Effect of Isometamidium hydrochloride on *Trypanosoma evansi* Infections in Rats

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Abstract Effect of Isometamidium treatment of rats infected with *Trypanosoma evansi* was studied under experimental condition. The infection resulted in 100 per cent mortality by 7 day post treatment (DPT) in the infected untreated rats (Gr.II) and Isometamidium-HCl exerted 90 percent anti-trypanosomal efficacy. Haematological studies of all the groups on 3 day post infection (DPI) and on 7 DPT showed that *T. evansi* infection significantly decreased haematological values, however Isometamidium treatment significantly ($P < 0.05$) improved Hb and PCV without any significant changes in TEC and TLC values. Serum AST and ALT activities were significantly ($P < 0.01$) higher in the treated rats compared to healthy rats. Treatment with Isometamidium-HCl significantly improved blood glucose level but decreased creatinine level compared to pre-treatment values. There was no significant change in BUN level of treated rats but the value was higher ($P < 0.01$) compared to healthy rats.

Keywords Hematology, Isometamidium, *T. evansi*

Introduction

Trypanosomosis caused by *T. evansi* is the most common and wide spread haemo-protozoal disease resulting in high

morbidity and mortality losses of domesticated livestock throughout the tropical and subtropical regions. In India, the disease condition caused due to *T. evansi* infection is commonly known as ‘surra’ and is prevalent in almost all the states, but with greater frequency in the arid and semi-arid zones (Bhatia and Shah 2001). Chemotherapy and chemoprophylaxis are the only practical methods available at present for the control of animal trypanosomosis (Chaka and Abebe 2003) but emergence of drug resistant strains limits the use of the currently available anti-trypanosomal drugs (Brun et al. 1998; Tuntasuvan et al. 2003). Hence sustainability of chemotherapy and chemoprophylaxis of this infection in India would depend on introduction of drugs which are hitherto not in common use.

Isometamidium hydrochloride effective against the African trypanosomosis has not been much used in the treatment of *T. evansi* infection, especially in India. This drug, first described by Wag et al. (1958), was obtained from the coupling reaction between m- amidino-benzenediazonium chloride and homidium chloride.

Different strains of *T. evansi* may be assumed to have variable responsiveness to a specific drug. Therefore, it is imperative to evaluate the efficacy of Isometamidium-HCl against the Indian strain of *T. evansi* before the drug is adopted for use in the control of trypanosomosis (surra) in India. *T. evansi* causes anaemia and hypoglycaemia (Bhatia 2000) in infected animals therefore the present study was undertaken to study the impact of Isometamidium hydrochloride treatment on haemato-biochemical parameters of rats experimentally infected with *T. evansi*.

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Materials and methods

Experimental Animals: Seventy Sprague Dawley rats (150–250 gm) and twenty albino mice (25–30 gm) and of either sex were purchased from the university's animal supplier. They were maintained separately in cages and were provided with pellet feed (Tetragon Chemie Pvt. Ltd., Howrah, West Bengal) and water *ad libitum*. The mice were used for isolation of *T. evansi*. Whereas 25 out of 70 rats were used for maintenance of the *T. evansi* isolate prior to and during the experiment and the remaining 45 rats were used for the experiment.

Isolation and maintenance of *T. evansi*

Collection of buffalo blood and inoculation in mice: With the help of a local veterinarian at Howrah, West Bengal five buffaloes clinically suspected for 'surra' were identified. Following aseptic measures one ml. of blood from each buffalo was drawn by disposable syringe and mixed with equal volume of Alsever's solution in separate vials (Soodan et al. 1995; Bhatia 2000). Two mice for each buffalo were intraperitoneally inoculated with 0.25 ml of the buffalo blood with the help of an insulin syringe. The mice were then brought to the laboratory for subsequent observation.

Examination of mice for appearance of parasitaemia: Blood sample of each inoculated mouse was examined by wet blood film (WBF) examination for presence of *T. evansi* parasites from 20 hours post infection (P.I.). The blood samples showing actively motile organisms with characteristic flagellar movement was considered as positive for the presence of *T. evansi*.

Maintenance of *T. evansi* isolate: Four to five drops (approximately 0.25 ml) of blood from the mouse detected positive for *T. evansi* was collected by tail pricking and diluted with equal volume of Alsever's solution. The diluted blood in equal volume (0.25 ml) was then intraperitoneally inoculated in two rats with the help of an insulin syringe. The blood from inoculated rats was examined daily for appearance of parasitaemia. Sub-inoculation of the blood from such rat was done at 72–96 hrs. intervals (when the parasitaemia was at the peak) till the end of the study.

Grouping of experimental rats: For the present experiment 45 Sprague Dawley rats were used. They were divided into three equal groups i.e. Gr. I to III. Group- I was kept as uninfected untreated control, Group-II as infected untreated control. Group-II and III were used for

infection with *T. evansi*. Subsequently, when teeming level of parasitaemia was noticed the rats of Gr. III were treated with isometamidium-HCl (I/P) at the dose rate of 1mg/ kg. body weight.

Artificial infection of rats with *T. evansi*: With the help of a disposable syringe five ml. of blood from two rats showing peak level of parasitaemia was collected aseptically by cardiac puncture and equal volume of Alsever's solution was added. The rats of Gr. II and Gr. III were inoculated (I/P) with 0.25 ml (10^4 number of parasites) with the help of a sterile insulin syringe.

Administration of Isometamidium-HCl in rats: The generic drug, Isometamidium-HCl obtained from Arochem Industries, Mumbai, India was evaluated in the infected groups of rats i.e. Gr.III, at the dose rate of 1 mg per kg body weight intraperitoneally. The drug was dissolved in the required volume of sterile water and when teeming level of parasitaemia was observed at about 72 hours post infection, the stipulated dose was administered in rats of Gr.III.

Monitoring of parasitaemia: Periodic examination of WBF of the rats in infected untreated group and those in the treated groups was done following treatment with Isometamidium-HCl. till 35 day post treatment (DPT) for detecting the presence of parasites.

Haematological/biochemical studies: For haematological /biochemical studies, blood and serum were collected from five rats of all the groups on two occasions i.e. on 3rd DPI and on 7th day post treatment (DPT).

Haemoglobin concentration (Hb): Haemoglobin concentration was estimated by Sahli's acid haematin method (Schalm et al. 1986) and the value is expressed in gram per deciliter (gm/dl).

Packed cell volume (PCV): Packed cell volume was estimated by Wintrobe's haematocrit method (Schalm et al. 1986) and the value is expressed in percentage (%) of total volume.

Total erythrocytes (TEC) and leucocytes count (TLC): TEC and TLC were measured by haemocytometer (Jain, 1993) and the values are expressed in million per cubic millimeter ($\times 10^6/\text{mm}^3$) and thousand per cubic millimeter ($\times 10^3/\text{mm}^3$), respectively.

Serum alanine and aspartate amino-transferase (ALT and AST) activities: Serum AST and ALT activities were estimated spectrophotometrically using 2,4-dinitro phenylhydramine (2,4- DNPH) according to the method of (Toro and Ackerman, 1975) and the values are expressed as international unit / litter (IU/L).

Serum glucose concentration: Serum glucose level was estimated spectrophotometrically by methods of Tietz 1976 and the value is expressed as gm/dl.

Creatinine (CRT) and blood urea nitrogen (BUN): Concentrations of BUN and CRT were estimated as per the methods described by Skeggs (1957), and Folin and Wu (1919), respectively and the values are expressed as mg/dl.

Statistical analysis: All the parameters for each group on different post-infection days were compared (Analyze-Compare Means) for obtaining the mean value along with standard error (S.E). Then they were analyzed separately (i.e. between groups and between post-infection days) by Duncan method (One way ANOVA) and the significance (p value) was recorded at 5 % ($p < 0.05$) level and 1 % ($p < 0.01$) level.

The complete statistical analysis was done with the help of Statistical package for Social Scientist (SPSS), Windows Version 10.0.

Results

Trypanocidal efficacy of Isometamidium - HCl in rats: *T. evansi* infection resulted 100% mortality in rats of Gr. II after 3 day post infection. In *T. evansi* infection of rat, Isometamidium-HCl at the dose rate of 1.0 mg/kg b.wt. was 90 per cent effective in preventing the mortality till 35 day post treatment. All the surviving rats were negative on wet blood film examination during the experimental period.

Haematological impact

Haemoglobin concentration: Trypanosomosis had significantly negative impact on Hb values of infected rats. The rats of Gr. II and Gr. III had significantly ($P < 0.01$)

lower Hb values than that of uninfected control (Gr. I) on 3 DPI (Table-1). Isometamidium treatment resulted in 12.7% improvement in Hb values ($P < 0.01$) of Gr. III on 7 DPT compared to 3 DPI while, the value was 6.06% ($P < 0.05$) lower than that of uninfected control rats.

Packed cell volume (PCV): *T. evansi* infection significantly ($P < 0.05$) reduced PCV in infected control and infected treated rats compared healthy control group of rat on 3 DPI. Packed cell volume of Gr. III significantly ($P < 0.01$) increased (20%) on 7 DPT as compared to pre-treatment value and it was also 13.5% higher ($P < 0.01$) than healthy rats (Table-1).

Total erythrocyte count (TEC): There was no significant ($P > 0.05$) difference in TEC values in different experimental groups during the study period (Table 1). The TEC values in Gr. III were lower than that of Gr. I on 3 DPI but this reduction was not statistically significant. There was 15.3% improvement ($P > 0.05$) in TEC values in Gr. III on 7 DPT as compared to 3 day post infection.

Total leucocytes count (TLC): Experimental trypanosomosis produced severe negative impact on TLC values in infected and infected treated rats (Table 2). The TLC values of Gr. II and Gr. III were significantly ($P < 0.01$) lower compared to control rats (Gr. I) during the entire study period. There was 52.5% reduction in TLC values of Gr. III as compared to Gr. I on 7 day post treatment. In infected treated groups of rats (Gr. III) TLC values did not differ significantly between pre and post-treatment study period.

Serum biochemical impact

Serum AST and ALT activities: Serum AST and ALT activities in infected and infected treated groups of rats were significantly ($P < 0.01$) higher compared to healthy rats during the study period (Table 2). There was significant

Table 1 Hb concentration, PCV and TEC levels during *T. evansi* infection in rats

	Hb (gm/ dl)				PCV (%)				TEC (X10 ⁶ / cmm)			
	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	P value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	P value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	P value
3 Day Post Infection	13.00 ^{xa} ± 0.14	10.00 ^c ± 0.26	11.00 ^{yb} ± 0.26	$P < 0.01$	36.00 ^{xa} ± 0.71	29.50 ^{cd} ± 0.76	35.00 ^{yab} ± 1.38	$P < 0.01$	4.50 ^{xa} ± 0.35	4.40 ^a ± 0.39	3.90 ^{xa} ± 0.31	$P > 0.05$
7 Day Post Treatment	13.20 ^{xa} ± 0.18	No rats survived	12.40 ^{xb} ± 0.18	$P < 0.01$	37.00 ^{xbc} ± 0.71	No rats survived	42.00 ^{xa} ± 0.89	$P < 0.01$	4.60 ^{xa} ± 0.28	No rats survived	4.50 ^{xa} ± 0.28	$P > 0.05$
P value	$P > 0.05$		$P < 0.01$		$P > 0.05$		$P > 0.05$		$P > 0.05$		$P > 0.05$	

Values bearing different superscript i.e. x, y, in a column and a, b, c... in a row differ significantly ($P < 0.05$)

Table 2 The effect of *T. evansi* infection on TLC, serum AST and ALT activities in rats

	TLC (10 ³ /cmm)				Serum AST activity (IU/ lit)				Serum ALT activity (IU/ lit)			
	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value
3 Day Post Infection	12.60 ^{xa} ± 0.31	5.60 ^b ± 0.27	5.60 ^{xb} ± 0.33	<i>P</i> < 0.01	25.00 ^{yc} ± 0.58	29.00 ^b ± 0.46	31.00 ^{ya} ± 0.42	<i>P</i> < 0.01	34.00 ^{xc} ± 0.33	38.00 ^a ± 0.94	36.00 ^{yb} ± 0.66	<i>P</i> < 0.01
7 Day Post Treatment	11.80 ^{xa} ± 0.31	No rats survived	5.60 ^{xb} ± 0.30	<i>P</i> < 0.01	28.00 ^{xd} ± 0.47	No rats survived	312.00 ^{xa} ± 0.58	<i>P</i> < 0.01	35.00 ^{xc} ± 0.37	No rats survived	98.00 ^{xa} ± 0.35	<i>P</i> < 0.01
<i>P</i> value	<i>P</i> > 0.05		<i>P</i> > 0.05		<i>P</i> < 0.05		<i>P</i> < 0.01		<i>P</i> > 0.05		<i>P</i> < 0.01	

Values bearing different superscript i.e. x, y.. in a column and a, b, c... in a row differ significantly (*P* < 0.05)

(*P* < 0.01) increase in AST and ALT activities in Gr. III on 7 DPT as compared to pre-treatment values. There were 1014.3% increase in serum AST activities and 180% increase in serum ALT activities in Gr. III than that of Gr. I on 7 day post treatment.

Serum glucose concentration: The blood glucose level of infected (Gr. II) and infected treated (Gr. III) rats declined significantly (*P* < 0.01) compared to healthy rats (Gr. I) both during pre-treatment (3 DPI) and post-treatment (7 DPT) study period. The blood glucose level of infected treated rats increased (17.6%) significantly (*P* < 0.01) on 7 DPT compared to 3 DPI but this value was 11.11% lower than healthy control rats (Table 3).

Serum creatinine (CRT) concentration: The serum concentration of creatinine increased significantly (*P* < 0.05) in rats of Gr. II and Gr. III on 3 DPI (Table 3). Infected treated rats (Gr. III) had 42.5% lower (*P* < 0.01) value of serum creatinine compared to healthy rats (Gr. I) on 7 DPT. There was 4.8% reduction (*P* < 0.05) in serum creatinine concentration of Gr. III on 7 DPT compared to the value recorded on 3 day post infection.

Blood Urea Nitrogen (BUN) level: The BUN level of healthy rats was significantly lower (*P* < 0.01) as compared to infected control (Gr. II) and infected treated groups (Gr. III) during the study period (Table 3). On 7 day post treatment BUN level of Gr. III reduced 4.3% than that of pre-treatment value and 46.7% decreased compared to healthy control rats.

Discussion

Isometamidium at intraperitoneal administration of 1 mg/kg body weight gave 90% anti-trypanosomal efficacy and the parasitaemia remained suppressed till 35 day post treatment. This finding did not commensurate with the earlier report (Toro et al., 1983) wherein the drug even upto 10 mg/kg body weight could not prevent mortality. This discrepancy could be attributed to the strain variation of rats in susceptibility to the parasite and strain variation of the parasite in susceptibility to the drug.

Haemoglobin concentration of the infected-treated rats decreased significantly as compared to healthy control, both during pre-treatment and post-treatment period. This

Table 3 The effect of *T. evansi* infection on Glucose, Creatinine and BUN levels in rats

	Glucose (mg/ dl)				Creatinine (mg/ dl)				BUN (mg/ dl)			
	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value	Gr. I (n = 5)	Gr. II (n = 5)	Gr. III (n = 5)	<i>P</i> value
3 Day Post Infection	90.00 ^{xa} ± 0.39	70.00 ^c ± 0.55	68.00 ^{yd} ± 0.57	<i>P</i> < 0.01	1.50 ^{xa} ± 0.12	1.80 ^a ± 0.12	1.80 ^{xa} ± 0.15	<i>P</i> > 0.05	15.00 ^{xc} ± 0.62	21.00 ^b ± 0.47	23.00 ^{xa} ± 0.57	<i>P</i> < 0.01
7 Day Post Treatment	90.00 ^{xa} ± 0.39	No rats survived	80.00 ^{xb} ± 0.40	<i>P</i> < 0.01	1.60 ^{xb} ± 0.13	No rats survived	0.92 ^{yc} ± 0.17	<i>P</i> < 0.01	15.00 ^{xc} ± 0.46	No rats survived	22.00 ^{xa} ± 0.65	<i>P</i> < 0.01
<i>P</i> value	<i>P</i> > 0.05		<i>P</i> < 0.01		<i>P</i> > 0.05		<i>P</i> < 0.05		<i>P</i> > 0.01		<i>P</i> > 0.05	

Values bearing different superscript i.e. x, y.. in a column and a, b, c... in a row differ significantly (*P* < 0.05)

finding commensurate with those of Prasad and Laha (1990), Chidi and Nnamdi (2001) and Adedapo et al. (2005). After treatment with Isometamidium the PCV value of Gr. III animals increased significantly ($P < 0.01$) compared to healthy rats as well as pre-treatment study period. The TEC value of Gr. III did not differ significantly during the study period. There might be normocytic-hypochromic anaemia in which haemoglobin synthesis in the rats was disturbed due to *Trypanosoma* infection but the normal rate of haemopoiesis was maintained. Anaemia might be due to haemodilution which could result from increased production of plasma immunoglobulin and a decrease in intravascular colloid-osmotic pressure (Bhatia 2000). In infected rats anaemia might be associated with coagulation disorders such as thrombocytopaenia and disseminated intravascular coagulation. (Boreham 1974). In rats of Gr. III, Hb and PCV increased significantly on 7 DPT compared to 3DPI. This increase might be due to superior efficacy of Isometamidium hydrochloride.

The total leukocyte count in the infected-treated rats decreased significantly compared to healthy rats during the study period. Severe leucopenia in trypanosomosis was also recorded earlier (Samaddar and Gill 1962; Samaddar 1969 and Silva et al. 1993). Trypanosomosis is well known as an immuno-suppressive disease (Soulsby, 1982) and changed surface glycoprotein of *Trypanosoma* repeatedly stimulates the immune system (Levine 1985) and thereby cause immune-exhaustion in the infected animals. Leucopenia in the experimental rats might be due to immune exhaustion, which caused reduction of leucocytes (lymphocytes and neutrophils), the major component of cell-mediated immune response.

Serum AST and ALT activities increased significantly in the infected-treated rats during the study period. Increased enzyme activities in *T. evansi* infection were also reported earlier (El Gendi and El Sayed 1984; Adedapo et al. 2005). The increased level of these two enzymes might be due to damage of liver tissue (Jones et al. 1982; Kaneko et al. 1997) in the infected rats.

Increased levels of serum creatinine (CRT) and BUN due to infection occur in *T. evansi* infection have been reported by Singla et al. (2004) and Adedapo et al. (2005). This may possibly be due to their increased production in the liver and/or decreased excretion through the glomerular filters which might have resulted from hepatotoxicity and nephrotoxicity (Gill 1977) due to parasite derived toxic factors. Isometamidium treatment could reverse this effect.

The drug although could lower the CRT level, the BUN level was remarkably elevated. Involvement of parasite factor in such elevated BUN level could be over ruled by the drug induced factor causing nephrotoxicity.

Blood glucose level of all the three infected-treated groups of rats decreased significantly as compared to healthy control rats during the study period. Hypoglycaemia due to trypanosomosis in the present study substantiated the finding of Sen et al. (1959) and Patel and Herangal (1985). Hypoglycaemia in the present study might be due to the rapid consumption of sugar by trypanosomes (Soulsby 1982) and disturbed sugar metabolism (Gill 1977) in the infected rats.

In rat model Isometamidium only at 1 mg/kg b.wt. gives the desired anti-trypanosomal efficacy by preventing mortality in 90 % of the infected rats till 35 day post treatment. The anaemia due to *T. evansi* infection in rat can be successfully cured by single such treatment with Isometamidium-HCl. Hepatotoxicity caused by *T. evansi* infection is aggravated by Isometamidium such treatment as revealed by elevated enzyme activities and the drug did not produce nephrotoxicity as revealed by kidney function test. Isometamidium-HCl could therefore be a promising alternative for the control of trypanosomosis in Indian livestock after its systematic evaluation in the target livestock species.

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