Histological Study of Azathioprine Effect on Liver in Insulin Resistant Rats

Ardeshiri R, Ghassemi F, Kargar H

Department of Biology, Jahrom branch, Islamic Azad University, Jahrom, Iran

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ABSTRACT

Background: Azathioprine with adverse effects on some organs, are widely used in the treatment of certain diseases. In this study, the effect of azathioprine on liver functional factors as one of the most important organs of body in mice resistant to insulin was investigated. Method: 56 Wistar rat weighing 200±20 gr were classified in 8 groups. Control (without treatment) and sham (140cc fructose 10% as a daily feeding). Groups 1 to 4 (diabetic) in addition froctose 10 % feeding, were injected (3.75, 7.5, 15 and 21) mg/kg/b.wt azathioprine interaperitoneal at 98th day. Groups 5 and 6 (adiabatic) were injected (15 and 21) mg/kg/b.wt azathioprine interaperitoneal. 24 hours after, blood samples were taken from all groups and their serum separated for biochemical analyzes. Then liver was separated and sections prepared for histological study. Data were analyzed by ANOVA and Duncan test (P<0.05). Results: The histological changes including lymphocyte invasion, hydropic changes in portal areas in diabetics treated groups with minimum does of drug and infiltration, cytoplasm granularity and necrosis of peri-portal area with the maximum dose was observed. In the control and non-diabetic treatment groups, slight changes were seen. ALP and FBS levels in all groups and and AST (dose depended) in treated diabetic groups and sham has seen a significant increase compared to control (p<0.05), but ALT and MD showed no significant change. Conclusion: Azotioprin is dose- dependent and had synergistic effects with diabetes. Therefore, diabetics are taking limits.

Key words: Azatioprin, Tissue damage, Liver enzyme

Introduction

Azathioprine (AZA) is an immune suppressive drug that treat diseases such as leukemia; acute lymphoblastic, inflammatory bowel disease and rheumatoid arthritis. Azathioprine with corticosteroids is the best option to prevent organ rejection. Despite the widespread use of this drug, AZA has been observed that has other effect such as suppressing the patient's lymphocytes, causing toxicity in the bone marrow, gastrointestinal tract and liver [2]. Toxic effects of the drug are the cause of production free radical in organs, tissues and oxidative injury. It act by selectively inhibiting the synthesis of purine nucleotides (adenine) and reducing DNA synthesis of a variety of immunologic and other specialized cells, including hepatocytes due to oral administration of AZA increase liver enzymes level as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Increased malondialdehyde and reduced glutathione levels, due to its effect on the creation of reactive oxygen radicals cause changes in tissues, tissue necrosis, enlarged mitochondria and the rough endoplasmic reticulum [1]. Diabetes (Type 2) or non-insulin dependent diabetes is the world's largest hormone disorder [16]. In this type of diabetes, skeletal muscle, liver and adipose tissue will resistance to insulin which can lead to decreased glucose uptake, increased hepatic glucose and lipid. Insulin resistance is associated with many disorders including high blood pressure, elevated blood lipids and renal disorders [13]. Diabetes can change metabolism and excretion of drugs and toxins, [11]. Furthermore, the role of the liver is in detoxification and metabolism of some drugs, such as carbon tetrachloride, thioacetamide [18], and aspirin [6], has been studied in rat. In the current study, effects of AZA on liver function in rats that resistant to insulin were evaluated.

Materials and Methods

In this study, 56 male Wistar rats weighing 20 ± 200 g in standard conditions (12 h dark, 12 h light and temperature 22 ±2°C) were kept and fed food and water intensive. LD50 was determined by taking a dose of medication for induction of diabetes, along with drinking water for 98 days, daily fructose solvent cc 140 (10%) were used. By shedding a drop of blood taken from the tail of mice after 12 hours of starvation on specific kits, blood glucose levels were
measured. The rats were divided in 8 sub-groups as follows:

Control group: no treatment, sham group: 140 cc fructose 10% as a daily feeding. Groups 1 to 4 (diabetic) in addition intake fructose 10%, were injected (3.75, 7.5, 15 and 21) mg/kg/b.wt AZA interaperitoneal at 98th day. Groups 5 and 6 (non-diabetic) were injected (15 and 21) mg/kg/b.wt AZA interaperitoneal at 98th day. 24 hours after drug injection, blood samples were taken from all groups, centrifuge with 2500 rpm for 10 minutes and their serum separated for biochemical analyzes. In this study, parameters including alanin aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, malondialdehyde and fasting blood sugar (FBS) measured. Then liver was separated and sections prepared for histological study. Specimen were fixed in 10% formalin, were placed in tissue processor and dehydrated in graded series of ethanol (70%-100%), then impregnated with paraffin and serial 4μ thickness sections were obtained and subjected to Haematoxylin and Eosin (H&E) stains. Sections were mounted with binocular light microscope (x40, x100) and photomicrograph was prepared.

Data were were statistically evaluated with Statistical Package for Social Sciences (SPSS), Version 17. Hypothesis testing methods included one-way analysis of variance (ANOVA) and all groups compared by Duncan test (p<0.05). All the results were expressed as mean ± S.D.

Results:

The histological changes including lymphocyte invasion, hydropic changes in portal areas in diabetics treated groups with minimum does of drug and infiltration, cytoplasm granularity and necrosis of peri-portal area with the maximum dose was observed (fig7: C-F). In the control (fig6: A) and non-diabetic treatment groups (fig 7: G,H), slight changes were seen. Alkalin phosphatase levels (Figure 2) and fasting glucose (Figure 5) in all groups and aspartate amino transferase (Figure 1) in diabetic treated groups increased significantly compared to the control group (P ≤ 0.05). Alanine amino transferase (Fig. 3) and malondialdehyde (Figure 4) in any groups compared to the control group showed no significant change. In the diabetic groups, at higher doses, indicating further damage (fig 6, 7).

*Common letters indicate no significant difference (p<0.05).
* Each column represents the mean ± SD
Fig. 3: Alanine transferase (ALT) in rat’s groups

Fig. 4: Malondialdehyde (MDA) in rat’s groups

Fig. 5: Fasting blood sugar (FBS) in rat’s groups
Fig. 6: Light micrographs of liver section (x40, x100), staining: H&E Control group (A): blood sinusoids (1) and hepatocytes (2) are normal Sham group (B): blood sinusoids (1) are dilated and congested with blood and hepatocytes are granulated (3)
Fig. 7: Histopathological effects of Azathioprin in rat's liver, Light micrograph of experimental groups 1-6 (C-H), sections (x40-x100), staining: H&E

C: Granularity in hepatocyte (1), D: Degeneration of hepatocyte (2) and moderate infiltration of inflammatory cells around portal triad(3), E: vein is widely dilated(4), and kupfer cells are in sinusoid(5), eosinophilic cytoplasm and inflammation (6), F: hydropic degenerating (7) and Inflammation cells (8), G:aggregation lymphocytes in the sinusoid (9), H: hepatocytes are more and less normal(10) and glandular around portal triad (11)

Discussion:

The toxicity of the drugs depends on the chemical nature of the drug and patient's factors. Some diseases affect the absorption, distribution, metabolism and excretion of drugs affecting modern society. Nowadays lifestyle-related diseases such as obesity, diabetes, hypertension and hyperlipidemia is increasing.

It can alter the toxicity of the drug, which leads to health crisis associated with drug use [2]. Based on the results this study are is consistent with the research [12,9], the effect of the drug which cannot be ignored also increasing serum alkaline phosphatase (ALP) in the treatment groups who received high doses of the drug, especially in diabetics rats comparing to control group showed the synergistic effect diabetes with drug [4].

Meanwhile, the drug appears to be dose-related acts but on the other hand no significant change in alanine amino transferase (ALT) and malondialdehyde (MDA) in this study were consistent with the expected results [17,15] at least with these doses. alanine aminotransferase in detecting liver damage is more important than the others because level of this enzyme in the cytoplasm of hepatic cells is several times higher than the serum's level of that and when the membrane was damaged, the enzyme was removed from the cells and its serumic concentration increases.

The extent of this increase is an indication of the degree of liver injury [8]. No significant changes in the enzyme levels above prove the claim. Given the dose-dependent and time-consuming toxins, can cause changes in tissue, in addition to the physiological state of the target tissue dose and time of exposure to the drug involved the effect of the drug [8].

Based on result of this study is to some extened justified. Aspartate aminotransferase increased in groups treated diabetes refers to diabetes complications though higher doses have been shown that the optimizer can work together and the sum effects of both drugs and diabetes on various organs in the body is the main cause of this result can be considered (Fig 1). When tissue damaged, these enzymes, from liver and other tissues as heart muscle leaking to serum and drug effects on other organs cannot be ignored [17]. lymphocytic invasion around the portal triad (Fig. 7) indicate the presence of smaller vessels in the fibrous mass that cannot be observed with an optical microscope.

According to studies, blood glucose and lipid peroxidation, glycosylation due to changes in the structure of proteins and lipids, changes in cell membrane structure and permeability changes, it leaking out of the cell and into the cytoplasm of some enzymes in the serum occurs [14].

Increase in AST and ALP in diabetic AZA-treated rats explains the leakage of these enzyme into circulation which suggests hepatocellular damage also happened in the vascular membrane and impaired liver enzyme levels (Fig. 2). The observed intensity of damage in tissues in treated diabetic (Fig. 1 to 4) comparing to control group (Fig 2), although it appears the damage is due to diabetic complications, but taking AZA, possibly intensified
and cell death (apoptosis) was observed in (fig. 7: E,F). Portal fibrosis and inflammation of the blood vessels around portal triad, can be hydropic liver cells and even cause cell death is induced [10]. The veins was widely dilated and the cytoplasm showed some degeneration. According to previous reports about AZA [4], seemingly obvious explanation could be that AZA selectively inhibit synthesis of purine nucleotides, which are required for DNA synthesis. It has been suggested that, in rat hepatocytes treated with AZA, ROS production could damage membranes and macromolecules at this level [10], although there are no convincing results supporting this hypothesis. Another potential source of ROS that could initiate oxidative stress may be related to production some metabolites as 6-mercaptourine that is toxin and ROS may be formed during their metabolism.

No significant differences in the levels of MDA as the most products of lipid peroxidation in the groups studied but according to some studies, after taking some toxins [5], or drugs such as AZA [2], MDA increased in blood or tissue, and antioxidants significantly reduced [13]. Probably short duration of the study, opportunity for complications due to oxidative stress were not enough, especially in diabetes(type II) that the effect is long lasting. According to the AZA metabolism purine antagonist and perhaps product free radicals, which inhibit the synthesis of nucleic acids, proteins, and lipids [3].

The low toxic effects AZA on hepatocytes in this study, justify widespread use in treatment comparing corticosteroids and cyclophosphamide. Although the physiological conditions of the exposure and the disease should be considered. Increase in fasting glucose in diabetes treatment due to resistance in skeletal muscle, liver and adipose tissue to insulin leading to reduced glucose uptake (increased glucose), increased hepatic glucose production is Lipogenesis and the effects of drugs on the synthesis of insulin receptors in cells can increase fasting blood sugar (FBS) [3]. Therefore, it should be more cautiously in diabetic patients.

Conclusions:

Results obtained showed azathioprine have less toxicity in rat hepatocytes and no induction stress oxidative but in diabetic rats, due to synergistic effects of AZA with diabetes medications can cause severe damage. Therefore, in such cases the drug has limitations.

Acknowledgment

We gratitude manager of Islamic azad university Jahrom branch and all those who helped us in doing this research.

References


