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CARBOHYDRATE AND GLYCOGEN CONTENT IN THE ABDOMINAL MUSCLES OF MICE TREATED WITH IMMUNEX DS AND HEPATITIS B VACCINE

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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: *Channa punctatus*, *Lytocestus indicus*, Histopathology..

INTRODUCTION

Immunostimulants are the products derived from natural or synthetic origin with different chemical characteristics and varied modes and mechanism of action (Petrunov et al., 2007). Immunostimulants can be administered before, with or after vaccines to amplify the specific immune response (Siwicki et al., 1998; Gautam et al., 2008' Jayadev et al, 2013). Immunostimulants can enhance the host defence during several infections (Blanca et al., 2007; Chen et al., 2009). Hepatitis is a disease caused by hepatitis B virus (HBV) and chronically infects 350 million people worldwide (Orito et al., 2001; Chan et al., 2002; Tong et al., 2005). Vaccines play most profounding role as

therapeuting agents (Michel and Tiollais, 2010). Investigations on laboratory animals reveal the strong association between biochemical and various diseases connected to oxidative stress and/or injury (Ye and Song, 2005). In recent years, a good number of commercial immunostimulants are used in aquaculture, poultry, livestock management and in mankind (Gautam et al., 2008). Immunex DS is an amalgam of essential immunoenhancers like β-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 *lactobacillus* and yeast. Due to the lack of a specific animal models (in vitro/in vivo) there is a great gulp to know the adverse reactions from hepatitis B virus infection (Dandri et al., 2006). Many viral and bacterial diseases induce alterations in the biochemical constituents and no information is available on the content of carbohydrate and glycogen in the abdominal muscle of mice infected with HBV. Hence, the present study is undertaken to estimate the level of carbohydrate and glycogen in the abdominal muscles of immunostimulated and vaccinated mice at different days of experiment.

MATERIALS AND METHODS

Eight groups (10 in each) (A, B, C, D, E, F, I and U) of male Swiss Albino mice (*Mus musculus albinus*; 6 to 8 weeks old; 23 to 26 g wt) were maintained in the present study. Mice were fed with standard balanced diet and water *ad libitum* and were properly maintained following the guidelines of CPSCEA. Immunex DS (IDS) was given orally (@150mg/mouse) with the help of a syringe fitted with a 3 inch 16guaze oral, blunt feeding needle to all the 7 groups (A, B, C, D, E, F and I) of mice. Mice of groups A, B, C, D, E and F received Gene Vac B vaccine on day 4 of experiment (@ 0.07ml/mouse, 0.1ml/mouse, 0.2ml/mouse, 0.4ml/mouse, 0.8ml/mouse and 1ml/mouse.). Mice of group U (10) served as controls (untreated with IDS + unvaccinated). Two mice from each experimental and control groups were necropsied, abdominal muscle tissue was separated and total carbohydrate and total glycogen content was estimated the method of Nicholas et al., (1956). Results were analysed statistically using student's t test.

RESULTS AND DISCUSSION

Results from groups A, B, C, D, E, F, I and U are shown in table 1. Carbohydrate and glycogen levels showed considerable increase from day 1 to 5 of experimental period in mice of group I (which received IDS only) when compared with controls; the increased values of carbohydrate and glycogen almost remained constant from day 1 to 5. Experimental mice which received various doses of HB vaccine along with immunostimulant showed remarkable changes in the estimated values of carbohydrate and

Table 1: Total carbohydrate (mg/100mg) and total glycogen (mg/100mg) content in the 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	
	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G
1	47.82	18.92	46.12	17.96	46.03	17.52	45.23	17.12	46.12	16.21	42.12	14.83	48.08	22.04	47.56	18.88
2	48.21	18.16	47.06	17.52	65.89	16.80	44.82	16.59	55.69	15.29	41.83	15.21	48.09	22.03	47.49	18.89
3	49.23	17.32	50.40	18.98	32.88	27.67	36.98	5.98	45.91	18.66	31.37	27.87	48.08	22.04	47.60	18.83
4	50.29	14.22	54.20	20.67	50.56	56.45	65.98	5.53	35.89	29.32	20.61	32.22	48.07	22.01	47.56	18.88
5	53.50	12.03	58.78	29.60	65.99	66.97	98.80	2.82	70.98	40.26	20.12	41.87	48.08	22.02	47.52	18.80

DN, Days of Necropsy ; C, Carbohydrate ; G, Glycogen; Values are expressed in the mean derived from 5 observations.

glycogen. It is of interest to note that mice which received low doses (@ 0.07 ml/mouse, group A; @ 0.1 ml/mouse group B) of vaccine showed increase of carbohydrate level from day 3 to 5 of experiment. Mice which received @ 0.2 ml/mouse (group C) and @ 0.4 ml/mouse (group D) showed heightened level of carbohydrate on day 4 and 5. Mice of group E (@ 0.8 ml/mouse) showed disturbed level of carbohydrate (increase on day 2 and 5 and decrease on day 1, 3, and 4).

Another interesting feature in the present investigation is that the mice received highest dose of vaccine (@ 1.0 ml/mouse) showed below normal values of carbohydrate from day 1 to 5. Whereas the level of glycogen decreased to below normal level from day 3 to 5 in group A, and on day 1 and 2 in groups B, C, E and F. Mice of group D showed below normal level of glycogen from day 1 to 5. The abnormality in the level of carbohydrate and glycogen might be due

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

	Experimental groups				Control groups			
Carbohydrate	**A**	**B**	**C**	**D**	**E**	**F**	**U**	**I**
Mean	49.81	51.30	52.27	58.36	50.91	31.21	47.54	48.08
t values	A–U t= 0.77@	B–U t= 1.75@	C–U t= 0.74@					
	D–U t= 0.96@	E–U t= 0.56@	F–U t= 3.36*					
	A–I t= 1.70@	B–I t= 1.37@	C–I t= 0.68@					
	D–I t= 0.91@	E–I t= 0.48@	F–I t= 3.47*					
	A–B t= 0.56@	A–C t= 0.37@	A–D t= 0.75@	A–E t= 0.18@	A–F t= 3.77*			
	B–C t= 0.14@	B–D t= 0.61@	B–E t= 0.06@	B–F t= 3.74*				
	C–D t= 0.47@	C–E t= 0.14@	C–F t= 2.65*					
	D–E t= 0.61@	D–F t= 2.22@						
	E–F t= 3.74*							

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

	Experimental groups				Control groups			
Glycogen	A	B	C	D	E	F	U	I
Mean	16.13	20.95	37.08	9.50	23.94	26.40	18.85	22.02
t values	A–U t= 2.09@	B–U t= 0.94@	C–U t= 1.06@					
	D–U t= 3.04*	E–U t= 1.06@	F–U t= 1.46@					
	A–I t= 4.42*	B–I t= 0.48@	C–I t= 0.88@					
	D–I t= 4.08*	E–I t= 0.40@	F–I t= 0.84@					
	A–B t= 1.86@	A–C t= 1.22@	A–D t= 1.98@				A–E t= 1.57@	A–F t= 1.92@
	B–C t= 0.93@	B–D t= 3.02*	B–E t= 0.56@				B–F t= 0.96@	
	C–D t= 1.58@	C–E t= 0.73@	C–F t= 0. 59@					
	D–E t= 2.54*	D–F t= 2.81*						
	E–F t= 0.34@							

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

to the stress cause by the injection of vaccine.

Statistical analysis showed that the decreased level of carbohydrate (Table 2) was found to be significant in group F when compared with controls (group U), immunostimulated (group I) and IDS treated + vaccinated mice (groups A, B, C and E); non-significant difference was found in groups A, B, C, D and E when compared with controls and immunostimmulated mice and among themselves. The difference in the level of glycogen (Table 3) was found to be non-significant in groups A, B, C, D, E and F when compared with controls (except in group D), immunostimmulated (except in groups A and D) and among themselves (except in between groups B and D, D and E and D and F).

The experimental mice probably abstaining from food might have experienced the abnormal

change in the intermediary metabolism leading to the delineation and/or reduction in the storage of carbohydrate and glycogen. These results compare well with those of Singh et al (1963), Lindell et al., (1964) and Klein (1966) during microbial infections. Wilder and Sword (1967) suggested abnormality in the metabolism of carbohydrates infected with *Listeria mono-cyto*genes. Also, these results are in agreement with that of Balaparameshwara Rao and Padmavathi (2004). Who found decreased level of carbohydrate in the liver of *Catla catla* under stress. The elevated and/or decreased level of carbohydrate and glycogen in all the experimental groups of mice was found to be significant when compared with controls (group U) and immunostimulated (group I) mice; mice treated with low or high doses of vaccine along with immunostimulated might have experienced cytotoxicity, necrosis and apoptosis during induced hepatitis B.

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