The Bioscan 6(2) : 229-232, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ABDOMINAL MUSCLE LDH IN MICE DURING IMMUNO-STIMULATION AND INDUCED HEPATITIS B

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KEY WORDS
Immunex DS
Gene Vac B vaccine
Lactate dehydrogenase (LDH)
Hepatitis B
Immunostimulation

Received on :
28.12.2010

Accepted on :
21.04.2011

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ABSTRACT
Lactate dehydrogenase (LDH) content in the biological systems is indispensably concerned with the tissue breakdown and cytotoxicity during various aliments. The prime objective in recent medical advances is to conceptualize the degree of cytotoxicity mainly in human diseases. The present work is centralized on the impact of Immunex DS on male Swiss albino mice innoculated with various doses of Gene Vac B vaccine. Six groups of male Swiss albino mice were immunostimulated by Immunex DS and innoculated with various single doses of Gene Vac B vaccine containing HbsAg, and compared the intense tissue break down and cytotoxicity in their abdominal muscles. The present findings clearly confirm elevated level of LDH which correlates with the extent of tissue break down and cell death during induced hepatitis B induction.

INTRODUCTION

Cytotoxic assays are widely used in molecular analyses during virological toxicity studies, xenobiotics and cell damage. Cell death involves apoptosis and necrosis. Lactate dehydrogenase (LDH) is an oxidative enzyme which exists in cell membranes and cytoplasm and released during apoptosis and necrosis, after the loss of membrane integrity during cell death. LDH is an efficient marker to assess cytotoxicity. During glycolysis LDH converts lactate into pyruvate. LDH is released from the cells into the culture supernatants immediately after cell damage, hence, the photospectrometrical assessment of leaked LDH levels hails a novel strata for studying the cell viabilities in cytotoxic research (Watanabe *et al*., 1995; Baba *et al*., 2005). Therefore, in the fields of toxicology, immunology and anti-cancer drug research and cyto-chemistry, it is indispensable to utilize LDH as a marker for degeneration of cells, apoptosis and other molecular aberrations. As the tissue break down occurs, it elevates the LDH, which can be a sign of certain pathological conditions like hemolysis, cancer, hepatitis, jaundice, meningitis, encephalitis, acute pancreatitis, HIV and myocardial infarction. Malignant and cancerous cells show high rate turn over with destroyed cells manifesting extreme heightened levels of LDH. Enhanced levels of this enzyme is found in cerebrospinal fluid during viral and bacterial meningitis. 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). The employing of these immunoboosters is an alternative to various drugs composed of chemicals and antibiotics which have adverse side effects (Gelina *et al*., 2009).

A wide range of commercial immunostimulants are available these days utilizing them in realms of aquaculture, poultry science, animal husbandry, livestock management apart from their immense usage in medical practices for health prospective towards mankind (Gautam *et al*., 2008). In the present investigations, a novel immunostimulant has been employed commercially known as "Immunex DS".

Immunex DS 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 lactobacillus and yeast (Nathanael *et al*., 2010). Immunex DS has a wide spectrum of effects to enhance body's immune response, aiding the body's strength, vigor and vitality. It increases the weight of the body by boosting up the natural stamina and vital energy. Immunex DS prevents the colonization of the bacteria and accumulation of pathogenic toxins in the 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 hepatocellular carcinoma (HCC) a hundred fold (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, erectile dysfunction, mild increase in transaminases and lactate dehydrogenase levels (LDH) and incidence of hepatocellular carcinoma (Wong and Goh, 2006;

Risco *et al*., 2009; Yeh *et al*., 1989; Madhuri *et al*., 2009; Kumada *et al*., 2010). Hence, a new dimension paved to understand the crux of cytotoxicity by knowing the level of Lactate dehydrogenase (LDH) in the abdominal muscles of Immunex DS administered mice during artificially induced hepatitis B condition achieved by inoculating them with various doses of Gene Vac B HbsAg vaccine.

MATERIALS AND METHODS

Male Swiss albino mice (*Mus musculus albinus*) (6-8 weeks of age, Average weight 23-31 g) were engaged in the present study. They were fed with standard balanced diet *ad libitum*. 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 mice (A, B, C, D, E and F) were orally intubated with a single dose of 150 mg of Immunex DS with the help of a syringe fitted with a 3 inch 16 gauze oral, blunt feeding needle. Later these six groups of mice were inoculated with various single doses of Gene Vac B HbsAg vaccine intramuscularly. One group of mice (c) was intubated orally with single dose of 150 mg of Immunex DS and served as immunostimulated control and another group (cc) of mice was neither immunostimulated nor inoculated served as normal controls for comparison. The mice (groups A, B, C D, E, and F) were immunostimulated on day 0 and innoculated with different doses of Gene Vac B HbsAg vaccine on day 7 (A, 0.07 mL/mouse; B, 0.01 mL/mouse; C, 0.2 mL/mouse; D, 0.4 mL/mouse; E, 0.8 mL/mouse; F, 1.0 mL/mouse) and waited for 72 hr, later from day 11 to 15, the experimental mice were sacrificed along with the mice of Immunex DS treated alone (group c) and normal ones (group cc). The abdominal muscles were severed and the samples were assayed for lactate dehydrogenase activity using standard methods.

RESULTS AND DISCUSSION

The mice of groups (D, E and F) showed lethargy, anorexia and aggressiveness. The surface of skin showed loss of hair and exposing the naked and inflamed skin with blisters and erythrema. The mice of group B developed exopthalmous condition at the phage end of the experimentation. The mice of groups D, E and F showed molted and malignant liver with extreme cirrhosis (Ahmed *et al*., 2010). Persistent spleenomegaly was observed in these groups of mice, often the texture of spleen became pale and covered by patches of white tumors. The gastro-intestinal tract was severely inflamed which is the outstanding feature manifested by groups B and F. Odematous thymus with heavy accumulation of mucilage was observed. The kidneys underwent Chronic Kidney Disease (CKD) and Macrocytic Glomerulo Nephritis (MGN) conditions in mice of groups D, E and F. The visceral peritoneum was lined by hemorrhagic blood clots. The content of Lactate dehydrogenase (LDH) observed in various groups of male *Swiss albino* mice is shown in Table 1 and Fig. 1.

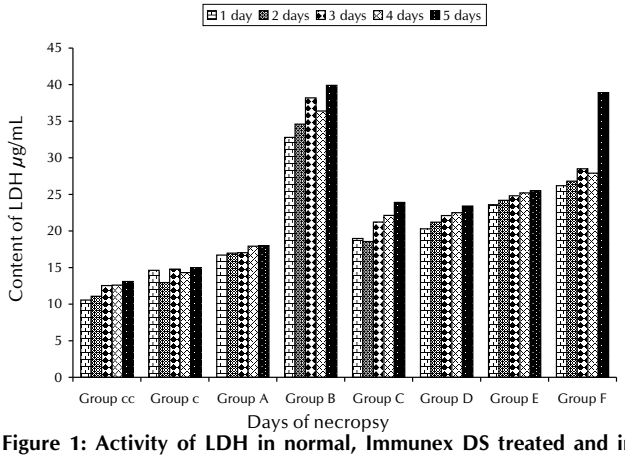

Figure 1: Activity of LDH in normal, Immunex DS treated and in various groups of immunostimulated and vaccinated mice

Group A: The mice of group A which were immunostimulated with 150 mg of Immunex DS and inoculated with 0.07 ml of Gene Vac B HbsAg showed high levels of LDH activity through out the experimental period than the Immunex DS treated (group c) and normal mice (group cc). On day 5, the LDH activity was high (18.0 mg/mL).

Group B: The mice of group B which were immunostimulated with 150 mg of Immunex DS and inoculated with 0.1 mL of Gene Vac B HbsAg showed exponentially higher level of LDH response, than the group c and group cc mice (even more than all the rest of the groups of mice).

Table 1: The content of LDH in abdominal muscles of control (group cc), immunostimulated (group c) and experimental (groups A, B, C, D, E and F) male swiss albino mice at different days of necropsy. Values are five expressed in mean derived from observations

Days of necropsy.	Group cc (untreated and uninno-culated)	Group c (treated with Immunex DS @ 150mg/ mouse and uninnocu-lated)	Group A (150 mg of Immunex DS/ mouse and infected with 0.07 mL of HbsAg/ mouse)	Group B(150 mg of Immunex DS/ mouseand infected with0.1 mL of HbsAg/ mouse)	Group C(150 mg of Immunex DS/ mouse and infected with 0.2 mL of HbsAg/mouse)	Group D(150 mg of Immunex DS /mouse and infected with 0.4 mL of HbsAg/mouse)	Group E(150 mg of Immunex DS/ mouse and infected with 0.8 mL of HbsAg/mouse)
1	10.56	14.62	16.70	32.8	18.98	20.3	23.6
2	11.07	12.96	16.98	34.6	18.56	21.2	24.2
3	12.53	14.79	17.03	38.2	21.23	22.1	24.8
4	12.61	14.32	17.93	36.4	22.12	22.5	25.2
5	13.09	14.98	18.00	39.9	23.90	23.4	25.5

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

Groups
A B C D E F c cc
Mean 23.77 23.44 12.89 35.10 19.32 19.20 22.14 11.97
A cc B cc C cc D cc E cc F cc
|____| |____| |____| |____| |____| |____|
t=14.63* t=35.72* t=20.13* t=20.40* t=22.45* t=29.30*
(p<0.05) (p<0.05) (p<0.05) (p<0.05) (p<0.05) (p<0.05)
A c B c C c D c E c F c
|____| |____| |____| |____| |____| |____|
t=13.89* t=35.59* t=19.96* t=20.07* t=21.9* t=29.23*
(p<0.05) (p<0.05) (p<0.05) (p<0.05) (p<0.05) (p<0.05)
A B A C A D A E A F
|____| |____| |____| |____| |____|
t=12.06* t=13.80* t=12.51* t=11.38* t=14.71*
(p<0.05) (p<0.05) (p<0.05) (p<0.05) (p<0.05)
B C B D B E B F
|____| |____| |____| |____|
t=35.26* t=35.18* t=35.02* t=34.97*
(p<0.05) (p<0.05) (p<0.05) (p<0.05)
C D C E C F
|____| |____| |____|
t=19.45* t=19.24* t=19.29*
(p<0.05) (p<0.05) (p<0.05)
D E D F
|____| |____|
t=18.76* t=19.64*
(p<0.05) (p<0.05)
E F |____|
t=22.14*
(p<0.05)

p value at 5% level of significance is 2.3060*; Statistically significant value.

Throughout the days of necropsy the LDH activity was high. It rose gradually from day 1 to 5. LDH activity was peak on day 5 (39.9 mg/mL).

Group C: The mice of group C which were immunostimulated with 150 mg of Immunex DS and inoculated with 0.2 mL of Gene Vac B HbsAg showed higher content of LDH than the immunostimulated mice (group c) and control mice (group cc) throughout the experimental period. The level of LDH was almost constant on day 1 (18.98 mg/mL) and 2 (18.56 mg/mL), but from day 3 (21.23 mg/mL) to 5 a steady enhancement was observed in experimental mice.

Group D: The mice of group D which were immunostimulated with 150 mg of Immunex DS and inoculated with 0.4 ml of Gene Vac B HbsAg manifested enhanced LDH release than the Immunex DS administered (group c) and normal mice (group cc). A gradual increase of LDH content was observed from day 1 to 5 of experiment.

Group E: The mice of group E which were treated with 150 mg of Immunex DS and inoculated with 0.8 mL of Gene Vac B HbsAg showed enhanced level than the immunostimulated (group c) and control (group cc) mice through out the days of sacrifice. A gradual ascension of the LDH value was vividly observed from day 1 (23.6 mg/mL) to 5 (25.5 mg/mL).

Group F: The mice of group F which were treated with 150 mg of Immunex DS and inoculated with 1.0 mL of Gene Vac B HbsAg revealed a very high levels of LDH throughout the days of experiment than the Immunex DS treated (group c) and normal (group cc) mice. In these experimental mice a steady increase of LDH level was observed from day 1 to 4. But on day 5 there was a sudden exponential hike was noticed and LDH activity was found at its pinnacle on day 5 (38.9 mg/mL).

Indiscriminately observing the pattern of the LDH activity in all the groups of mice in accordance with the experimental design, certain facets came into light. All the mice in experimental groups (A, B, C, D, E and F) showed a heightened LDH activity. This finding clearly gives an evidence of cytotoxicity, necrosis and apotopsis during induced hepatitis B. This clearly gives an insight of heavy tissue destruction in the abdominal muscles of experimental mice which were suffering from abdominal pain manifested by highly inflamed muscle on autopsy (Baba *et al*., 2005). Another interesting feature of these findings is that the experimental mice of group B (which received Immunex DS @ 150 mg/mouse and inoculated with 0.1 mL of Gene Vac B HbsAg), showed a markedly heavy increase of LDH activity than the rest of all the experimental groups. Though a low dose of antigen was given the cell destruction was very vast than the other groups of mice indicating a halted immune response of the animal which was treated by Immunex DS. This gives a clear breakthrough towards cytopathecity and immune response of the individual. Work is having a similitude dimension of the previous investigations in human where in chronic hepatitis and liver dysfunction there is a slight to heavy raise of LDH level in serum (Yamada *et al.,* 1967).

The elevated LDH values were found to be significant in all the experimental groups (A, B, C, D, E and F) when compared with controls (group cc) and immunostimulated (group c) mice and among themselves (Table 2).

ACKNOWLEDGEMENTS

The author express his deep gratitude and esteem honor to his research director, Prof. V. Viveka Vardhani who is his guide, philosopher and above all a great teacher. I devotedly dedicate this paper to my beloved granny P. Esther Vedanayakam, for her unbounded love and celestial concern.

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