Histopathological changes and immunosuppression induce by diazepam in mice

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Abstract

In order to investigate the histological changes and immunosuppression effects of diazepam in mice, forty white mice of both sexes were divided into four groups equally. 1st group was immunized twice with *pasturella multocida* (bacterines) with two weeks intervals. 2nd group was immunized as in the 1st group and at same time administrated orally with 0.6 mg/kg b.w of diazepam daily for 8 weeks. 3rd group was administrated with diazepam as in the 2nd group while 4th group was served as control negative group. Immunological examination revealed that the diazepam inducing depresses of the both arms of immune response, the cell mediated immunity and humeral immunity as comparing with vaccinated non-treatment animals. The pathological examination revealed that the diazepam induced large multiple granulomatous liver lesions consist form aggregation mononuclear cells particularly macrophages and lymphocytes. In addition, section of kidney showed marked inflammatory cells infiltration particularly mononuclear cells and neutrophils in the interstitial tissues was seen. In spleen there was congestion of blood vessels with mononuclear cells in their lumen and depletion of white pulp as well as proliferation of megakaryocytes, in addition to gliosis in the brain parenchyma was seen. The immunized animals showed mild pathological changes characterized by aggregation mononuclear cells around central vein in addition to proliferation of kupffer cells, Spleen show proliferation of lymphocytes in the periartirilar sheath as well as protein aqueous materials deposition around white pulp. We concluded that diazepam induced Histological changes in the internal organs of mice and stimulated the immune response diminished its toxic effects.

Key words: Diazepam, Histological, Immune suppression.

دراسة التغيرات المرضية النسيجية والمناعية المحدثة بواسطة عقار الديازپام في الفئران

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الخلاصة

أجريت هذه الدراسة لتحديد السمية المرضية النسيجية والتسمم المناعي لمادة الديازپام في الفئران الممنعة والغير الممنعة لقاحيا. تم اختيار 40 فأر من كلا الجنسين قسمت عشوائيا الى أربعة مجاميع متساوية وعولجت كالتالي. المجموعة الأولى منعت بلقاح الباستوريلا أما المجموعة الثانية لقحت بلقاح الباستوريلا وجرعت فمويا بمادة الديازپام لمدة ثمانية أسابيع 0,6 ملغم/كغم أما المجموعة الثالثة جرعت فقط بمادة الديازپام، أما المجموعة الرابعة هي مجموعة السيطرة حيث جرعت 0,25 مل من الماء خلال فترة التجربة. أظهر الفحص المناعي الخلوي والخلطي تأثير الديازپام في خفض الاستجابة المناعية في الحيوانات الممنعة مقارنة مع المجاميع الممنعة غير المعاملة بالديازپام. أما الفحص النسيجي أشار الى ان الديازپام يتسبب في أحداث أفات حبيبية مع تجمع الخلايا الوحيدة النواة، أما الكلية فتميزت بأرتشاح الخلايا الالتهابية في النسيج الخلالي. أما الحيوانات الممنعة والمعالجة بمادة الديازپام فقد كانت التغيرات المرضية معتدلة وتميز الفحص النسيجي بتجمع خلايا وحيدة النواة في الطحال. نحن أثبتنا أن مادة الديازپام لها تأثير سام على الأعضاء الداخلية وانخفاض الاستجابة المناعية في الفئران. الكلمات المفتاحية: الديازپام، التغيرات النسيجية، انخفاض المناعة.

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 epileptics), 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 amnesic properties (3). All benzodiazepines, including diazepam, effect on peripheral benzodiazepine receptor sites (PBR) or translocation protein receptor (TSPO) (4). Evidence for a direct immunomodulatory action for BZD emerged from recent studies demonstrating the presence of (TSPO) on immune/inflammatory cells (5). Diazepam overdose causes many problems in paradoxical side effects can occur, including nervousness, irritability, excitement, worsening of seizures, insomnia, muscle cramps and in some cases, rage. These adverse reactions are more likely to occur in children, the elderly, and individuals with a history of drug or alcohol abuse and or aggression (6). Diazepam may increase, in some people, the propensity toward self-harming behaviors and, in extreme cases, may provoke suicidal tendencies or acts. Patients with severe attacks of apnea during sleep may suffer respiratory depression (hypoventilation), leading to respiratory arrest and death (7). Acute hepatic injury may be caused by a direct toxic effect of the drug or its metabolites on the hepatocyte, producing a predictable dose-dependent effect, or by idiosyncratic drug reactions, which occur unpredictably in a small number of cats exposed to a particular drug such as diazepam (8).

Materials and methods

Diazepam using in water as a solvent, diazepam has an 0.6mg/kg b.w for white mice (8). Forty white mice of 7-8 weeks in age, supplied from animal house were used in this study. The animals were divided into four each groups (10 mice) and was treated as following: G1, was immunized with *pasturella multocida* (bacterin), two doses, with two weeks intervals. G2, was immunized as in 1st group and at the same time it was given daily diazepam, via oral route 0.6mg/kg, b.w for 8 weeks. G3, was administrated with diazepam as in 2nd group. G4, was administrated daily with 0.3ml of sterile normal saline orally for 8 weeks and it was served as control negative group.

Results

Immunological examination

Skin test: At 24 hrs. post testing, the results showed that the mean values of skin thickness against bacterin was high in 1st group (0.94 ± 0.01) as comparing with 2th group (0.25 ± 0.01) and these values were decrease at 48hr post-examination in the 1st group (0.82 ± 0.01), 2nd group (0.12 ± 0.01). (table 1).

Table (1) values skin test in immunized groups at 24 and 48 hours. (measured in mm).

Group	Group	24 h mean $\pm$ SE	48 h mean $\pm$ SE
1	<i>pasturella</i> vaccine	0.94 ± 0.01 Ab	0.82 ± 0.01 Ba
2	diazepam + <i>pasturella</i> vaccine	0.25 ± 0.01 Ad	0.12 ± 0.01 Bd

L.S.D = 0.16

The results of passive haemagglutination examination in the 2 weeks revealed that the serum Abs titers in 1st group was (307.20 ± 86.82) higher than in 2nd group (115.2 ± 0.01), while the these values at 30 days post-the serum Abs titers in 1st group (665.6 ± 153.61) was higher than in 2nd group (256.0 ± 0.01) (table: 2).

Table: 2. values serum Ab titers at 14 and 30 days post-immunized animals

Group	Group	4 weeks mean $\pm$ SE	8 weeks mean $\pm$ SE
1	<i>Pasturella</i> vaccine	307.20 ± 86.82 bB	665.6 ± 153.61 Ba
2	diazepam + <i>pasturella</i> vaccine	115.2 ± 0.01 Ad	256.0 ± 0.01 Bd

L.S.D = 490.70

The current study showed that the mean values of skin thickness in immunized animals with *p. multocida* (bacterin) was higher than those value in immunized animals treatment with diazepam, these result may be indicated that the diazepam depressed the cell mediated immune response with reduction in delayed type hypersensitivity (DTH) which considered one arm of CMI that mediated by CD4+T cells and CD8+T cells cytokine production (11). Also these observation may be supported idea that diazepam have immunosuppression effects. Also our investigation was in agreement with (12), who reported that the diazepam decrease in macrophage spreading and phagocytosis was

reported as a result of pre- and postnatal diazepam treatment in rats (13), also immunosuppress may result from inhibitory for the phagocytic functions and the antibody synthesis(14) who said diazepam was found to depress both primary antibody and delayed-type hypersensitivity responses in normal mice treated with diazepam is (15).The lower values of DTH reaction in immunized animals treated with diazepam as comparing with only immunized animals may be due to diazepam type binding in sites benzodiazepine receptors (PBR) in all endocrine steroid genic tissues, organs and cells of the immune system, such as macrophages and lymphocytes. Thus, the PBR may be a possible primary target for the immunotoxicity effects of BDZ (16).also immune toxic effects might be related to cortisol production. The present finding showed that the Abs titer in general, were high at day 60 as comparing with those values at 30 days post-immunization ,these results may be due to exposure the animals to posture dose of antigen at two weeks post-first dose which lead to stimulate the secondary immune response and the values of immune response were high in secondary immune responses as comparing with primary immune response (17), also these result may be indicated that diazepam have toxic effects on humeral immune response. Our results are in accordance with those of(17) who stated that many benzodiazepines induced prolonged impairment of cellular immune functions in experimental animals after chronic low-dosage administration. It has been substantiated that stressful stimuli in man as well as in animals lead to suppression of the humeral and cellular components of the immune system. The central nervous system is known to be involved in the regulation of stress-induced immune responsiveness (17)and administration ofbenzodiazepine with inhibition of IL-2. (17) Found that exposure to low doses of BDZ resulted in long-lasting reduction of TNF-alpha, IL-1, IL-6, IL-2 diazepam induced severe inhibitory effects on the proliferative responses of both B- and T-cells.

Histopathological diazepam:

At4weeks post-treated with diazepam

Brain: the main lesions in the brain of animals treated with diazepam were increase number of astrocytes (fig.1)

Liver: There is inflammatory cells infiltration particularly neutrophil in dilated sinusoid (fig.2) in other section large multiple granulomatous lesion consist form aggregation mononuclear particularly macrophages and lymphocyte scatter in the liver parenchyma and around congested blood vessels in addition necrosis of hepatocyte (fig.3).

Kidney: main pathological lesion characterized congestion of blood vessels (fig.4).

Spleen: Spleen showed precipitate protinicious materials in red pulp wall as well as congestion blood vessels, edema and depletion of lymphocytes in the white pulp (fig.5).

At 8 weeks post- treated with diazepam:

Brain: Microscopic section revealed mononuclear cells aggregate around blood vessels in brain parenchyma together with edema around neuron (fig.6).

Kidney; Microscopic section revealed fibrous connective tissue around blood vessels and renal tubules that showed cellular degeneration characterized by enlargement of renal epithelial cells and vacuolar degeneration. also there is sloughing of the epithelial lining cells of renal(fig.7).

Spleen: the pathological examination of the spleen showed congestion blood vessels with mononuclear cells in their lumen and depletion of white pulp as well as proliferation of megakaryocytes (fig.8).

Immunized animals at 4weeks treated with diazepam:

Brain: No clear lesions were seen in the brain (fig.9).

Liver: aggregation mononuclear cells around central vein in addition to proliferation of kupffer cells (fig.10).

Kidney: No clear lesions were seen in the kidney.

8 weeks post- treated with diazepam

Liver: main lesion characterized in liver mice treated by diazepam with immunized by aggregation mononuclear cells around bile duct and portal vein (fig.11).

No clear lesions were seen in the kidney (fig.12), and brain.

Fig:1 Histopathological section in brain of mouse treated with diazepam at (4)weeks shows focal microglial cells proliferation and perineuronal congestion and edema arrow (H&E stain 40X).

Fig:2 Histopathological section in liver of mouse treated with diazepam at (4)weeks shows inflammatory cells infiltration particularly neutrophil in the dilated sinusoid and single necrosis of hepatocytes arrow (H&E stain 40X).

Fig:3 Histopathological section in liver of mouse treated with diazepam at (4)weeks shows large multiple granulomatous lesion and around congested blood vessels in addition to necrosis of hepatocytes arrow (H&E stain 40X).

Fig:4 Histopathological section in kidney of mouse treated with diazepam at (4)weeks shows congestion blood vessels and RBCs in the lumen renal tubule arrow (H&E stain 40X)

Fig: 5 Histopathological section in spleen of mouse treated with diazepam at (4)weeks shows precipitate proteinaceous substance in red pulp wall as well as congestion blood vessels, edema .also depletion lymphocytes which left space in white pulp arrow (H&E stain 40X) (H&E stain 40X).

Fig:6 Histopathological section in brain of mouse treated with diazepam at (8)weeks shows mononuclear cells aggregate around blood vessels in brain parenchyma together with edema around neuron arrow (H&E stain 40X)

Fig:7 Histopathological section in kidney of mouse treated with diazepam at (8)weeks shows fibrous connective tissue proliferation around blood vessels and renal tubule with mononuclear cells infiltration, as well as vacuolar degeneration of epithelial lining cell of renal tubules arrow (H&E stain 40X)

Fig:8 Histopathological section in spleen of mouse treated with diazepam at (8)weeks shows congestion red pulp and proliferation megakaryocytes and depletion of lymphoid tissues of white pulp arrow (H&E stain 40X)

Fig: 9 Histopathological section in brain of immunized mouse treated with diazepam at (4)weeks shows no clear lesions in the brain at 30 days post treated arrow (H&E stain 40X).

Fig:10 Histological section in liver of immunized mouse treated with diazepam at (4)weeks shows aggregation mononuclear cells around central vein in addition to proliferation of kupffer cells arrow . (H&E stain 40X)

Fig11 Histopathological section in liver of mouse immunized with diazepam at (8)weeks shows aggregation mononuclear cells around bile duct and portal vein arrow (H&E stain 40X).

Fig:12 Histopathological section in kidney of mouse treated with diazepam and immunized at (8)weeks shows dilated of bowman capsule and proliferation fibrous connective tissues between renal tubules and around glomerular infiltrated with mononuclear cells arrow . (H&E stain 40X)

Discussion

Main pathological lesion in the liver may be due to drug toxicant these results that agreed with (18) they reported that toxicity in liver characterized by the presence of hepatic cells undergo necrosis and others undergo degenerative changes and it appeared as a constant feature of all examined sections and that because of the accumulative ability of diazepam these results similar to that noticed by (19). Our result finding seen in the spleen showed protienaceous materials in red pulp as well as congestion blood vessels these results may be the spleen is considered one of the lymphoid tissue play an important role in the immune system which affected with sedative drugs lead immune suppression this agrees with (20) who found Lymphocytic depletion was noticed in both red and white pulp of the spleen in rats treated with diazepam (0.1 mg) daily for 4 weeks. Our result finding seen in the brain of animals treated with diazepam were proliferation focal microglial cells also microscopic section revealed mononuclear cells aggregate around blood vessels in brain parenchyma these results may be attributed to the toxic effect of diazepam overdose causes CNS and respiratory depression which may lead to coma and death. Experimental data show that acute administration of high doses of diazepam results in a cascade of oxidative changes and significantly diminishes cell antioxidant defense, especially the intracellular levels of reduced glutathione (21). ,also (22) prenatal treatment with benzodiazepines affects the development of the immune system. in addition to, edema may be due to a breakdown of tight endothelial junctions which make up the blood-brain barrier (BBB) (23) also agreement with (24) referred that the BBB has a very important function in maintaining the fluid environment of nervous

system. Our result finding seen main pathological lesion characterized vacuolar degeneration in the glomerular tuft with dilatation in bowman space all these lesion may be accumulate main active metabolite of diazepam is called desmethyl diazepam in Kidneys which consider important excretory organ, which, along with the liver, also accumulate toxicants these agreement with (25). The lesions in the organs in immunized animals are mild lesions than organs animals' toxicity by benzodiazepine. This result may indicate the vaccine stimulate the immune system that this lead to activation immune cells that Contribute in protective the body from effects the diazepam this suggest Explains as follows the immunize ability of outer membrane proteins which activated CD4 T-cell in turn liberate lymphokines such as IL-4, IL-6 for B-cells and IL-2 for T-cells also INF- α FOR macrophages and all lymphokines, and the activated immune cells play a role in produce highly production against infection (26). Also the cytokine Contribute in repair tissue damage and supported this idea Cytokines are critical controllers of cell, and hence tissue, growth, migration, development and differentiation. The family includes the inflammatory cytokines such as the interleukins and interferon's, growth factors such as epidermal and hepatocyte growth factor and chemokine's such as the macrophage inflammatory proteins, MIP-1a and MIP-1b. They do not include the peptide and steroid hormones of the endocrine system. Cytokines have important roles in chemically induced tissue damage repair, in cancer development and progression, (27), the complex relationships that occur between tissues and drugs, and subsequently between cytokines and the tissue responses that modulate the therapies to which drugs are applied (28); (29).

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