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RESEARCH ARTICLE

Effect of Arsenic on the Disposition Kinetics of Sulphadimidine and Protective efficacy of Pipali (*Piper longum*) in Cockerels

RAM SAJAN, S.P. SINGH, RACHNA VARMA, G. K. CHOUDHARY and ATUL PRAKASH

For author affiliations, see end of text.

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ABSTRACT

This study was designed to evaluate the effect on pharmacokinetics of sulphadimidine in cockerels fed on subclinical dose of arsenic (100 ppm) in diet and to determine the protective efficacy following simultaneous dietary medication with *Piper longum* (pipli) (100 ppm) in a 12 weeks feeding trial. Pharmacokinetic parameters were determined after single i.v. dose (50mg/kg) of sulphadimidine in cockerels of three groups fed on 0 ppm (control), arsenic (100ppm) and arsenic plus powdered dried fruits of *Piper longum* (100 ppm each)), respectively, in ration for 12 weeks. The elimination half-life ($t_{1/2}\beta$, h), AUC (μg mL^{-1}h) and MRT (h) were significantly (p<0.05) higher in arsenic treated group than arsenic + pipali and control groups, respectively. The distribution half-life ($t_{1/2}\alpha$, h), Vd_{area} (L/kg) and clearance (Cl, mL/kg/h) were significantly (p<0.05) reduced in arsenic treated group in comparison to arsenic plus pipali and control groups. Dosage regimens at 8 and 12 h interval for therapeutic plasma concentration (25μg/mL) of sulphadimidine revealed significant (p<0.05) reduction in priming and maintenance doses in arsenic treated cockerels in comparison to control. However, the priming and maintenance doses were higher in cockerels of arsenic plus pipali treated group than the group treated only with arsenic. It, is, thus concluded from this study that arsenic altered pharmacokinetics of sulphadimidine in cockerels and simultaneous treatment with pipali (100ppm) revealed protective efficacy against arsenic induced pharmacokinetic effects following 12 weeks feeding in cockerels. These findings may be correlated for the pharmacokinetics of other drugs in other species of animals.

Keywords: *Pharmacokinetics, Sulphadimidine, Arsenic, Cockerels, Piper longum, Intravenous (i.v.)*

Sulphadimidine is an antimicrobial agent belonging to the sulfonamides group. It competes with para-aminobenzoic acid (PABA) for the catalytic site of the enzyme dihydropteric acid synthetase and thus subsequently inhibits the synthesis of folic acid [1].

Elimination of unchanged sulphonamides and their metabolites mainly occur in urine and feaces. The disposition kinetics of sulphadimidine has been well documented in healthy as well as in animals with hepatic and renal dysfunction. Sulphadimidine has been taken as a model drug in arsenic toxicity.

Pipali (*Piper longum*) contains piperine, the peper alkaloid, possesses several pharmacological actions like anti-infertility, CNS depressant, anti-inflammatory, inhibition of hepatic drug metabolizing enzymes and enhancement of drug bioavailability [2]. Fruit extract of *Piper longum* was found to possess hepatoprotective action in rodents in carbon tetrachloride induced hepatotoxicity [3].

Arsenic is one of the most common toxicants responsible for environmental pollution. In India, underground water is the most prominent source of arsenic poisoning. The absorption of 80-90% of arsenic both trivalent and pentavalent takes place from stomach and small intestine and only a small fraction from skin and lungs [4].

Arsenic is eliminated through faeces, urine, sweat, milk, skin and lungs; however, renal excretion is the major route of elimination with half life of 3-5 days in human beings [5, 6]. Accidental exposure to large doses of inorganic arsenic caused acute toxicity manifested by gastroenteritis, gastrointestinal hemorrhage, bloody diarrhea, coma and death in sheep [7]. Inorganic and organic arsenicals are toxic to the liver and kidney [8, 9, 10].

Table 1. Blood concentration (μg/mL) of free sulphadimidine in control and treated cockerels following single i.v. administration (50 mg/kg) (n= 7; mean ± SE)

Time (hr)	(Mean±SEM)		
	Control (0ppm)	Arsenic (100ppm)	Arsenic (100ppm) + Pipali (100ppm)
0.08	108 ± 4.3	105.0 ± 2.22	108 ± 2.59
0.17	95.85 ± 3.41	95.2 ± 0.30	93.85 ± 0.88
0.25	83.42 ± 4.08	77.85 ± 0.50	85.71 ± 1.28
0.50	72.57±1.84	67.85 ± 0.63	76.35 ± 2.98
1	52.85 ± 2.96	55.71 ± 1.01	66.14 ± 5.05
2	42.42 ± 2.39	48.28 ± 1.80	55.85 ± 5.25
4	27.42 ± 1.81	37.71 ± 1.84	43.42 ± 4.33
8	20.07± 1.29	32.71 ± 1.84	30.57 ± 2.96
12	15.42 ± 1.13	25.71 ± 0.52	19.41± 1.81
16	7.84 ± 0.244	19.14 ± 0.67	10.67± 1.22
20	4.81 ± 0.38	14.28 ± 1.14	5.07± 0.64
24	1.55 ± 0.19	6.80 ± 0.37	2.58 ± 0.52
28	ND	1.65 ± 0.19	ND
32	ND	ND	ND

N.D. = not detected

The main objective of the present study was to evaluate the protective efficacy of pipali *(piper longum)* in arsenic intoxicated cockerels. The arsenic altered metabolizing enzyme system of the body might change the disposition kinetics of sulphadimidine, the model drug in this study.

MATERIALS AND METHODS

Chemicals

Pure analytical grade of arsenic trioxide (As_2O_3) procured from loba chemie was used in this study. The fruits of pipali (*Piper longum*) were collected from Research and Development Farm, Medicinal and Aromatic Plant, Department of Horticulture N.D.U.A. & T., Kumarganj, Faizabad and identified taxonomically, pulverized and used in this investigation.

Animals

Day old male white leghorn chicks were procured from government poultry farm chak ganjaria, Lucknow and were reared for four weeks for acclimatization before the start of the study. Four weeks chicks were used in this study and kept in deep litter system of housing and maintained on grower ration procured from U.P. state Agro Industries Corporation limited, Lucknow. Feed and water were provided *ad libitum* throughout the study.

Medicated ration was prepared for about a week period and kept in close container. Pipali fruits were pulverized to prepare 100ppm pipali medicated ration by mixing 600mg of powder of pipali fruits in about 500 g of ration and then more feed was added to make 6 kg feed.

Arsenic (100ppm) treated feed was prepared by mixing 660.17mg of arsenic trioxide (containing arsenic equivalent to 500mg) thoroughly in 5 kg feed. All the birds were fasted over night prior to the start of the experiment.

Animal Treatment

Twenty-one, four weeks old male white leghorn chicks, were randomly divided into three groups i.e.I (control), II (arsenic 100 ppm), and III (arsenic 100 ppm + pipali 100 ppm) of seven birds each, the pharmacokinetics pattern of sulphadimidine was estimated after injecting a single dose (50 mg/kg, I.V., because it will achieve 25μg/ml therapeutic concentration) in these groups after 12 weeks of feeding trial. Blood sample were collected from wing vein in heparinised sterilized tubes before and after 0.08, 0.17, 0.25, 0.50, 1, 2, 4, 8, 12, 16, 20, 24, 28 and 32 h of drug administration.

Analytical method

The concentration of free and total sulphonamide in plasma (μg/ml) was estimated according to the method of [11] as described by [12] and measured by AUTOCHEM 2011 at 580nm. Dosage regimen was calculated using information of single dose trial [13].

The pharmacokinetic analysis of plasma concentration time profile of sulphadimidine for each bird was performed with the aid of PHARMAKIT, (M/s Clyde Soft, Glasgow, and U.K.,).

Statistical Analysis

Comparison of pharmacokinetic parameters of treated and control groups were done employing one way analysis of variance mentioned by [14].

RESULT

Mean plasma concentration (μg/mL) of sulphadimidine following single dose (50 mg/kg) i.v. administered in different groups of chicks (control, 100 ppm arsenic and 100ppm arsenic + 100ppm pipali) is presented in Table 1 and disposition curve in fig 1. The minimum therapeutic concentration of sulphdimidine (25μg/mL) was maintained for 12 h, 8 h and 4 h in

Table 2. Pharmacokinetic parameters after single i.v. administration sulphadimidine (50 mg/kg) in control, arsenic intoxicated and arsenic plus pipali medicated cockerels (n= 7; mean ± SE)

Kinetic parameters	Units	Control	Arsenic (100ppm)	Arsenic (100ppm) + Pipali (100ppm)
A	µg /mL	69.22 ± 3.94	67.54 ± 3.37	70.89 ± 7.44
B	µg/mL	48.08 ± 3.91	60.83 ± 1.74	72.17 ± 7.5
α	h^{-1}	2.14 ± 0.23	3.96 ± 0.52	3.26 ± 0.627
β	h^{-1}	0.102** ± 0.011	0.076** ±.001	0.114** ± 0.002
AUC	µg/mL/h	29.17*± 60.94	812.94* ± 33.8	672.19* ± 80.62
$t_{1/2}$ α	h	0.347* ± 0.041	0.156* ± 0.031	0.168* ± 0.055
$t_{1/2}$ β	h	7.50*±1.35	9.12* ± 0.25	6.085* ± 0.09
K_{10}	h^{-1}	0.235 ± 0.013	0.209 ± 0.04	0.340 ± 0.07
K_{12}	h^{-1}	1.054 ± 0.106	2.189 ± 0.307	0.783 ± 0.105
K_{21}	h^{-1}	0.962 ± 0.136	2.412 ± 0.58	2.706 ± 1.340
Cl	mL/kg/h	0.99** ± 0.007	0.061** ± 0.002	0.083** ± 0.010
MRT	h	10.11* ± 1.78	12.74* ± 0.357	7.83* ± 0.317
Vdarea	L/Kg	1.013* ± 0.077	0.803* ± 0.040	0.716* ± 0.102

* 5% level of significance
** 1% level of significance

chicks of arsenic 100 ppm, arsenic + pipali and control groups respectively. The sulphadimidine was detected up to 28 h in arsenic treated groups whereas other groups were found to have sulphadimidine in the plasma only up to 24 h of post administration (Table 1 and Fig 1). The plasma concentration time profile of sulphadimidine in all groups indicated that disposition of the drug was appropriately fitted to a two compartment open model.

The value of various pharmacokinetics parameters computed from plasma levels of free sulphdimidine in control and treated birds following single i.v. administration at the dose of 50 mg/kg is given in Table 2.

The mean value of extrapolated drug concentration during distribution (A, µg/mL) and elimination (B, µg/mL) phase did not reveal any variation among control and treated groups. The mean value of elimination rate constant (β, h^{-1}) was significantly (p<0.01) lower in arsenic treated group in comparison to arsenic plus pipali and untreated control groups, however there was no significant different in distribution rate constant (α, h^{-1}) among treated and control groups. The mean value of elimination half life ($t_{1/2}$ β, h) was significantly (p<0.05) higher than other groups as it was 9.12 ± 0.25 in arsenic 100 ppm group in comparison to 6.085 ± 0.09 and 7.50 ±1.35 in arsenic + pipali and control groups, respectively. The distribution half life ($t_{1/2}$ α, h) is significantly (p<0.05) higher as the value was 0.156 ± 0.031, 0.168 ± 0.055 and 0.347 ± 0.041in arsenic, arsenic plus pipali and control group, respectively.

A and B = extrapolated zero time plasma drug concentration during distribution and elimination phases respectively; α and β = distribution and elimination rate constants, respectively; AUC = area under plasma concentration time curve; $t_{1/2}$ α and $t_{1/2}$ β = distribution and elimination half lives, respectively; K_{10} = rate constant of drug elimination from central compartment; K_{12} and K_{21} = micro-rate constant of drug transfer from central to peripheral, peripheral to central compartment, respectively; MRT = mean residential time; Cl = total body clearance; Vd_{area} = volume of distribution from AUC.

There was no significant difference in rate transfer constant from peripheral to central (K_{21}, h^{-1}) and central to peripheral (K_{12}, h^{-1}) compartments among control and treated groups. Area under curve (AUC, µg mL^{-1} h) was significantly (p<0.05) higher in arsenic group as compared to other groups and the mean value of AUC was 812.94 ± 33.8 in arsenic and 672.19 ± 80.62, 529.17 ± 60.94 in arsenic plus pipali and untreated control groups respectively. The volume of distribution (Vd_{area}, L/kg) was higher (p<0.05) in control as compare to treated groups. The mean value of (Vd_{area}, L/kg) was 1.013* ± 0.077, 0.803* ± 0.040 and 0.716* ± 0.102 in control, arsenic 100pmm and arsenic plus pipali group, respectively.

The retention time was higher in arsenic treated group as compared to other groups and the value of

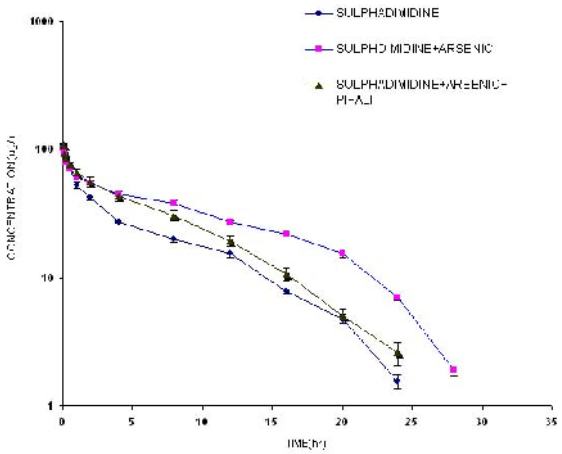

Fig 1. Mean + S.E. Plasma cocentration vs time plot of sulphadimidine,sulphadimidine +Lead,sulphadimidine+Lead + Tulsi following single dose (50 mg/kg) i.v. administration in cockerels (n=7)

Table 3. Intravenous Dosage regimens of sulphadimidine to maintain desired therapeutic concentration of 25 µg/mL at 8 and 12 h interval in control, arsenic intoxicated and arsenic plus pipali medicated cockerels.

Group	Interval (h)	D (mg/kg)	C_{ss} (min) Following D (µg/mL)	C_{ss} (max) Following D (µg/mL)	Dm (mg/kg)	C_{ss} (min) Following Dm (µg/mL)	C_{ss} (max) Following Dm (µg/mL)
Control	8	56.46	43.29	101.90	32.08	25	57.90
	12	85.93	34.89	123.03	61.56	25	88.14
Arsenic	8	37.03	54.36	100.65	17.03	25	46.28
	12	50.38	41.45	104.43	30.38	25	62.98
Arsenic + Pipali	8	44.62	41.78	104.02	26.69	25	62.23
	12	70.40	33.54	131.73	52.48	25	98.18

mean residential time (MRT, h) was 12.74 ± 0.357 in arsenic treated birds which was significantly higher than 10.11*± 1.78 in control and 7.83*± 0.317 in arsenic + pipali treated group. The clearance (Cl, mL/kg/h) of sulphadimidine was slower in arsenic treated groups than arsenic plus pipali and control groups as it was 0.061 ± 0.002, 0.083 ± 0.01and 0.099 ± 0.007 in arsenic, arsenic plus pipali and control groups, respectively.

The dosage regimens for minimum plasma therapeutic concentration of 25 µg/ml at 8 and 12 h intervals for control and treated groups are given in Table-3. The priming dose of sulphadimidine (mg/kg) following single i.v. administration is proposed to be 56.46 (mg/kg) for control, 37.03(mg/kg) for arsenic and 44.62 (mg/kg) arsenic plus pipali fed chicks at 8 h interval and at 12 h interval the priming dose was least (50.38 mg/kg) for arsenic and 70.40 mg/kg for arsenic plus pipali and 85.93 mg/kg for control groups. The maintenance doses ($D_{m,}$ mg/kg) were least in arsenic 100ppm (46.28 and 62.98), arsenic plus pipali (62.23 and 98.18) and control (57.90 and 88.14) groups at 8 and 12h intervals.

DISSCUSSION

Disposition kinetics of sulphadimidine revealed that the minimum therapeutic concentration of sulphadimidine was maintained for 12 h in cockerels treated with arsenic 100ppm group, which was significantly higher than other groups. The sulphadimidine was also detected for longer duration that is up to 28 h in arsenic treated cockerels whereas rapid elimination as observed in healthy and arsenic + pipali treated groups. This indicates a significant variation in arsenic and treated group regarding the duration of therapeutic concentration rate of elimination of the drug as also suggested by other pharmacokinetics parameters in this study.

The distribution half-life ($t_{1/2}\alpha$) explains the distribution of sulphadimidine faster in control in comparison to arsenic and arsenic + pipali groups. The slow distribution of drug in arsenic treated cockerels may be attributed to the alteration in transportation of drug as a result of toxic effect of arsenic on capillaries [15] and other tissues causing disruption in the transport of drug across membrane.

The mean value of sum of K_{12} and K_{21} are also higher than K_{10} (elimination rate constant). Thus the elimination of the drug is slower than its distribution in the body. This might be due to renal insufficiency caused by arsenic toxicity [8, 10]. The mean value of elimination rate constant (β, h^{-1}) was also significantly lower in arsenic treated cockerels than other groups suggesting slow rate of elimination of sulphadimidine, this might be due to nephrotoxic effect of arsenic [8, 10].

The volume of distribution (Vd_{area}) was higher in control as compare to treated groups, which might be due to the disruption in transformation of the drug across the biomembranes in body tissue of arsenic treated cockerels.

Area under the curve (AUC) of concentration time profile characterizes the relative availability of drug in the body. The value of AUC (µg/ml/h) of sulphadimidine was highest in arsenic treated group followed by arsenic + pipali and control groups. The effectiveness of drug depends not only on the plasma concentration of drug achieved but also duration for which these concentrations exist in the body [16].

The MRT was higher in arsenic treated group as compared to arsenic + pipali and untreated control explaining the retention of sulphadimidine for longer duration in arsenic treated cockerels. The clearance (Cl, ml/Kg/h) of sulphadimidine was slower in arsenic treated cockerels than arsenic + pipali and control groups. The value of clearance in this study also indicates the slow elimination of drug in arsenic intoxicated cockerels.

The main objective of pharmacokinetics study of a drug is to calculate its dosage regimen. On the basis of minimum plasma therapeutic concentration of sulphadimidine as 25 µg/mL, the priming dose (D, mg/kg) were computed to be 37.03, 44.62 and 56.46 at 8 h and 50.38, 70.40 and 85.93 at 12 h intervals for arsenic, arsenic plus pipali and control groups respectively following i.v. administration. Similarly the maintenance dose (Dm, mg/kg) was least in arsenic group followed by arsenic plus pipali and control group at 8 and 12 h intervals. Indicating the priming and maintenance dose level were less in arsenic treated cockerels than healthy and pipali treated cockerels.

On conclusion, it may be suggested that the arsenic altered the metabolism and excretion of drug that might be due to nephrotoxic and hepatotoxic property of arsenic and that may interfere the distribution, metabolism and excretion of sulphadimidine. Sulphadimidine was retained for longer duration in the

body therefore, require less priming and maintenance dose in arsenic intoxicated cockerels in comparison to pipali plus arsenic and untreated healthy cockerels. Pipali have protective effect on different organ especially liver and kidney [3].

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CURRENT AUTHOR ADDRESSES

Ram Sajan, Department of Animal Husbandry, U. P. Government., India.

S.P. Singh, College of Veterinary & Animal Sciences, Patnagar., India.

Rachna Varma, College of Veterinary Science & Animal Husbandry, Faizabad., India.

G. K. Choudhary, College of Veterinary Science & Animal Husbandry, Faizabad., India. E-mail: drgovindvet@yahoo.co.in (Corresponding author)

Atul Prakash, Ph D Scholar, Indian Veterinary Research Institute, Izatnagar, India.