

ALKALINE PHOSPHATASE AND ACID PHOSPHATASE LEVELS IN THE ABDOMINAL MUSCLES OF IMMUNOSTIMULATED MICE DURING HEPATITIS B

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ABSTRACT

The investigations were conducted in 8 groups of male Swiss Albino mice (6-8 wks old; 23-26 g wt). In mice of group I (10), Immunex DS (IDS) was orally administered (@150mg/mouse) as a single dose. In mice (10) of group A, B, C, D, E and F, IDS was given orally @150mg/mouse (single dose) (on day 0) and Gene Vac B vaccine was infected on day 4 of experiment @ 0.07ml/mouse, 0.1ml/mouse, 0.2ml/mouse 0.4ml/mouse, 0.8ml/mouse and 1ml/mouse. Another group (U) of mice (10) was kept untreated (with IDS and uninfected) as controls for comparison. Two mice from each experimental (A, B, C, D, E, and F) and control groups (U) were necropsied after day 7 of vaccine treatment. Abdominal muscle tissue was separated and analysed for total carbohydrate and total glycogen using standard methods. The level of total carbohydrate increased and glycogen decreased markedly from day 1 to 5 of experiment in group A. Mice of group B showed a decrease in the content of carbohydrate and glycogen on day 1 to 2 and a gradual increase from day 2 to 5 of experiment. In mice of group C, the level of carbohydrate decreased on day 1 and 3 and glycogen on day 1 and 2 with an increased content of carbohydrate and glycogen on day 2, 4, 5 and 3, 4 and 5. The content of carbohydrate and glycogen decreased on day 1, 2, 3 and increased on day 4, 5 in mice of group D. Mice of group E decreased level of carbohydrate on day 1, 3 and 4 and glycogen on day 1, 2 and 3 and an increased level of carbohydrate and glycogen on day 2 and 5 and 4 and 5. Mice of group F showed a gradual decrease in the level of carbohydrate from day 1 to 5 and and glycogen on day 1, 2 increased level of glycogen from day 3, 4 and 5 when compared with the control and immunostimulated mice group U and I. The altered level of carbohydrate and glycogen in all the experimental groups might be due to their disturbance in their metabolism.

Key words: ALP, ACP, Abdominal muscles, Mice, Immunostimulant, Hepatitis.

INTRODUCTION

Hepatitis B is a major global health problem. This disease is the leading cause of acute and chronic liver disease and there are 350 million people surviving as hepatitis B virus (HBV) carriers (Lavanchy, 2004 ; Milich and Liang 2003). Ogata et al., (1993) found that Hepatitis B e Antigen (HBeAg) can induce the disease and

acts as an immunoregulatory protein in chimpanzees. Induction of inflammatory response including innate as well as adaptive immune response directed by multiple proteins of HBV is found in an immunocompetent adult (Guidotti et al., 1996; Baron et al., 2002). One way to suppress inflammatory and/or stimulation of phagocytosis is the application of

immunostimulants which increase resistance to bacterial and viral infections by stimulating nonspecific immune mechanisms in mice (Petrunov et al., 2007). The protective effect of immunostimulant against various bacterial and viral infections has been demonstrated (Petrunov, 2004 a and b ; Petrunov et al., 1996). Increased activities of serum enzymes (Alkaline phosphatase, Aspartate transaminase) was found in rats treated with artesunate when compared with controls; both hepatotoxicity and hemotoxicity caused alteration in the level of serum enzymes (Omotuyi et al., 2008) Dehydroepiandrosterone (DHEA) treatment is found to be effective in altering the activities of tissue enzymes like phosphatases in mice and rats (Marrero et al., 1990). Studies on trypanosomiasis in rats treated with honey showed increase of serum alkaline phosphatase activity (Ekanem et al., 2005). Increase of AST and ALT, superoxide dismutase, catalase and glutathione peroxidase was found in liver and kidney of ethanol treated rats (Saravanan et al., 2002). Significant alteration was found in the level of phosphatases in heart, liver and skeletal muscles of mice treated with lead (Satyalatha and Vardhani, 2004). The relation between phosphorylase and glycogenolysis has well documented in abdominal muscles and liver of mice (Lyon and Porter, 1963; Beis and Newsholme, 1975). Enzymes like alkaline and acid phosphatase are produced predominantly in liver and also by several other parts of the body and their levels in serum/plasma are considered as markers for identifying the toxicity of that particular tissue (Wang et al., 1981; Chu and Lin, 1998). Alkaline phosphatase is the key enzyme involved in dephosphorylation of Lipopolysaccharides (LPS) during physiological conditions (Poelstra et al., 1997). Oral administration of sources of ALP was used for the treatment of various inflammatory disease of gastrointestinal tract. Intestinal Alkaline Phosphatase (IAP) detoxifies LPS and other bacterial toxins (Narisawa et al., 2003; Chen et al., 2010). Increased activity of ALP was reported in mice treated with a traditional herbal formulation triphala against induced hepatic toxicity (Sabina et al., 2013). Since the HB Virus induces the chronic disease leading to

much physiological disturbances in liver, serum and abdominal muscles, a new vista has been opened to study the level of phosphatases in the abdominal muscles of mice treated with immunostimulant during induced hepatitis.

MATERIALS AND METHODS

Male Swiss albino mice (*Mus musculus albinus*) (6 - 8 weeks old; 23 - 26g wt) were fed with standard balanced diet and water *ad libitum*. According to the guidelines of CPCSEA - proper acclimatization, care, housing and hygiene were properly maintained during the experimentation. Eight groups of experimental mice were selected in the present study. Immunex DS (IDS) (@150mg/mouse) was orally administered with the help of a syringe fitted with a 3 inch 16 gauze oral, blunt feeding needle in mice (ten) of group I. In mice (ten) of group A, IDS (@150mg/mouse) was orally administered on 0 day and Gen Vac B Vaccine (0.07 ml/mouse) was infected on day 4 of experiment. In mice (ten) of group B, C, D, E and F IDS (@150mg/mouse) was given orally on day 0 and Gen Vac B Vaccine (@0.1ml/mouse, 0.2ml/mouse, 0.4ml/mouse, 0.8ml/mouse and 1ml/mouse) was infected on day 4 of experiment. Another group (U) of mice (ten) was kept as controls for comparison (untreated with IDS + uninfected) Two mice from each of the experimental and control groups (after day 7 of vaccine treatment) were necropsied, abdominal muscle tissue was separated and analysed for phosphatases following the method of Bessey et al., (1946). Results were statistically analysed using student's t test.

RESULTS AND DISCUSSION

The observations in groups A, B, C, D, E and F are shown in Table 1. ALP and ACP levels showed considerable increase during the entire experimental period in all the experimental groups of mice when compared with controls. It is interesting to note that in mice treated with immunostimulant, the level of ALP and ACP almost remained constant from day 1 to 5 of experiment. Initially (on day 1) there was no difference in the activity of ALP and ACP in all the experimental groups (group A, B and C).

Table 1: Alkaline phosphatase (µmoles of PNP formed/min/mg of protein) and Acid phosphatase (µmoles of PNP formed/min/mg of protein) content in abdominal muscles of experimental. (Group A - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.07 ml/mouse), (Group B - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.1 ml/mouse), (Group C - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.2 ml/mouse), (Group D - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.4 ml/mouse), (Group E - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 0.8 ml/mouse), (Group F - treated with Immunex DS @ 150 mg/mouse and infected with Hbs Ag @ 1ml/mouse) and control (Group I - treated with Immunex DS @ 150 mg/mouse) (Group U - untreated and uninfected) male swiss albino mice at various days of experimental period.

DN	Group A		Group B		Group C		Group D		Group E		Group F		Group I		Group U	
	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
1	18.21	13.12	18.63	13.98	19.22	14.98	20.21	15.12	20.82	16.20	21.56	17.21	18.64	14.73	16.46	12.23
2	17.82	14.98	19.12	14.23	22.56	15.21	29.82	15.82	21.02	19.23	21.83	19.86	18.63	14.72	16.45	12.24
3	20.70	22.90	20.12	25.62	35.54	19.56	42.60	20.91	32.89	27.89	28.53	22.99	18.64	14.73	16.43	12.23
4	25.90	29.80	22.00	33.53	42.50	29.67	57.56	29.88	36.78	39.98	37.76	25.98	18.64	14.73	16.46	12.22
5	34.60	42.00	25.19	34.29	58.56	39.65	67.29	32.33	41.22	48.26	43.22	29.33	18.64	14.70	16.44	12.23

DN, Days of Necropsy ; ALP, Alkaline phosphatase ; ACP, Acid phosphatase.
 Values are expressed in the mean derived from 5 observations.

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

ALP	Experimental groups						Control groups	
	A	B	C	D	E	F	U	I
Mean	23.44	21.01	35.67	43.49	30.54	30.58	16.44	18.64
tvalues	A — U t= 2.23@		B — U t= 3.84*		C — U t= 2.70*			
	D — U t= 3.13*		E — U t= 3.40*		F — U t= 3.27*			
	A — I t= 1.12@		B — I t= 1.99@		C — I t= 2.39*			
	D — I t= 2.87*		E — I t= 2.86*		F — I t= 2.76*			
	A — B t= 0.65@		A — C t= 1.25@		A — D t= 1.86@		A — E t= 0.88@	
	B — C t= 2.02@		B — D t= 2.57*		B — E t= 2.21@		B — F t= 2.13@	
	C — D t= 0.69@		C — E t= 0.62@		C — F t= 0.61@			
	D — E t= 1.35@		D — F t= 1.33@					
	E — F t= 0.006@							

P value at 5% level of significance is 2.306.* - Statistically significant values. @ - Statistically non –significant values.

Table 3: t values obtained in different experimental groups (A, B, C, D, E and F) of mice

ACP Mean t values	Experimental groups				Control groups			
	A	B	C	D	E	F	U	I
	24.56	24.33	23.81	22.81	30.81	23.07	12.23	14.72
	A — U t = 2.33*		B — U t = 2.72*		C — U t = 2.42*			
	D — U t = 2.97*		E — U t = 2.96*		F — U t = 5.04*			
	A — I t = 2.77*		B — I t = 2.16@		C — I t = 1.90@			
	D — I t = 2.27@		E — I t = 2.34*		F — I t = 3.88*			
	A — B t = 0.16@		A — C t = 0.06@		A — D t = 0.13@		A — E t = 1.00@	A — F t = 0.09@
	B — C t = 0.07@		B — D t = 0.26@		B — E t = 0.79@		B — F t = 0.25@	
	C — D t = 0.16@		C — E t = 0.83@		C — F t = 0.14@			
	D — E t = 1.05@		D — F t = 0.06@					
	E — F t = 1.11@							

P value at 5% level of significance is 2.306.* - Statistically significant values. @ - Statistically non-significant values.

There was a gradual increase of ALP and ACP from day 1 - 5 of experiment in groups B and C (except the ALP value on day 2 in group B). Peak values of ALP and ACP were found on day 5 of experiment in both groups B and C. In case of groups D, E and F the activity of ALP increased from day 1 to 5 and maintained a constant value on day 1 and 2 in case of group F where as ACP activity increased from day 1 to 5 in groups E and F (except in group D on day 1 and 2 there was no change). The increase of enzymes may be influenced by the stress caused by immunostimulant in case of group I and due to the immunological stress by vaccine in case of

groups A, B, C, D, E and F. Statistical analysis showed (table 2 and 3) a significant increase of ALP and ACP in all the experimental groups (A, B, C, D, E and F) of mice when compared with controls (group U) and immunostimulated (group I) mice (except the level of ALP in group A when compared with group U and the level of ACP in groups B, C and D when compared with group I). Also, there was no significant difference in level of ALP and ACP in all the experimental groups when compared among themselves (except the level of ALP in group B when compared with group D).

Reduced tolerance to vaccination can result from inherited factors such as glycogenolytic defect in the muscle. Whether, and to what extent, these factors are responsible for altered enzyme activities in muscle is unknown.

There is an evidence, that it is possible for a muscle fiber to change its properties during new activities (Pette and Vrbova, 1989; Irintchev and Wering, 1987). The existence, of such an adaptive mechanism is reported in young rats (Kugelberg, 1976). It is interesting to consider such mechanisms playing a central role in the alteration of phosphatase levels in experimental mice treated with immunostimulant and/or vaccine. In the present investigation, the metabolic/pathogenic changes that occur in host immunity due to vaccine might have caused impairment in the synthesis of phosphatases. These results are in agreement with those of Satyalatha and Vardhani (2004) who also reported significant alteration in the level of phosphatases in mice treated with lead and also, in those treated with nematode infective larvae and lead.

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