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Protective effect of Immunex ds against Hepatitis B in a mouse experimental model

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ABSTRACT

Heart DNA was estimated in immunostimulated, immunostimulated + vaccine treated and normal male swiss albinomice. One group (I) mice treated with immunex DS (IDS) (@ 150mg/mouse); six groups of experimental (A, B, C, D, E and F) were treated with IDS + hepatitis B vaccine and one group (N) was kept as normal's (untreated with IDS + vaccine). Two mice from all the groups I, N and A to F were sacrificed on day 1, 2, 3, 4 and 5 of experimental period. Experimental mice (groups A to F) which received IDS + vaccine showed an increase of heart DNA when compared with controls (group N) and decrease in comparison with immunostimulated mice (group I).

Key Words: Heart, DNA, Immunostimulant, Hepatitis, Mice.

INTRODUCTION

Immunostimulants are able to reinforce natural resistance to cope with different viral and bacterial infections ; they are employed in the development, and/or in the clinical practice of the non-specific and specific immunity (Marinova et al., 2000; Petrunov, 2004). Use of immunostimulants in mice models is a wide discourse to assess the immune response and immunocompetence. Immunostimulants stimulate macrophages and T-lymphocytes and improves cellular immunity by increasing. Secretion and proliferation of T-cells (Demirci et al., 2005; Citarasu et al., 2006; Halder et al., 2009). Immunostimulants are used as alternatives to drugs, chemicals and or antibiotics in aquaculture (Gelina et al., 2009). Acute infection with hepatitis-B virus begins with general ill-health, loss of appetite, nausea, vomiting, tiredness, severe asthenia, pains in muscles and joints, arthalgia, dark urine, skin rashes, the skin becomes yellow in colour and itchy, body aches, mild fever and progress to severe jaundice, gall bladder and bile obstruction. Hepatitis-B incidence shows its impact on liver, kidney etc. ; they become pale, necrotic, inflamed and develop an unique pathological condition known as membranous glomerulonephritis (Zhang et al., 2010). Animal models can be employed to understand the mode of replication of virus, disease diagnosis and pathogenesis and invention and improval of specific drugs to cure hepatitis B (Zhan Gao et al, 2010; Chayama et al., 2011). Lack of a specific animal model led to great gulf to understand the immunopathogenicity of hepatitis B virus infection and its genesis into liver fibrosis and liver carcinoma (Jin et al., 2011). Production of free radical and calcium overload are considered as the two major events implicated in the development of myocardial ischemia and reperfusion injury (Hearse and Bolli, 1992; Maxwell and Lip, 1997; Piper et al., 1998). The oxidative stress (an imbalance between the production of radical species and the protection by many antioxidant system) can lead to electrophysiological, biochemical and mechanical disturbances and dramatically impairing the ability of the heart to recover from the initial ischemic insult (Hearse and Tosaki, 1987; Bolli, 1991). Mouse models are increasingly adopted to study cardiovascular human diseases and identify new therapeutic targets; the most frequently used include Myocrdial infarction (MI) or ischemia-reperfusion by ligation of the left coronary artery (Kumar et al., 2005; Nilles and London, 2007). Infections are invariably associated with loss of body constituents,

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rapid utilization of body store of nutrients and redistribution between various physiologic and metabolic compartments. A decrease in plasma concentration of aminoacids and proteins occur due to deamination for the glucose synthesis, proliferation of lymphocytes and granulocytes and production of antibodies (Bhatnagar et al., 2008). Several factors may influence the synthesis and release of biomolecules during infections. These observations led to study the effect of the immunostimulant against hepatitis B on DNA level of heart in male swiss albino mouse model.

MATERIAL AND METHODS

Eight groups (10 in each group) of male swiss albino mice (*Mus musculus albinus*) (6-8 weeks; 23-31g) were fed with standard balanced diet and water ad libitum and maintained according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiments in animals). 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 (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). Heart samples were collected and analyzed for DNA according to Diphenylamine method (Burton, 1956). Results were analyzed to understand the statistical significance.

RESULTS AND DISCUSSION

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

Group I: Mice of group I (treated with immunostimulant) showed higher level of DNA from day 1 (784.5 mg/ml) to 5 (786.0 mg/ml) of experimental period than the control (214.4 mg/ml) mice (group N, untreated with IDS and vaccine). In group I, higher level of DNA was found on day 3 (786.0 mg/ml) and 5 (786.0 mg/ml) when compared to other days of experimental course.

Group A: Lower value of DNA was found when compared to controls and immunostimulated (group I) mice throughout the experimental period. The content of DNA increased gradually from day 1 (116.7 mg/ml) to 5 (204.1 mg/ml) of experimental period.

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

Group C: Higher and lower value of DNA was exhibited throughout the experimental period when compared with control (group N) and immunostimulated (group I) mice respectively. The DNA levels increased gradually from day 1 (166.5 mg/ml) to 5 (380.5 mg/ml).

Group D: Higher and lower value of DNA was recorded when compared to controls (Group N) and immunostimulated (group I) mice throughout the experimental period. The content of DNA increased gradually from day 1 (239.4 mg/ml) to 5 (268.0 mg/ml) of experimental period.

Group E: Increased and decreased DNA was found during the entire experimental period when compared with normal controls (group N) and immunostimulated (@ 150 mg/mouse) (group I) mice. There was a gradual increase of DNA from day 1 (240.0 mg/ml) to 5 (270.0 mg/ml).

Group F: Higher and lower value of DNA was found when compared with control (group N) and immunostimulated (group I) mice from day 1 to 5 of experimental period. The DNA level increased gradually from day 1 (529.6 mg/ml) to 5 (660.0 mg/ml); highest value of DNA was recorded on day 5.It is of interest to note that though there was a decreased value of DNA in groups A, B, D, E and F when compared to immunostimulated mice (group I), the DNA level increased significantly from day 1 to 5 of infection period. The lowest value of DNA was found on day 1 (116.7 mg/ml) in group A, and the highest value of DNA was found on day 5 (660.0 mg/ml) in group F. Statistical analysis (table 2) showed significant differences in heart DNA 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) showed significant increase of heart DNA (except in group A) when compared with controls and significant decrease when compared with immunostimulated mice. These results suggest that the treatment of Immunex DS significantly altered the content of DNA in heart. The present investigations provide that hepatitis B infection affect the DNA metabolism in the heart of mice. The viral infection altered the tissue DNA level in heart in a significant manner. There are many possible mechanisms by which the heart DNA could increase or decrease. The viral antigens, endotoxins or metabolites may have various effects on DNA. The increase of heart DNA in all the experimental groups of mice might be due to increase in the rate and amount of DNA synthesis. The specific alterations of DNA synthesis in the heart of experimental groups confirm that of Mudge et al., (1976) who found marked myocardial amino acid metabolisms in chronic ischemic heart disease patients.

Table – I. Table 1. DNA (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 N (untreated and uninfected)	Group I (treated with IDS @150mg/ mouse and unvaccinated)	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	214.4	784.5	116.7	260.0	166.5	239.4	240.0	529.6
2.	214.2	784.6	163.7	264.0	259.4	256.8	260.0	558.8
3.	214.2	786.0	173.5	268.0	319.9	258.0	264.0	593.8
4.	214.3	785.6	184.9	270.0	325.5	260.0	268.0	640.0
5.	214.4	786.0	204.1	274.0	380.5	268.0	270.0	660.0

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

	Experimental groups					Group Control		
Mean t-value	**A** 169.4	**B** 267.2	**C** 290.3	**D** 256.4	**E** 260.4	**F** 596.4	**N** 214.3	**I** 785.3

A N t= 3.36* | B N t= 24.4* | C N t= 2.32* | D N t= 1.27 | E N t= 9.4* | F N t= 17.5* | N I t= 644.0*

A I t= 46.2* | B I t= 240.6* | C I t= 15.1* | D I t= 126.4* | E I t= 109.0* | F I t= 8.67*

A B t= 7.5* | A C t= 3.42* | A D t= 6.2* | A E t= 1.61 | A F t= 16.5*

B C t= 0.69 | B D t= 2.29 | B E t= 1.24 | B F t= 15.0*

C D t= 1.02 | C E t= 0.90 | C F t= 7.8* | D E t= 0.63 | D F t= 15.3* | E F t= 15.0*

Heart DNA level showed significant increase in comparison with controls (except in group A) and significant decrease in comparison with IDS treated animals. These results suggest that the treatment of Immunex DS significantly altered the content of DNA in heart. Though the experimental groups (A, B, C, D, E and F) of animals were injected with vaccine, the orally administered IDS might have enhanced the level of heart DNA (this enhancement is in comparison with normal animals, group N). These results compare well with that of Petrunov et al., (2007) and Park et al., (2008) who also confirmed that immunostimulants are able to reinforce body's natural immunity to cope with various bacterial and viral infections in humans and mice. Sakunthala et al., (2014) also reported tissue injury and/or inflammation and disturbed protein and DNA level in the gut of various groups of mice treated with immunostimulant and various doses of Gene Vac B vaccine. The significantly decreased level of DNA in the heart in mice of group A (received the lowest dose of vaccine) in comparison with controls, IDS treated (group I) mice and other experimental groups (B, C, D, E and F) suggest that the synthesis of DNA is impaired. However, in groups B, C, D, E and F there was an increase in the level of DNA from day 1 to 5 of experimental course. These results confirm that of Jin et al., (2011) who suggested increase of liver protein and DNA in mice injected with hepatotoxin like CCL(4) and Divya Teja and Viveka Vardhini (2015) who found significant alteration in the level of DNA in abdominal muscles of mice treated with immunostimulant and hepatitis B vaccine. Jasmine Gold and Viveka Vardhini (2016) suggest the immunostimulant stimulated the immune response in heart with regard to marked alteration in the level of protein. Although the mechanism of the action of immunostimulant and Gene Vac B vaccine is not known, it is of interest to note that mice of group A showed decreased level of DNA, and rest of the other groups showed increased level of DNA in the heart (in groups B to F). It is of interest to note that the immune system of mice which received IDS (groups A to F) responded almost similarly in the metabolism of DNA in heart.

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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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