic variables. A value of 2301.66 ± 53.43 µg.h.ml^{-1} was observed following single dose i.v. administration of sulphadimidine in cattle calves which could be compared with AUC reported earlier in calves (2564.16 ± 333.37 µg.h.ml^{-1}) suggested maintenance of drug for long duration in the body [7].

It is concluded on the basis of pharmacokinetic parameters obtained in the present study that sulphadimidine was rapidly distributed in the body and therapeutic concentration (50µg) was maintained up to 12 h in Indian cattle calves.

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