ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Adverse Effect of Diazepam on Immunization of Mice Fed Diet Supplement with Chitosan

Dr. Khalil H. Al – Jeboori and Ali H. Ali,
Dept. of Pathology, College of Veterinary Medicine, Univ. of Baghdad, Iraq.

Manuscript Info

Manuscript History:

Received: 22 March 2014
Final Accepted: 26 April 2014
Published Online: May 2014

Key words:

****Corresponding Author***

Dr. Khalil H. Al – Jeboori and

Abstract

The objective of the study was to know the impact of diazepam on the immune response and the role if immune stimulation with or without Chitosan on the toxic effect of diazepam. For this purpose 60 mice both sexes, and aged (7-8) weeks were randomly divided into 4 groups and treated as follows. The first group (n=15) as immunized by the killed vaccine of Pasteurella multocida injected (0.1ml) I/P at a dose of 3×10^8 cfu/ml two doses at two weeks interval and administrated daily with diazepam for a period of eight weeks. The second group (n=15) was administrated orally with diazepam (0.6 mg/kg B. W.) for a period of eight weeks by stomach tubes and vaccinated as in the first group and fed a diet containing Chitosan (1 gm/kg). The third group (n=15) was vaccinated with Pasteurella vaccine and considered as a positive control. The forth group (n=15) was fed diets containing Chitosan and immunized with P. multocida vaccine two doses at two weeks intervals. At 28 days post immunization cell mediate immunity response determined. The mean thickness of skin at 24 and 48 hrs were 1st group (0.25±0.01) (0.12±0.01); 2nd group (0.73±0.03) (0.55±0.04); 3rd group (0.51±0.02) (0.32±0.03) and 4th group (1.10±0.09) (0.75±0.04) respectively. The humeral immune response was measured at 30 and 60 days after immunization results were shown in the 1st group (115.2±37.32) (256.0±70.11); 2nd group (179.2±31.35) (716.8±125.42); 3rd group (307.20±86.82) (665.6±153.61) and 4th group (819.2±125.42) (1638.4±250.84) respectively.

Conclusion:

The diazepam has side effects on immune response, while Chitosan stimulating the immune response which help in preventing the adverse effects of diazepam induce in mice, also the vaccine neutralized the adverse effects of drug with or without of Chitosan in this study.

INTRODUCTION

Diazepam is a member of a group of medications that belong to a group known as benzodiazepines it is commonly used to treat anxiety, panic attacks, insomnia, seizures (including status epileptic), muscle spasms (such as in tetanus cases), restless legs syndrome, alcohol withdrawal, benzodiazepine withdrawal, opiate withdrawal syndrome and Meniere's disease (1). It may also be used before certain medical procedures (such as endoscopies) to reduce tension and anxiety, and in some surgical procedures to induce amnesia (2). It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties (3). Incorrect uses of the diazepam with overdose may be led to impairment in immune system principal against opportunistic bacterial infections or decrease infinity program vaccination (4). Chitosan has widely been investigated for many biomedical and pharmaceutical applications. It is insoluble in water, but becomes soluble and cationic in aqueous acidic solution (PH<6.5) (5). Due to the few researches on the effects of diazepam on the immune response and influence strength

of the immune response against toxic effects of diazepam. The aims of the present study are to determine the influence of the augment immune response with Chitosan against diazepam toxic effects.

Materials and methods

Experimental Design:

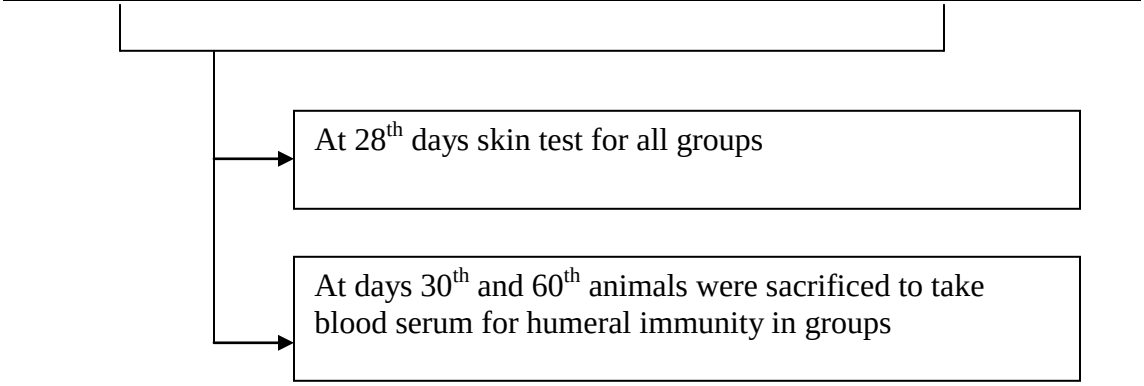
Sixty healthy mice of both sexes 7-8 weeks old were randomly divided into four groups and treated as follows:

1. First group: (n=15) was administrated with diazepam 0.6mg/kg b.w orally daily but at the same time immunized with P. multocida vaccines, I/p with 3×10^8 cfu/ml two doses, 2 weeks intervals.
2. Second group: (n=15) was administrated with diazepam 0.25 ml orally containing 0.6mg/kg b.w daily for eight weeks and received (1gm/kg of diet) Chitosan and immunized with P. multocida vaccines (I/p with 3×10^8 cfu/ml two doses, two weeks intervals).
3. Third group: (n=15) was immunized I/p with 3×10^8 cfu/ml of P. multocida vaccine two doses at two weeks intervals.
4. Fourth group: (n=15) was received Chitosan and at the same time immunized with P. multocida vaccines as the 3rd group.

Experimental design

Total number 60 mice divided into 4 groups

Group 1	Group 2	Group 3	Group 4
N=15 Diazepam Pasteurella Vaccine 60 days	N=15 Diazepam Vaccine Chitosan 60 days	N=15 Pasteurella Vaccine 3×10^8 cfu/ml 60 days	N= 15 Pasteurella Vaccine Chitosan 60 days



Parameter of study:

Delayed type hypersensitivity test (DTH):

This test was carried out at the 28th day post 1st immunization and the procedure was adopted from (6), as following: The right footpad of immunized groups were injected intradermal with 0.1ml of Whole Cell Sonicated Antigen of Pasteurella multocida (WCS Ags) (0.5mg/ml protein concentration) and the left footpad of the same animals were intradermal injected with o.1ml of PBS. The thickness of the skin in the both sides were measured at 24hrs and 28hrs, using standard electronic digital calipers.

Passive haemagglutination test (PHA Test):

Procedure of PHA:

Precisely 50 µl of normal rabbit serum (NRS) were added to wells of micro titer plate (96 U-shape wells), then added 50 µl of serum sample to first well in rows, then made two fold serial dilutions of serum by pipetting 50 µl of mixture transferred to next wells until last well, then add 50 µl of sensitized RBCs ,incubate at least for 2 hrs at room temperature, then read the reaction, and the plate covered with aluminum paper and incubated and reading repeated after 18hr/4C° (7). The positive reaction appeared when RBCs agglutinate and form carpet shape. The negative reaction appeared when RBCs precipitate at the bottom as dot like without agglutination.

Results and Discussion

Immunological examination:

Cellular immune response (delayed type hypersensitivity DTH):

The results revealed a significant difference ($p<0.05$) between the immunized groups in thickness of indurated area (Table: 1).The 4th group was (1.10mm±0.09, 0.75mm±0.04) showed significantly higher values ($p<0.05$) at both 24hrs and 48hrs respectively while the 2nd group was (0.73mm±0.03, 0.55mm±0.04) revealed significantly ($p<0.05$) thicker than those of the 3rd group was (0.51mm±0.02, 0.32mm±0.03) while the 1st group (0.25mm±0.01, 0.12mm±0.01) in both post of 24hrs and 48hrs respectively. In the mean time the only 4th group showed significantly higher ($p<0.05$) values in both post 24hrs and 48hrs.

Group No.		**Skin test (mm)**	
		24 hrs	**48 hrs**
G1	Diazepam Vaccine	0.25±0.01	0.12±0.01
G2	Diazepam Vaccine Chitosan	0.73±0.03	0.55±0.04
G4	Chitosan Vaccine	1.10±0.09	0.75±0.04
G3	Pasteurella Vaccine	0.51±0.02	0.32±0.03

Table (1): (Mean ± SE) of skin test in different immunized groups at 24 and 48 hours.

Humeral examination: Passive haemagglutination test:

The passive haemagglutination test for immunity was significantly increased with the time progress in the first month and second month in all groups. The mean titer antibodies of passive haemagglutination in all groups (Table 2) recorded after the first month of vaccination in the 1st group (115.2±37.32), 2nd group (179.2±31.35), 3rd group (307.20±86.82) and 4th group (819.2±125.42) respectively, while the titer antibody test values after second month were in the 1st group (356.0±70.11), 2nd group (716.8±125.42), 3rd group (665.6±153.61) and 4th group (1638.4±250.84) respectively. However the titer antibodies to 4th group recorded significantly ($p<0.05$) higher values than all other groups in their titer antibodies after the first and second month post vaccination.

Group No.		**Abs titers**	
		30 days	**60 days**
G1	Diazepam Vaccine	115.2±37.32	256.0±70.11
G2	Diazepam Vaccine Chitosan	179.2±31.35	716.8±125.42
G4	Chitosan Vaccine	819.2±125.42	1638.4±250.84
G3	Pasteurella Vaccine	307.20±86.82	665.6±153.61

Table (2): (Mean ± SE) of the titers Abs in haemagglutination test in immunized groups and control group at days 30-60.

The results of skin test and haemagglutination test indicate that Whole Cell Sonicated Antigens (WCS Ags) of P. multocida have the capability to enhance the immune system. Delayed type hypersensitivity (DTH) is the essential type of CMI and it is mediated by CD4+ T-cells and CD8+ T-cells cytokines production, these results consistent with (8) who found that (WCS Ags) were stimulating the (DTH) reaction. DTH was used to test if the prior exposure

to an antigen had occurred when a small quantity of extracted antigen are reinjected intradermal, an obvious mark response is elicited represented by the thickness (indurations), and swelling due to monocytes infiltration into the site of the lesions within 24 to 48 hrs. This reaction has been shown to be absolutely dependent on the presence of memory T-cells for CD4+ fraction have been modulated a response (9). Also a contemporary debate regarding focusing on the role of the Th1 and Th2 cells in the reaction and it has been postulated that the Th1-cells are the inducer of a DTH response since it secretes interferon gamma (IFN-γ) a potent stimulator of macrophages, while the Th2 cells are either not involved or acting as a down regulator of cell mediated immune response (10). In addition to, the interleukins IL-1 and TNF-α stimulate lymphocytes migration into the skin following intradermal inoculation, the IL-1 and TNF – α and β all increase binding of lymphocytes to micro vascular epithelium presumably these two phenomena are linked because the interleukins recruit lymphocytes to inflammatory sites (11). The results of this study consisted with (12) who explained the ability and activity of skin test depend on the cells to recognized the antigen and the secrete of IL-1 by macrophages which enhanced proliferation and differentiation of other T-cell into Th1-cells which secret IL-2 as a chemoatractive factor to attract macrophages around the area of activated T-cells. Also secrete INF-γ which enhancing the cytolysis activity of accumulated macrophages leading to a skin thickness (indurations). The present investigation also showed that immunized animal treated with diazepam revealed low antibody titers as compared with other immunized groups, these results may give indication that diazepam depressed the skin test and humeral immune response and these evidences are inconsistent with (13) who suggested an immune toxic effect of diazepam and alprazolam on both humeral and cell-mediated immune responses in native non-stressed animals. The low values of (DTH) in immunized-treated animals with diazepam may be induced defect in CD4+ and CD8+ T-cells by diazepam, these propositions supported the idea that maintained by (14) who found that the immunodeficiency induced by sedative drugs therapy, is primarily related to CD4+ and CD8+ T-cell depletion and induced immunosuppression. The results agreement with (15) who found that depress both primary antibodies to sheep red blood cells and delayed-type hypersensitivity responses in normal mice through drug treated. Diazepam is inhibited in vitro for the phagocytic function and the antibody synthesis (16). The present study showed that increase levels values of DTH and serum Abs titers in immunized animals treated with Chitosan compare with other immunized group, these results may give indication that Chitosan is an immune stimulators, this suggestion was in consistence with (17) who suggested that the protein antigens alone are weak stimulators of the immune system and thus require adjuvant system such as Chitosan enhanced both humeral and cell mediated immune responses. The present results of this study may indicate that the immunized animals treated with Chitosan and diazepam may be neutralized the effects of diazepam by activation of the immune system from during cells mediated immunity (CMI) and humeral immunity so that leads to remove the dead cells and prevent formation of free radical, these results agreement with (18) who suggested that Chitosan can scavenge free radicals.

The references:

1. **Pupchem. (2006).** National Institute Of Health: National Library Of Medicine. Retrieved 2006-03-11.
2. **Drummond, H.R. (1988).** Fatal intoxication of a young drug addict with diazepam. Arch toxikol, 31:147-51.
3. **Riss, J.; Cloyd, J.; Gates, J. and Collins, S. (2008).** "Benzodiazepines In Epilepsy: Pharmacology and Pharmacokinetics" Scand 118 (2): 69-86.
4. **Huemer, H.P.; Lassnig, C.; Nowotny, N.; Irschick, E.U.; Kitchen, M. and Palvic, M. (2010).** Diazepam leads to enhanced severity of orthopox virus infection and immune suppression. J. vaccine.31; 28 (38):6152-8.
5. **Sinha, B.; Herrmann, M. and Krause, K.H. (2000).** Is staphylococcus aureus an intracellular pathogen – Response. Trends Microbial; 8:343-44.
6. **Hudson, L. and Hay, F.C.(1980).** Practiced immunology, 2nd. Ed. Blackwell Scientific Publication, London.
7. **Herbert, W.J. (1978).** Passive heamagglutination with special reference to the tanned cell technique. Ch. 20, In: Weir, D. M. "Handbook of Experimental Immunology". (3rd Ed.) Vol. II, Cellular Immunology. Blackwell Scientific Publication, 20:1-20.
8. **Fjallbrant, H.M.; Ridell, M. and Lorsson, L.O. (2001).** The tuberculin skin test in relation to immunological in vitro reaction in BCG-vaccinated health care worker. Eur. Respir. J. 18:376-380.
9. **Dietert, R.R.; Bunn, T.L.; and Lee, J.E. (2010).** The delayed type hypersensitivity assay using protein and xenogeneic cell antigens, Methods Mol Biol.; 598:185-194.
10. **John, D. and Mac Arthur, C.T. (1997).** Decrease in cell mediated immunity-a marker for allostatic load effect on immune function. Research network on socioeconomic status and health. File://I:/ cell-mediated immunity-htm.
11. **Tizard, I. R. (1992).** Veterinary immunology W.B. Saunders Comp. Philadelphia, Pennsylvania, USA.

12. **David, A.Z.; Kenneth W.H.; Jeffrey, S. and John, W.G. (2006).** Chitosan solution enhances both humeral and cell-mediated immune response to subcutaneous vaccination. 37:21-27.
13. **Mediratta, P.K. and Sharma, K.K. (2002).** Role of benzodiazepine-GABA receptor complex in stress-induced modulation of leukocyte migration inhibition factor. Indian J. Pharmacol. ; 29:228-232.
14. **Mackall, C.L.; Hakim, F.T. and Grees, R.E. (1999).** T-cell regeneration: all repertoires are not created equal. Immunol Today. 18:245-251.
15. **Desctes, J.; Tedone, R. and Evreux, C. (1982).** Suppression of humeral and cellular immunity in normal mice by diazepam. Immunol Lett5:4143.
16. **Covelli, V.; Maffione, A.B.; Nacci, C.; Tato E. and Jirillo, E. (1998).** Stress, neuropsychiatric disorders and immunological effects exerted by benzodiazepines. Emu-nopharmacol Immunotoxicol 20:199-209.
17. **Reed, Y.; Tanioka, S.; Tanaka, M.; Tanigawa, T.; Kitamura, Y. and Minami, S. (2009).** Effects of chitin and Chitosan particles on BALB/c mice by oral and parenteral administration. Biomaterial, 18:591-595.
18. **Kim, J.H.; Lee, J.; OH, B.; Kimm, K. and Koh, I. (2007).** Prediction of phosphorylation sites using SVMs. Bioinformatics. Nov 22;20 (17):3179-84.