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Protein level in the heart of mice during immunostimulation and hepatitis infection

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ABSTRACT

Six different doses of Gen Vac B Hbs Ag vaccine were given orally to 6 varied groups (A, B, C, D, E and F) of male swiss albino mice after immunostimulation. Heart protein level in all the experimental groups of mice were studied and compared with controls (group N) and immunostimulated (group I) mice 0n day 1, 3, 4 and 5 of experimental period. Mice treated alone with immunex DS (group I) showed a very high level of protein throughout the experimental period when compared to all the experimental groups (A, B, C, D, E, F, G and H). Mice of groups A (which received the lowest dose of Gene Vac B @ 0.07 ml/mouse) and E (received Gene Vac B @ 0.08 ml/mouse) showed high protein level throughout the experimental period when compared among the experimental groups. However, mice of group A showed heightened protein response compared to group E. Experimental mice of group C (which received single dose of IDS and inoculated with 0.2 ml of Gene vac B vaccine) showed very lowest protein levels in comparison with IDS treated mice (group I) and with the experimental groups.

Key words: Heart, protein, immunostimulant, hepatitis, mice.

INTRODUCTION

Use of immunostimulants along vaccines proved the enhancement of resistance against a variety of diseases in birds (Kumar et al., 2010; Thacker, 2010). Supplementation of immunostimulants in diet also proved the enhancement of innate immunity to resist the ill effects of pathogens during stress, reproduction, migration etc., in birds (Bricknell and Dalmo, 2005). Immunostimulants are used as alternatives to drugs, chemicals and/or antibiotics in aquaculture (Gelina et al., 2009). Plant products (compounds like alkaloids, flavonoids, quinones, terpenoids) show antioxidant, anti-neoplastic, anti-ulcer and anti-inflammatory properties (Dashputre and Naikwade, 2010).

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The acute illness of hepatitis B causes liver inflammation, vomiting, jaundice and chronic hepatitis-B may eventually leads to liver cancer or cirrhosis, which responds insensitive to present chemotherapy (Malmstrom, et al., 2010). Severe inflammation in liver dramatically enhances the risk of hepatocellular carcinoma (liver cancer) with a decrease in complete antioxidant activity and increase in oxidative stress and enzymatic enhancement of ALK, SGPT, SGOT and LDH in humans (Mastoi et al., 2010; Kumada et al., 2010). The heart of rat perfused with medium containing glucose and insulin increases the rate of tricarboxylate-cycle turnover, impairs glycolysis and suppresses the oxidation of pyruvate formed by glycolysis (Randle et al., 1970). Coronary heart disease, including ischemic heart disease, sudden cardiac death, and myocardial infarction, is the leading cause of death for men and women in the United States (Roger et al., 2012). About 9,35,000 Americans suffer from myocardial infarction every year with 610,000 new cases and 325,000 recurrent attacks (Roger et al., 2011). Lujan et al., 2012; Madhuri, D and Viveka Vardhani, V, 2015) described the use of an intact, conscious and unrestrained mouse model of myocardial

ichemia reperfusion and infarction. Rats and mice are normally used in cardiovascular research because they are less expensive and are easier to handle and to house compared to large animals (Recchia and Lionetti, 2007). Malliaras et al., 2013; Nathanael P. J. R and Vardhani V. V, 2014) evaluated that cardiomyocyte proliferation and progenitor cell recruitment underlie therapeutic regeneration after myocardial infarction in the adult mouse heart. Hence, a new vista has been opened to study the protein level in the heart during hepatitis infection and immunostimulation.

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. Proper acclimatization, care, housing and hygiene were properly maintained. Eight groups (10 in each group) of mice were maintained. Six groups of each mouse (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 a single dose of 150mg of IDS/mouse and another group (N) 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 N). Tissue samples of heart were collected and analyzed for protein following the method of Lowry et al. (1951). Results were submitted to students the “t” test for understanding the significant changes.

RESULTS AND DISCUSSION

Results are shown in table-1. Mice which were treated with immunostimulant (group I) showed higer level of heart protein than in controls and other experimental groups (except in group A) of mice during the entire experimental period. Mice of all the experimental groups (A to F) which were treated with immunostimulant + vaccine showed increased level of heart protein in comparison with controls and decreased level (except in group A) in comparison with immunostimulated mice (group I).

Group A: Higher value of protein was found when compared to controls and immunostimulated (group I) mice throughout the experimental course. The content of protein increased gradually from day 1 (330.0 mg/ml) to 5 (341.8 mg/ml) of experimental period.

Group B: Increased and decreased level of protein was found throughout the experimental period compared to controls (group N) and immunostimulated (group I) mice.

Group C: Higher and lower value of protein was recorded throughout the experimental period when compared with control (group N) and immunostimulated (group I) mice respectively. The protein levels increased gradually from day 1 (116.6 mg/ml) to 5 (174.2 mg/ml).

Group D: Higher and lower value of protein was found when compared to controls (Group N) and immunostimulated (group I) mice throughout the experimental period. sThe content of protein increased gradually from day 1 (166.0 mg/ml) to 5 (200.0 mg/ml) of experimental period.

Group E: Increased and decreased protein content was exhibited throughout the experimental period when compared with normal controls (group N) and immunostimulated (@ 150 mg/mouse) (group I) mice. There was a gradual increase of protein from day 1 (239.4 mg/ml) to 5 (430.0 mg/ml); highest value of protein was recorded on day 5.

Group F: Higher and lower value of protein was found when compared with control (group N) and immunostimulated (group I) mice from day 1 to 5 of experimental period. The protein level increased gradually from day 1 (109.2 mg/ml) to 5 (245.3 mg/ml) showing peak level on day 5.

It is of interest to note that though there was a decreased value of protein in groups D, E and F when compared to immunostimulated mice (group I), the protein level increased significantly from day 1 to 5 of infection period. The lowest value of protein was found on day 1 (109.2 mg/ml) in group F, and the highest value of protein was found on day 5 (430.0 mg/ml) in group E. Statistical analysis (table 2) showed significant changes in heart protein of various experimental groups of mice. Experimental animals of groups A, B, C, D, E and F (oral administration of immunostimulant as a single dose + vaccination) and G and H (oral administration of immunostimulant as repeated dose + vaccination) showed significant increase of heart protein when compared with controls and significant decrease (except in groups A and E) when compared with immunostimulated mice. These results suggest that the treatment of Immunex DS significantly altered the content of protein in heart.

The present study provides evidence that hepatitis B infection affect the heart protein in mice. The viral infection altered the tissue protein in heart in a significant manner. There are many possible mechanisms by which the heart protein could increase or decrease. The viral antigens, endotoxins or metabolites may have various effects on protein. The increase of heart protein in all the experimental groups of mice might be due to increase in the rate and amount of protein synthesis. Thomas and Kalyanaraman (1997) reported that the loss of balance between ROS and anti-oxidants led to the oxidative damage of cellular macro molecules like lipids, proteins and nucleic acids; this may cause pathogenicity leading to cardiovascular and metabolic dysfunction of almost all the vital organs. These observations correlate with that of Sun et al., (2002) who postulated that the oxidative stress in mice

Table 1. Protein (mg/ml) content in the heart of control (group N - untreated and uninfected), IDS treated (group I, @ 150 mg/mouse) and experimental (group A, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.07 ml/mouse; group B, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.1 ml/mouse; group C, treated with IDS @ 150 mg/mouse and infected with Gen Vac B vaccine @ 0.2 ml/mouse) group D, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.4 ml/mouse; group E, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 0.8 ml/mouse; group F, treated with IDS @ 150 mg/mouse and infected with Gene Vac B vaccine @ 1 ml/mouse) male swiss albino mice at different days of experimental period. Values are expressed in the mean derived from five observations.

Days of necropsy	Group C (untreated and uninfected)	Group I (treated with IDS @150mg/ mouse and uninfected)	Group A (150mg of IDS/mouse and infected with 0.07ml of Hbs Ag/mouse)	Group B (150mg of IDS/mouse and infected with 0.1ml of Hbs Ag/mouse)	Group C (150mg of IDS/mouse and infected with 0.2ml of Hbs Ag/mouse)	Group D (150mg of IDS/mouse and infected with 0.4ml of Hbs Ag/mouse)	Group E (150mg of IDS/mouse and infected with 0.8ml of Hbs Ag/mouse)	Group F (150mg of IDS/mouse and infected with 1 ml of Hbs Ag/mouse)
1	112.5	309.9	330.0	136.8	116.6	166.0	239.4	109.2
2.	112.0	311.0	332.5	145.9	118.5	170.0	256.8	149.9
3.	112.4	312.0	335.0	152.2	120.4	184.0	379.9	175.3
4.	112.0	312.6	338.0	158.9	157.5	186.0	392.1	181.5
5.	112.2	313.0	341.8	162.8	174.2	200.0	430.0	245.3

Table 2. 't' values obtained in different experimental groups (A,B,C,D,E and F) of mice.

	Experimental groups						Control group	
	A	B	C	D	E	F	N	I
Mean	335.0	151.3	137.4	**181.2**	**339.6**	**172.2**	**112.2**	**311.7**

A N t= 118.1*; B N t= 9.41*; C N t= 2.37*; D N t= 12.6*; E N t= 6.62*; F N t= 3.8*; N I t= 379.3*

A I t= 0.83@; B I t= 5.65*; C I t= 5.81*; D I t= 2.35*; E I t= 0.86@; F I t= 8.8*

A B t= 4.4*; A C t= 18.3*; A D t= 26.7*; A E t= 0.11@; A F t= 10.4*

B C t= 1.2@; B D t= 4.36*; B E t= 5.43*; B F t= 1.26@

C D t= 3.64*; C E t= 5.6*; C F t= 1.84@; D E t= 4.5*; D F t= 0.53@; E F t= 4.43*

may cause inflammation thereby disturbing the metabolic function of the liver.

Mice of group A which were treated with the lowest dose of Gene Vac B vaccine (0.07 ml/mouse) exhibited elevated level of protein in the heart throughout the experimental period (except on day 5 in group E) compared to mice which received IDS alone (group I) and other immunostimulated + vaccinated groups. This shows that the lowest dose of vaccine stimulated the immune system to elicit the highest level of protein; the host immune system might have disturbed slightly in comparison with the other experimental groups which received various higher doses of antigen. These results correlate with Borzi et al., (1992) who reported secondary immune response during the vaccination of cDNA Hbs Ag in humans. Mice of group F which received highest dose of vaccine (1 ml/mouse) showed lowest level of protein on day1 which was progressively

increased by day 5;however, these values are lower than the experimental groups, D (received vaccine @ 0.4 ml/mouse) and E (received vaccine @ 0.8 ml/mouse). These results suggest that the higher dose of vaccine might have caused abnormality in the metabolism of proteins in this mouse model. Davis (2006) suggested that anti-viral, anti-bacterial and anti-parasitic therapy will operate in the host immune system to clear the pathogenic load in many experimental animal models.

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Conflict of Interests

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

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