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SERUM CHOLESTEROL IN IMMUNOSTIMULATED MICE DURING INDUCED HEPATITIS B

V. JASMIN GOLD AND V. VIVEKA VARDHANI*
Department of Zoology and Aquaculture,
Acharya Nagarjuna University, Nagarjuna Nagar, Guntur - 522 510, A.P., INDIA
e-mail: vadlamudi_vv@yahoo.co.in

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***Corresponding author**

ABSTRACT
The level of serum cholesterol showed considerable decrease during the entire experimental period (1 to 5 days) of infection in all experimental groups (immunostimulated + vaccinated) of mice when compared with controls (group c, unimmunostimulated + unvaccinated; group I, immunostimualted). The decrease of serum cholesterol was influenced by the retained Gen Vac B Hbs Ag infective virus and the immunological damage caused by the infectious viral organisms within the host system.

INTRODUCTION

The recent decades shined out by producing novel category of drugs known as immunostimulants, which reinforce and activate the immune system (Petrunov *et al.,* 2007). Immunoboosters are employed in experimental animals as alternative to various drugs composed of chemicals and antibiotics (Gelina *et al.,* 2009). A number of commercially available immunostimulants are being used in aquaculture, poultry science, animal husbandary, live stock management apart from their immense usage in medical practices for health prospective towards mankind (Gautam *et al.,* 2008). In the present investigations, a novel immunostimulent, Immunex DS (IDS) has been employed. IDS is unique and endeavored with essential immuno enhancers like beta-carotenes, L-Lysine, Dl-methionine essential fatty acids, livamisol hydrochloride, vitamins like A, D3, E, C and B12 minerals like zinc, cobalt, manganese, selenium and probiotics like bacillus and yeast. IDS is able to enhance body's immune response. It increases the weight of the body by boosting up the natural stamina and vital energy. IDS prevents the colonization of the bacteria and accumulation of pathogenic toxins in gastro-intestinal tract (Rao *et al.,* 2010). Hepatitis B accounts for 0.5 to 1.2 million deaths annually and is the most common cause to increase the risk of hepatocelluar carcinoma (Beasley et al., 1981; Milich and Liang, 2003; Tong *et al.,* 2005). Acute infection of hepatitis B initiated with general ill-health, loss of appetite, anorexia, arthalgia, gall bladder obstruction, severe jaundice, malena condition of stools and abdominal pain. Chronic infection with hepatitis B is manifested by heavy inflammation of liver, cirrhosis and heavy fat depositions on the surface of liver (Wong and Goh, 2006; Risco *et al.,* 2009; Yeh *et al.,* 1989). Hepatitis B is the leading cause of liver cancer in the world and frequently leads to cirrhosis and liver failure. The currently available antiviral drugs are unable to eradicate hepatitis B virus (HBV) infection because of the non-protective immune response against infected hepatocytes and the persistence of viral covalently cloud circular DNA (CCC DNA) in the liver of infected patients. Goals of treatment are to suppress viral replication to the lowest possible level, and thereby to halt progression of liver disease and the onset of complications can be prevented by treating them with immunostimulants then by raising the resistance.

Serum cholesterol is a soft, waxy substance found in the blood and cells of the body. It is in fact an integral part of cell membranes and is a vital ingredient in many physiological processes. The two main types of cholesterol include: LDL (low density lipoprotein) cholesterol (bad cholesterol) and HDL (high density lipoprotein) cholesterol (good cholesterol). The presence of good cholesterol is beneficial in maintaining organ health and providing the body with necessary energy; the presence of bad cholesterol can lead to blockages in arteries and heart and lungs problems. For this reason, the medical community has developed standards to understand a healthy level of good cholesterol in the blood. Diet high in cholesterol and saturated fatty acids could significantly elevate plasma cholesterol levels and also increase the risk of cardiovascular disease. Repeated systemic *Escherichia coli* in conjunction

with hypercholesterolemia, leads to development of oxidative stress in mice (Dutta, *et al*., 2009). Serum cholesterol levels were elevated in high cholesterol diet fed mice, compared to those fed with normal laboratory diet. Alvarez and Ramos (1986) studied the alterations in the concentrations of total cholesterol, high-density lipoprotein cholesterol, tryglycerides and apoproteins A and B in serum of 54 patients in Spain. Sepsis caused the concentrations of total cholesterol, high density lipoprotein cholesterol and apoproteins A and B in serum to decrease, whereas, tryglycerides increased. However, these changes are not related to the infectious agent, in the underlying illness or the clinical situation of the patients. Wattanasuwan, *et al*. (2001) evaluated the effect of acute myocardial infraction on the total cholesterol/HDL cholesterol ratio on the low density lipoprotein cholesterol / high density lipoprotein cholesterol ratio in humans and found that acute myocardial infraction does not effect cholesterol ratios. Biochemical characterization of the serum and aorta in inbred C57BL/6Cr mice fed with high cholesterol diet was investigated by Kunitomo *et al*. (1984). Serum total cholesterol and free cholesterol levels of mice fed with the high cholesterol diet were increased about 80% and 110% respectively, compared with the control.

Gene Vac B (Recombinant Hepatitis-B vaccine) is a non infectious recombinant DNA hepatitis B vaccine; it contains purified surface antigens of the virus obtained by culturing genetically engineered Hansenula polymorpha yeast cells having the surface antigen gene of the Hepatitis B Virus. The Hepatitis-B surface antigen (HBs Ag) expressed in the cells of Hansenula polymorpha is purified through several chemical steps and formulated as a suspension of the antigen adsorbed on aluminium hydroxide and thiomersal is added as preservative. No information is available on the level of serum cholesterol in mice treated with immunostimulant during hepatitis. Hence, a new vista has been opened to estimate the level of serum cholesterol in mice treated with IDS during the induction of hepatitis.

MATERIALS AND METHODS

Male swiss albino mice *(Mus musculus albinus)* (6-8 weeks; 23-31g) were employed in the present study. They were fed with standard balanced diet and water *ad libitum* and taken care according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiments in animals). Proper acclimatization, care, housing and hygiene were properly maintained. Eight groups (10 in each group) of mice were maintained. Six groups of each mice (A, B, C, D, E and F) were orally intubated with a single dose of 150 mg Immunex DS (IDS) / on day 0 and waited for 72 hours, and vaccinated with various single doses of Gen Vac B Hbs Ag vaccine (A, 0.07 mL/mouse; B, 0.01mL/mouse; C, 0.2mL/mouse; D 0.4mL/mouse; E, 0.8mL/mouse; F, 1.0mL/mouse) intramuscularly. One group (I) of mice was intubated orally with single dose of 150mg of IDS/mouse and another group (c) which was neither immunostimulated nor vaccinated served as normal controls for comparison. Later from day 11 to 15, the experimental mice were sacrificed along with the mice of IDS treated (along group I) and normal ones (group c). Blood was collected and serum was separated following standard method.

Table 1: Serum Cholesterol (mg/dl) in control (group C), immunostimulated (group I) and experimental (groups A, B, C, D, E and F) male swiss albino mice at different days of experimental period. Values are expressed in mean derived from 5 observations

Days of Necropsy	Group c (untreated and uninfected)	Group I (treated with IDS @150 mg/ mouse and unvaccinated)	Group A (150 mg of IDS/mouse and infected with 0.07 mL of Hbs Ag/ mouse)	Group B (150 mg of IDS/mouse and infected with 0.1 mL of Hbs Ag/ mouse)	Group C (150 mg of IDS/mouse and infected with 0.2 mL of Hbs Ag/ mouse)	Group D (150 mg of IDS/mouse and infected with 0.4 mL of Hbs Ag/ mouse)	Group E (150 mg of IDS/mouse and infected with 0.8 mL of Hbs Ag/ mouse)	Group F (150mg of IDS/mouse and infected with 1mL of Hbs Ag/ mouse)
1	177.3	588.9	111	110	57	106	120	95
2	174	584	112	112	58	107	121	96
3	170	582	114	114	59	108	122	98
4	178	586	116	115	60	110	124	102
5	172	580	117	116	61	111	125	106

Table 2: "t" values obtained in different experimental groups (A, B, C, D, E and F) of mice. Experimental groups

	Experimental groups						Control groups	
	A	B	C	D	E	F	c	I
Mean	114.0	113.4	59.0	108.4	122.4	99.4	174.2	584.1
t value	A c		B c		C c			
	t = 35.0*		t = 36.8*		t = 79.0*			
	D c		E c		F c			
	t = 41.3*		t = 31.2*		t = 33.6*			
	A I		B I		C I			
	t = 275.0*		t = 285.9*		t = 360.7*			
	D I		E I		F I			
	t = 300.0*		t = 280.4*		t = 218.6*			
	A B		A C		A D		A E	A F
	t = 0.31@		t = 48.1*		t = 4.0*		t = 6.0*	t = 7.1*
	B C		B D		B E		B F	
	t = 50.5*		t = 4.1*		t = 6.6*		t = 6.7*	
	C D		C E		C F			
	t = 48.6*		t = 30.3*		t = 21.1*			
	D E		D F		E F			
	t = 11.0*		t = 4.5*		t = 11.2*			

P value at 5% level of significance is 2.306*; * - statistically significant value; @- statistically non-significant value

Serum samples were analyzed for cholesterol following CHOD-PAP (cholesterol oxidase peroxidase) method.

RESULTS AND DISCUSSION

Results are shown in Table 1. In all the experimental groups (A, B, C, D, E and F) of mice treated with immunostimulant + different doses of vaccine, it is found that the level of serum cholesterol decreased markedly when compared with control (untreated with IDS + unvaccinated) (group c) and immunostimulated (treated with IDS) (Group I) mice throughout the experimental period (day 1 to 5). Also, it is found that mice which were treated with immunostimulant (Group I) showed higher values of cholesterol in comparison with controls (Group c) and other experimental groups (A, B, C, D, E and F) of mice from day 1 to 5 of experimental period.

Group A

The mice of group A which were immunostimulated with 150 mg of IDS and vaccinated with 0.07 mL of Gen Vac B Hbs Ag showed low levels of serum cholesterol throughout the experimental period than the IDS treated (group I) and normal mice (group c). The level of serum cholesterol on day 1 (111.0 mg/dL) is found to be increased progressively by day 5 (117.0 mg/dL) of experimental period; still these values are lower than controls (group c and I).

Group B

The mice of group B which were immunostimulated with 150 mg of IDS and vaccinated with 0.1mL of Gen Vac B Hbs Ag showed low levels of serum cholesterol than the group I and group c throughout the days of necropsy. The cholesterol activity was low. There was a progressive increase of serum cholesterol from day 1 (111.0 mg/dL) to 5 (116.0 mg/dL) of experimental period. However, these values were found to be below normal.

Group C

The mice of group C which were immunostimulated with 150 mg of IDS and vaccinated with 0.2 mL of Gen Vac B Hbs Ag showed lower levels of serum cholesterol than the immunostimulated (group I) and control (group c) mice throughout the experimental period. A gradual increase of cholesterol content was observed from day 1(57.0 mg/dl) to 5 (61.0 mg/dL) of experiment and these values were below normal range.

Group D

The mice of group D which were immunostimulated with 150 mg of IDS and vaccinated with 0.4 mL of Gen Vac B Hbs Ag showed lower levels of serum cholesterol than the immunostimulated mice (group I) and control mice (group c). The level of serum cholesterol on day 1 (106 mg/dl) is found to be increased progressively by day 5 (111.0 mg/dl); still these levels were below normal level.

Group E

The mice of group E which were treated with 150mg of IDS and vaccinated with 0.8 mL of Gen Vac B Hbs Ag showed low levels of cholesterol when compared with the immunostimulated (group I) and control (group c) mice throughout the days of experiment. A gradual increase of serum cholesterol value (below normal value) was vividly observed from day 1 (120.0 mg/dL) to 5 (125.0 mg/dL).

Group F

The mice of group F which were treated with 150 mg of IDS and vaccinated with 1.0mL of Gen Vac B Hbs Ag revealed low levels of serum cholesterol throughout the days of experiment in comparison with IDS treated (group I) and normal (group c) mice. There was a progressive increase of cholesterol from day 1 (95.0mg/dL) to 5 (106.0 mg/dL) of experimental period. However, these values were below normal values.

Immune mechanisms against microbial infections may result in nutritional and metabolic disorders; these disorders inturn lead to alterations in the level of serum cholesterol as suggested by Kitagawa (1992) in mice during inflammation. Cabana *et al*. (1982) also suggested that changes in plasma lipids (due to microbial injury) may be another form of biochemical derangement that develops in the mammalian system responding to acute inflammation or tissue injury of gut in mice during Immunex DS treatment as suggested by Sakunthala (2012) confirm the present investigations that the synthesis of serum cholesterol disturbed in mice.The observations of serum cholesterol levels are in agreement with the decreased serum cholesterol levels in mice during parasitic infections (Khovidhunkit *et al.,* 2004) and *Moringa stenoptala* (folk medicine) treatment (Ghebreselassie (2011). It was reported that the mechanism of cholesterol reduction may be through the lowering of plasma concentrations of low density lipoprotein. Also, it was suggested that the decrease in cholesterol might be mainly as reflection of altered metabolic efficiency induced by the treatment of Immunex DS and vaccine. Microbial and parasitic infections which cause inflammation/injury may alter the level of serum cholesterol (Wang *et al.,* 2005; Spanakis *et al.,* 2006). An interesting feature in the present findings is that the experimental mice of group C (which received IDS @ 150 mg/mouse and inoculated with 0.2mL of Gen Vac B Hbs Ag) showed a marked decrease of serum cholesterol than the rest of all the experimental groups. The decrease of serum cholesterol in all the experimental groups of mice was not found to be dose dependent of vaccine. It is of interest to note that in groups A and B the level of serum cholesterol is exactly similar on day 1 (110.0 mg/dL), 2 (112.0 mg/dL) and 3(114.0 mg/dL) of experimental period. Comparatively higher level of cholesterol (still lower in comparison to control groups c and I) is recorded in group I on day 1 (120.0 mg/dL) to 5 (125.0 mg/dL) of experimental period. Statistical analysis (Table 2) showed significant decrease of serum cholesterol in all the experimental groups of mice when compared with controls (group c) and immunostimulated (group I) mice and among the experimental groups themselves (except the cholesterol level in between groups A and B).

The present findings clearly indicate that the administration of Gen Vac B vaccine results in decrease in the synthesis of cholesterol in mouse host. The decreased cholesterol levels in all the experimental groups of mice are attributed to the administrate dosage of vaccine in the host system and/or to the metabolic disturbances and immunobiochemical reactions of the host. Krishna Rao and Vardhani (1995) reported decrease of serum cholesterol in helminthic infection in mice (when there is low level of worm load in the host system). The present findings also suggest that the injection of vaccine (intramuscularly) might have caused shock in the muscle and eventually disturbing the cholesterol physiology.

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