



Nucleotide content in spleen of male Swiss albino mice during experimental Ancylostomiasis

Md. Arjuman Akhil¹ and V. Viveka Vardhani^{2*}

¹Department of Biochemistry, Acharya Nagarjuna University, Nagarjunanagar-522 510, Andhra Pradesh. INDIA

²Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjunanagar-522 510, Andhra Pradesh. INDIA

E-mail: vadlamudi_vv@yahoo.co.in

ABSTRACT

Three groups (10 in each) (6 to 8 weeks old) of experimental mice were orally infected each with 500 (group A) 1000 (group B) and 2000 (group C) infective larvae of *Ancylostoma caninum*. Group (D) was kept as uninfected control for comparison. Two mice from each of the experimental groups were sacrificed on day 1, 4, 9, 16 and 30 after infection. Two mice from control group were also sacrificed on the same designated days. Spleen was separated and the content of DNA and RNA was estimated following standard methods. DNA and RNA levels in spleen of experimental groups (A, B and C) are lower and higher than control group during 1 to 30 days of infection respectively. The synthesis of DNA and RNA was influenced by three varied doses of infection; the occurrence of abnormal physiological changes in spleen indicates the influence of infection in the lymphoid organ.

Keywords: DNA, RNA, *Ancylostoma caninum*, Infection, Spleen.

INTRODUCTION

Approximately 24% of the world's human population (over 1.3 billion people) is found to be infected with (WHO, 2002) hookworms (*Ancylostoma duodenale* and *Necator Americanus*). *Ancylostoma caninum* (Canine hookworm) is the best studied of all hookworms. Children and young adults are at greater high risk of intestinal helminthosis (Hotez, 2004). Helminthic infections are identified with poor sanitation (Wongstitwilairoons *et al.*, 2007). Socio-demographic variables have associated with a

particular helminthic infection (Belizario, 2011; Batbatan, 2012).

Infective larvae of hookworms exhibits burrowing behaviour at 37°C suggesting that the larvae attracted to the body heat of host animal (Bhopale *et al.*, 2001) and also dissolved gases like CO₂ and H₂CO₃ (Peteronujevic and Rogers, 1983), which are released by host during respiration (Vetter *et al.*, 1985). The main pathology associated with hookworm infection is the loss of blood and anemia, diarrhoea and intestinal pain, which is specifically problematic in immunocompromised humans and animals because they are not able to eliminate the hookworms as effectively as individually with normally functioning immune system (Crompton and Savioli, 1993; Albonico *et al.*, 1999). Anemia, anorexia, emaciation and weakness developed in chronic diseases of ancylostomiasis (Gonta, 1993).

The adult hookworm live in the small intestine of dog (natural host). It was found that during

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ancylostomiasis in mice the migratory behaviour and survival pattern of larvae was influenced by the passive immunity acquired with the transfer of sensitized peritoneal exudates cells (Vardhani and Johri, 1983).

In mammals spleen is the major lymphoid organ and it is an important hemopoietic organ. Spleen possess a combination of phagocytic and antibody forming activities which are of great importance in immunity to organisms or antigen which get into blood (Taliaferro, 1956). No information is known on the changes in nucleotide content during hookworm infection in lymphoid organs. The present investigation is designed to study the levels of DNA and RNA in spleen of male swiss albino mice during *A. caninum* infection.

MATERIALS AND METHODS

Infective larva of *A. caninum* were obtained from an infected dog and doses of inoculum was prepared by method of Sen *et al.*, (1965) and Scott (1928). Experiments were conducted with male swiss albino mice (body weight 25-31 g) and infected per OS. Three groups A, B and C of mice 10 in each were infected with 500, 1000 and 2000 larvae respectively. A separate group (D) of mice (10) was kept as uninfected control. Two animals from each infected and control groups were sacrificed on day 1, 4, 9, 16 and 30 of experimental period. Spleen samples from infected and uninfected mice were taken and analysed for the estimation of DNA and RNA by methods of Diphenylamine method Burton (1956) and Orcinol method.

RESULTS

Group-A (500 dose):

DNA content:

The content of DNA was found to be lower than the controls throughout the experimental period. The increase of DNA was in ascending fashion from day 1 to 9 (peak value at day 9) but declined on day 16 (which was still lower than control). On day 30, the value of DNA was 1.82 µg/mg (Table-1).

RNA content:

The level of RNA in experimental mice was found to be higher than control throughout the experimental tenure (except on day 16 - 2.49 µg/mg). The level of RNA showed gradual rise from day 1 (3.47 µg/mg) to 9 (4.29 µg/mg) which was a zenith point; the RNA level decreased on day 16 (2.49 µg/mg) (less than control) and slightly increased on day 30 3.66 µg/mg.

Group-B (1000 dose):

DNA content:

The content of DNA was found to be increased in experimental mice throughout the experimental period (exception day 16 (4.66 µg/mg) when compared with controls (Table-1).

RNA content:

The level of RNA increased gradually from day 1 (3.62 µg/mg) to 9 (4.74 µg/mg) and decreased on day 16 (3.43 µg/mg) and 30 (3.33 µg/mg) (still RNA levels found to be higher than control group).

Table 1. DNA (µg/mg) and RNA (µg/mg) content in the spleen of central (group D, uninfected) and infected (group A, 500 dose; B, 1000 dose; group C, 2000 dose) mice at different days of experiment. Values of expressed in the mean derived from 5 observations.

Days of Necropsy	Experimental groups						Control group uninfected	
	Group A		Group B		Group C		Group D	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
1	1.80	3.47	2.00	3.62	2.40	3.84	2.67	3.0
4	1.86	3.80	2.05	4.19	2.47	3.95	2.66	3.1
9	1.99	4.56	2.20	4.74	2.39	4.90	2.67	3.0
16	1.51	2.51	4.66	3.43	2.32	4.09	2.66	3.1
30	1.82	3.66	1.88	3.33	2.30	3.82	2.67	3.0

Table 2. 't' values obtained for experimental (Infected with 500, A; 1000, B and 2000, C) doses of *A. caninum* (larvae/mouse) and control (uninfected D) group of mice.

Spleen	Experimental groups			Control groups
	A	B	C	N
DNA :				
Mean :	1.79	2.55	2.37	2.66
t-value :	A D	B D	C D	
	_____	_____	_____	
	t= 11.03*	t= 0.20@	t= 8.65*	
	A B	A C	B C	
	_____	_____	_____	
	t= 1.42@	t= 10.51*	t= 0.33*	
RNA :				
Mean :	3.60	3.86	4.12	3.04
t-value :	A D	B D	C D	
	_____	_____	_____	
	t= 1.69@	t= 3.08*	t= 5.17*	
	A B	A C	B C	
	_____	_____	_____	
	t= 0.61@	t= 1.37@	t= 0.79@	

t' value at 5% level of significance is 2.306

*Statistically significant value

@Statistically non-significant values

Group-C (2000 dose):

DNA content:

The content of DNA in the experimental mice was found to be lower than the control from day 1 (2.40 µg/mg) to 30 (2.30 µg/mg) except on day 4 (2.47 µg/mg).

RNA content:

The level of RNA in test mice was found to be higher than control throughout the experimental period. There was a slight increase from day 1 (3.84 µg/mg) to 4 (3.95 µg/mg) and on day 30 (3.82 µg/mg); peak response was noticed on day 9 (4.90 µg/mg).

The mean values of DNA and RNA from spleen and their 't' values for 30 days of infection period is shown in [table 2](#). The decreased DNA levels was statistically significant in group A when compared with group C. Significant increase in group B when compared with control (D), and in between groups A and B, and B and C.

The increased RNA levels were statistically significant in groups and non-significant in group A significant in groups B and C when compared with control (D); no significant different

difference was found when compared among themselves.

DISCUSSION

The increase of RNA and decrease of DNA (with few experiments) in spleen (in all the three experimental groups) suggests the occurrence of abnormal metabolic changes due to the pathogenic effects of various single oral doses of infective larvae. The major cause that impair DNA in mammals tissues are due to the large amount of reactive oxygen mutagen species generated by activated lymphocytes, inflammation and oxidative stress (Schwizer, 1996; Beekman and Ames, 1997; Tarakalakshmi, Y and Viveka Vardhani, V, 2014). Dukan and Nystrom (1999) suggested the denaturation of DNA due to their reaction with reactive oxygen species/free radicals. Flagg *et al.*, (1994) explained that melonaldehyde (one of the end product of lipid peroxidation) reacts with nucleic acid base of DNA resulting in tissue damage and breakage of DNA and RNA. The change in the DNA and RNA activity reveals the strong association between the synthesis of nucleic acids and ancylostomiasis which is also connected to oxidative injury.

Many studies of both human and laboratory animals have documented association between impaired different DNA and RNA activity and various diseases like lung cancer, leukaemia and malaria influenced by oxidative stress (Ye and Song, 2005; Chikezie *et al.*, 2009 and Tarakalakshmi, Y and Viveka Vardhani, V, 2014).

In the present study, the oxidative stress caused due to infection might have resulted in bringing alteration in the level of nucleic acids in spleen. The alteration in the level of DNA and RNA in the spleen of all the three groups was not in accordance with the number of larvae administered but did depend on the period of infection, this would explain the involvement of lymphoid organs in the sensitized mouse system (due to host parasitic interaction). This has been supported by the work of Vardhani (2003) who reported significant changes in serum enzymes during ancylostomiasis in mice.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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