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Effect of Carbontetrachloride-Induced Hepatopathy on the Disposition Kinetics of Closantel in Goats

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ABSTRACT

Clinically healthy adult black Bengal female goats were used to investigate the pharmacokinetics of closantel following intravenous administration (10 mg/kg) both in healthy and in liver damaged goats. Induction of liver damage was done by subcutaneous injection of carbontetrachloride at 0.75 mL/kg on three occasion at 48 h interval. After administration of drug, blood samples were collected in heparinized test tubes at pre-determined time. The drug concentration in plasma was measured by double beam UV–Vis spectrophotometer at 222 nm. *In vitro* plasma protein binding of closantel was also carried out. C_{max} (118.82 ± 4.85 μg/mL) and C_{min} (4.69 ± 0.72 μg/mL) of closantel were recorded at 0.08 and 48 h respectively after i.v. administration to healthy goats, while the above values were 144.67 ± 2.50 and 3.93 ± 0.05 μg/mL at 0.08 and 48 h respectively in liver damaged goats. The $t_{½}\beta$, Cl_B and Vd_{area} values were 12.92 ± 1.30 h, 0.008 ± 0.0005 L/kg/h and 0.15 ± 0.01 L/kg respectively in healthy goats, while these values were 12.84 ± 0.38 h, 0.008 ± 0.001 L/kg/h and 0.16 ± 0.005 L/kg respectively in liver damaged goats. Binding capacity and association rate constant of closantel were not altered significantly in liver damaged animal compared to healthy animal. Liver damaged condition did not alter significantly the pharmacokinetic parameters of closantel.

Keywords: *Closantel, Disposition kinetics, Liver damage, Goats*

Helminthosis is one of the important diseases of domestic animal in India, although the magnitude of incidence may vary in different geographical location. Worms cause various disorders viz., blood loss, nutritional deficiencies, urticaria, allergic manifestation, intestinal obstruction, loss of production, interference of immune system, damage to internal organs, release of toxic substances and secondary bacterial infections. Hence, control of both external and internal parasites of has immense economical and clinical importance.

Closantel N-[5-chloro-4(4-chlorophenyl) cyanomethyl]-2-methylphenyl]-2-hydroxy-3, 5-diiodobenzamide] is a typical member of the halogenated salicylanilide group of compound. It is a truly ectendoparasiticidal as it kills both ecto- and endo-parasites. It has been found to be effective against trematodes [1] and ecto-parasites [2].

The liver is the main metabolic organ in the body. The efficacy as well as disposition kinetics of anthelmintics used in liver damage condition may be interfered with due to altered metabolism. Besides flukes and round worms, some other parasites may damage the liver and cause cirrhosis during their larval migratory phase. Wherever the larva lodges, chronic inflammatory changes arise with resulting fibrosis. The effects of liver disease on pharmacokinetics of drugs are unpredictable, but clearly, the elimination of many potent drugs is impaired in patients with chronic liver disease. There are two distinct approaches to study the biotransformation of drugs by the diseased liver. One is measuring the activity of drug metabolizing enzymes in liver biopsies, and the other is determining the disposition kinetics of drugs in patient with liver disease [3].

In view of the above, the present research was undertaken to determine disposition kinetic behaviour of closantel in healthy and carbontetrachloride-induced liver damaged animals following intravenous administration.

MATERIALS AND METHODS

Chemicals

Closantel, a halogenated salicylanilide derivative (Technical grade, purity > 90%) was obtained as a gift from Sarabhai Zydus Animal Health Pvt. Ltd., Vadodara, Gujarat, India. All other chemicals used in this experiment were obtained from E. Merck (India) and Sigma Chemical Co (U.S.A.).

Animals

Twelve clinically healthy adult black Bengal nulliparous female goats weighing between 7 – 10 kg of 1 – 1½ year old were used. They were divided into group I and group II, each containing six animals. The animals were kept in individual custom made stainless steel cage (size - 48″ × 48″ × 36″) in temperature (22 ± 2°C) controlled animal room having provision of artificial light. The animals were acclimatized with the laboratory condition for 7 days. They were fed with balanced feed and water was supplied *ad libitum.* The animals were dewormed with albendazole at 7.5 mg/kg orally 30 days prior to the onset of study. The lower part of the neck of each animal was shaved and the jugular vein was exposed. The animals were fasted overnight prior to the start of the experiment.

All the procedures adopted in the study were approved by the 'Animal Ethical Committee' of West Bengal University of Animal and Fishery Sciences, India.

Animal Treatment

A single dose of closantel dissolved in 2.5 mL of glycerinformal was administered to each animal of group I through the left jugular vein at 10 mg/kg. Blood samples were collected from the right jugular vein in heparinized test tubes before and after 0.08, 0.16, 0.33, 0.5, 0.66, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h of drug administration.

Blood samples were (3.5 mL) collected at the above mentioned time period except at 0.08, 0.16, 1 and 2 h where 6 mL of blood were collected for protein binding study of closantel.

Plasma was then separated by centrifugation at 3000 rpm for 30 min. One mL of plasma was utilized for analysis of closantel and 2 mL of plasma for estimation of plasma protein binding of closantel.

Liver of each goat of group II was damaged by subcutaneous administration of carbontetrachloride mixed with equal volume of liquid paraffin following overnight fasting. Carbontetrachloride at 0.75 mL/kg was administered on three occasions at 48 h interval. The intensity of liver damage was assessed by performing serum bromosulphophthalein clearance test, icterus index, and serum aspartate and alanine aminotransferase activity.

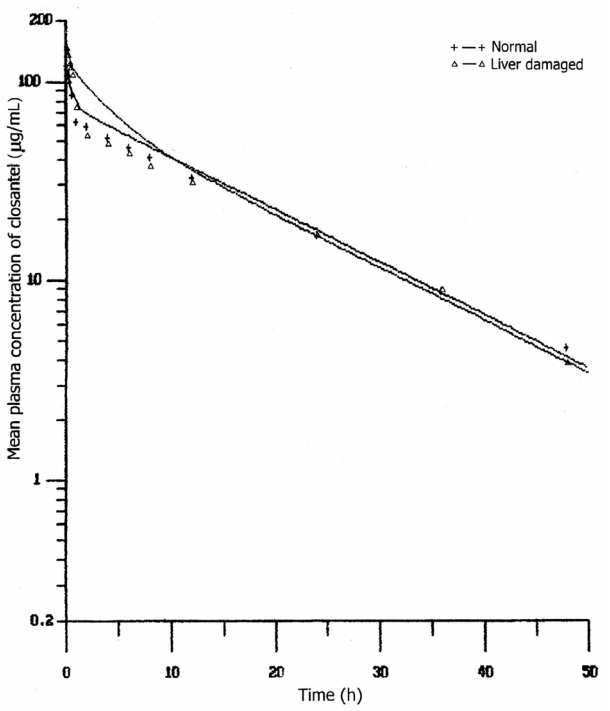

Fig 1. Semilogarithmic plot of mean plasma concentration (µg/mL) of closantel against time with comuterised best-fit line following single i.v. administration at 10 mg/kg in normal and liver damaged goats.

Bromosulphophthalein (BSP) clearance test and icterus index were done by the method of Oser [4]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured as per method described by Yatazidis [5].

Closantel was administered intravenously at 10 mg/kg to each of liver damaged goat of group II after 48 h of last dosing of carbontetrachloride. Blood samples were collected and plasma was separated as previously.

Analytical Method

To a centrifuge tube containing 1 mL of plasma, 10 mL of acetonitrile (acidified with concentrate sulfuric acid) was added, shaken vigorously for 2 min and centrifuged at 5000 rpm for 20 min. The supernatant was collected and the absorbance was read in double beam UV–Vis spectrophotometer at 222 nm wavelength against blank prepared with plasma collected at '0' h (before administration of drug). A standard curve of closantel in control plasma at different concentration was prepared. Concentration of closantel present in each

Table 1. Bromosulphophthalein (BSP) clearance, icterus index, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum of goat following carbontetrachloride - induced liver damage (Mean±SEM, n = 6).

Day	Biochemical Parameters			
	BSP (min)	Icterus index (unit)	AST (unit/mL)	ALT (µg pyruvic acid/mL/h)
0	3.32 ± 0.36	4.64 ± 0.10	340.00 ± 11.67	92.00 ± 7.83
2	8.49 ± 0.27*	8.74 ± 0.18*	589.67 ± 12.44*	143.67 ± 4.67*
4	14.25 ± 0.32*	14.66 ± 0.27*	663.33 ± 21.10*	176.33 ± 6.94*
5	20.80 ± 0.23*	20.14 ± 0.26*	925.00 ± 12.58*	235.33 ± 4.17*
7	18.90 ± 0.41*	19.61 ± 0.21*	829.50 ± 6.22*	204.33 ± 2.29*

* $p < 0.05$ compared to '0' day

blood samples was then calculated from standard curve prepared earlier and expressed as μg/mL. The coefficient of variance of the accuracy and precision of this method was less than 9.50 % of the mean. The sensitivity of the method was 0.5 μg/mL. The limit of detection of closantel was 1 μg/mL. To avoid photodegradation of closantel, the analytical process was done in dark room.

The plasma protein binding of closantel was determined by equilibrium dialysis technique, as described by Sisodia *et al*. [6] and Banerjee *et al*. [7], and modified by Mandal *et al*. [8]. The protein content of plasma of each sample was also estimated by Biuret method [9]. The protein binding was then expressed as percentage. The binding capacity, dissociation and association constants were also calculated by the method of least regression technique described by Pilloud [10].

Plasma drug concentration versus time for each goat was analyzed using computerized curve fitting programme 'PHARMKIT' (supplied by Department of Pharmacology, JIPMER, Pondicherry, India). The programme fits the line to a biexponential equation for both healthy and liver damaged groups of animal. Pharmacokinetic parameters were calculated from the computerized curves according to Gibaldi and Perrier [11].

Statistical Analysis

Students *t*-test was applied to test the level of significance in drug concentration, kinetic and different biochemical parameters in different groups of animals.

RESULTS

All the biochemical parameters determined from serum sample collected at 2, 4, 5 and 7th day of administration of carbontetrachloride were significantly increased from the control values (sample collected before administration of carbontetrachloride) (Table 1). This indicated that carbontetrachloride at the recommended dose level damaged the liver.

Table 2. Pharmacokinetic parameters of closantel after single dose i.v. administration to normal and liver damaged goats at 10 mg/kg (Mean ± SEM, n = 6).

Parameters	Normal goat	Liver damaged goat
C_0 (μg/mL)	117.51 ± 7.27	148.40 ± 2.69*
α (h^{-1})	1.10 ± 0.09	0.96 ± 0.04
$t_{½}α$ (h)	0.66 ± 0.06	0.73 ± 0.03
β (h^{-1})	0.06 ± 0.005	0.05 ± 0.001
$t_{½}β$ (h)	12.92 ± 1.30	12.84 ± 0.38
AUC (μg. h/mL)	1258.80 ± 71.45	1222.19 ± 7.63
Vd_c (L/kg)	0.08 ± 0.003	0.07 ± 0.001*
Vd_{ss} (L/kg)	0.20 ± 0.04	0.14 ± 0.003
Vd_{area} (L/kg)	0.15 ± 0.01	0.16 ± 0.005
Cl_B (L/kg/h)	0.008 ± 0.0005	0.008 ± 0.001
K_{el} (h^{-1})	0.10 ± 0.006	0.12 ± 0.001*
K_{12} (h^{-1})	0.52 ± 0.04	0.47 ± 0.02
K_{21} (h^{-1})	0.62 ± 0.06	0.43 ± 0.01*
f_c	0.56 ± 0.02	0.44 ± 0.01*

* $p < 0.05$ compared to normal goat

Abbreviations: C_0, Zero time plasma drug concentration; α, Distribution rate constant; $t_{½}α$, Biological half-life (distribution phase); β, Elimination rate constant; $t_{½}β$, Biological half-life (elimination phase); AUC, Total area under the drug concentration versus time curve; Vd_c, Apparent volume of central compartment; Vd_{ss}, apparent volume of distribution at steady state; Vd_{area}, apparent volume of drug distribution; Cl_B, Total body clearance of drug; K_{el}, First order elimination rate constant for disappearance of drug from central compartment; K_{12}, First order rate constant for transfer of drug from central to peripheral compartment; K_{21}, First order rate constant for transfer of drug from peripheral to central compartment; f_c, Fraction of drug retained in the central compartment.

Kinetics

The mean plasma concentrations of closantel in healthy and carbontetrachloride-induced liver damaged goats at different time intervals have been incorporated in Fig 1. The maximum and minimum concentration of closantel in healthy goat were 118.82 ± 4.85 and 4.69 ± 0.72 μg/mL at 0.08 and 48 h while in liver damaged goats, the maximum and minimum concentrations were 144.67 ± 2.50 and 3.93 ± 0.05 μg/mL at the same time, respectively. The maximum concentration of closantel in liver damaged goats was higher than that of healthy goats but the concentrations for different times between both groups of animals were not altered significantly.

Kinetic parameters of closantel in normal and liver damaged goats are presented in Table 2.

Protein Binding

Results obtained in relation to plasma protein binding of closantel are presented in Table 3. The binding capacity, association constant and dissociation constant of closantel with plasma protein have been presented in Table 4.

DISCUSSION

Disposition kinetics of closantel in healthy and hepatopathic animal after single dose intravenous administration at 10 mg/kg showed a biphasic declination of the concentration in both the groups of animal suggestive of "two compartment open model" kinetics of closantel. Swan *et al*. [12] also reported two compartment model with first order rate constant of closantel in sheep following single dose intravenous administration.

It would be evident from Table 2 that $t_{½}β$ and Cl_B values of closantel in animals of damaged liver were not altered significantly compared to healthy goats. Swan *et al*. [12] observed long elimination half life of 17.0 ± 4 day in adult sheep after i.v. administration. The shorter $t_{½}β$ (12.92 ± 1.30 and 12.84 ± 0.38 h in normal and liver damaged goats respectively) value in goat might be attributed to species variation which has been substantiated by Hennessy *et al*. [13] during their study on comparative pharmacokinetics of closantel in sheep and goat following intraruminal or intramuscular administration. The values of AUC and Vd_{area} were also unaffected in

Table 3. Plasma protein bound (%) of closantel in normal and liver damaged goats after single dose i.v. administration at 10 mg/kg (Mean ± SEM, n = 6).

Time (h)	Normal goat	Liver damaged goat
0.08	98.17 ± 0.04	98.16 ± 0.17 NS
0.16	98.15 ± 0.19	98.43 ± 0.22 NS
1	98.23 ± 0.16	98.19 ± 0.07 NS
2	98.12 ± 0.08	97.76 ± 0.19 NS

NS Non-significant compared to normal goat

Table 4. Plasma protein binding capacity (β_I), dissociation rate constant (K_β) and association rate constant (K'_a) of closantel in normal and liver damaged goats after single dose i.v. administration at 10 mg/kg (Mean ± SEM, n = 6).

Parameters	Normal goat	Liver damaged goat
β_I (mol/gm)	$1.74\times10^{-7} \pm 0.05\times10^{-7}$	$1.71\times10^{-7} \pm 0.11\times10^{-7}$ NS
K_β (L/mol)	$0.14\times10^{-7} \pm 0.01\times10^{-7}$	$0.16\times10^{-7} \pm 0.02\times10^{-7}$ NS
K'_a (mol/L)	$0.76\times10^{7} \pm 0.06\times10^{7}$	$0.68\times10^{7} \pm 0.08\times10^{7}$ NS

NS Non-significant compared to normal goat

liver damaged goats. Further, these values also suggested very limited distribution of closantel in the body. The values of K_{12} and K_{21} in both groups of animals also indicated that closantel has lower affinity to accumulate in tissue compartment. Halogenated salicylanilides are extensively plasma bound and poorly distributed to tissues [12]. In the present experiment, the extensive binding of closantel of plasma albumin (> 97%) has been observed. Results obtained in relation to plasma protein binding of closantel showed that the equilibrium association constant was greater compared to dissociation constant. The binding to plasma albumin has also been characterized by very high affinity and high capacity. Therefore, the extensive plasma binding of closantel is responsible for long elimination half-life which has also been reported in other salicylanilide compound to other species. Michiels *et al*. [14] reported that 90% of closantel is excreted unchanged in faeces and urine in sheep and cattle. A reductive monodiiodination reaction appears as the main metabolic pathway for closantel in sheep resulting in the formation of 3, 5 monoiodoclosantel isomers [14]. Similar results have been confirmed in cattle and goats [15]. It is expected that the metabolism of drug might be interfered in hepatic damage as liver is the main metabolic organ in the body. Therefore, unaffected pharmacokinetic parameters of closantel in liver damaged goat may suggest extra hepatic metabolism of closantel in goats.

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