Study of Azathioprine Effects on Serum Markers of Insulin Resistance in Male Rats

Abbas Houshmandi, Farangis Ghassemi, Rouhollah Ardeshiri, Marzie Eilani

1Department of Biology, Islamic Azad University, Jahrom, Fars, Iran.
2Young Researchers Club, Islamic Azad University, Jahrom Branch, Jahrom, Iran.

ABSTRACT

Background: Diabetes is the most common metabolic disease in humans, which can lead to kidney damage; Azathioprine is widely used in the treatment of other autoimmune diseases, cancer, and organ transplantation. In this study, the effect of azathioprine on renal tissue and serum markers of insulin resistance in rats were studied.

Methods: In this research, 56 adult male wistar mouse with 80 to 90 days old and weighing approximately 200 ±20g were diveded in 8 groups with 7 members as follow: Control group (without sugar and drug dose), sham (140cc fructose 10% as a daily feeding) and 1 to 4 experimental groups that in addition of 140cc fructose 10% as a daily feeding, were injected [3.75, 7.5, 15 and 21] mg/kg/bw azathioprine both in single dose and interaperitoneal at 96th day. 24 hours after drug administration, blood samples were taken from all groups. Its serum separated for biochemical study. Using statistical software, SPSS [18], data were analyzed by One Way ANOVA and Duncan test at the significant level of 0.01.

Results: Results showed that serumic amounts of blood glucose (FBS), alkalinphosphotase (ALP) and cholesterol (LDL) in all experimental groups has significant difference than control group (p<0.01). Direct and total bilirubin, aspartate aminotransferase (AST), urea nitrogen, creatinine and cholesterol (HDL) were increased in diabetic groups. In other hand,in all experimental groups, total protein and triglyceride (TG) were decreased significantly and no change in malondialdehid (MDA), albumin and alanine aminotransferase(ALT) levels were observed.

Conclusion: According to above results, azathioprine in diabetic patients spatially in high dose, cause liver damage and using this drug should be restricted.

Key words: diabetes, azathioprine, kidney.

Introduction

In developing countries, one of the major problems in diabetic nephropathy and progressive renal failure is the end [16] that lead to dialysis and eventually a kidney transplant disease mortality [13] that such symptoms can be these include: increasing the thickness of the glomerular basement membrane, the rise and spread of renal interstitial tissues and increasing in creatinine, level. Our current knowledge is limited about diabetic nephropathy. However, the studies that have been done in this area, have found that factors such as glycemic control, blood pressure and improve kidney function in diabetic nephropathy will eventually recover [1]. Glycosylation and lipid peroxidation leading to increased oxidative stress due to excessive production of reactive oxygen species (ROS) and decreased antioxidant system is [8, 16]. Increased production of free radicals cause lipid peroxidation and damage to cell membranes, proteins and nucleic acids and tissue damage by oxidation, resulting in decreased glomerular filtration and renal damage [16]. Azathioprine is immune suppressive drugs to treat diseases such as leukemia; acute lymphoblastic, inflammatory bowel disease and rheumatoid arthritis are used. Azathioprine with corticosteroids, the best option is to prevent organ rejection [3].

Drug Azathioprine is a drug that can inhibit the synthesis of purine bases and prevent re-replication in cells that do not synthesize DNA and RNA through the action takes place. Toxicity caused by the use of these drugs has been demonstrated in organs such as bone marrow, liver and gastrointestinal tract and pancreas. Cytotoxic effect of the drug on the production of free radicals in the body and organs of the patient [14]. Many disorders, including hypertension, insulin resistance, elevated blood lipids and renal dysfunction is associated with diabetes, metabolism and excretion of drugs and other toxic changes [15]. Therefore the aim of this project examined the effects of some factors on kidney function azathioprine in mice resistant to insulin.

Materials and Methods
In this study, 56 male Wistar rats weighing 200 ± 20 gr selected under standard conditions (12 h light and 12 h dark and the temperature of 2 ± 22 °C and humidity of 55-50 percent) were maintained. To create insulin resistance, mice were treated for 98 days with fructose (10%) daily and were divided into eight groups as follows:

Control group: no treatment group
Diabetic control group: daily cc 140 solution of fructose (10%) received group
Treated diabetes: after receiving the oral solution of fructose (10%) for 98 days, respectively, kg / bw mg / (3/5, 5/7, 15 and 21) received intra-peritoneal injection drug azathioprine.

To validate these findings, biochemical markers were measured using specific kits (6). Mean values obtained analyzed by statistical software SPSS-18 and using (ANOVA) and Duncan test for comparing groups (05/0 ≤ P). The results were presented as Mean ± SEM.

Results:

The results show that the mean serum uric acid(fig 4), BUN(fig 2) and creatinine(fig3) in diabetic treated and control groups (as diabetes showed a significant increase. However, no differences were observed in non-diabetic treatment groups (05/0 ≤ P) in the control group, mean serum urea nitrogenor BUN (as diabetes) shows a significant increase compared to the control group and groups that have received only azathioprine(fig 1) (05/0 ≤ P). In all treatment groups, including diabetic and non-diabetic serum LDL(fig 9) and fasting glucose(fig 10) compared with the control group (05/0 ≤ P) shows the concentration of HDL in sham group and some diabetic groups treated, decrease but the non-diabetic treatments didn’t change significantly and increased compared to the diabetic groups(fig 8). Albumin(fig 5) and malondialdehyde(fig3) did not change significantly (Table 1). Serum triglycerides (fig 7), total bilirubin (fig11) significantly increased in diabetic treated group (05/0 ≤ P) and total protein(fig 6), levels in the treated diabetic group some treatments are non-significant decrease compared to controls.
Fig. 2: Creatinine level in serum.

Fig. 3: Malondialdehyde level in tissue.

Fig. 4: Uric acid level in serum.

Fig. 5: Albumin level in serum.
Fig. 6: Total protein level in serum.

Fig. 7: Triglyceride level in serum.

Fig. 8: HDL cholesterol level in serum.

Fig. 9: LDL cholesterol level in serum.
Discussion:

Diabetes is the most common endocrine diseases, metabolic disorders can be applied to this disease. In hyperglycemia, most cells cannot use glucose for nutrition [10]. Evidence indicates that hyperglycemia is an important factor for kidney damage. Increased levels of uric acid, BUN, creatinin, indicating kidney damage in diabetic [10] into the tubular secretion of creatinin and glomerular filtration on urine. Some drugs can despite normal kidney function, decreased tubular secretion may increase serum creatinine [14]. In addition to drugs, other substances such as glucose, uric acid, ketones and bilirubin levels can also cause a sharp rise in serum creatinine are false changes [14]. Ammonia from the amino acid is converted to urea in the liver by a cyclic mechanism and inner medullary nephron reabsorption in the urine-collecting tubes. Increased urea and uric acid in the control group (diabetic) can be related to the metabolism of the drug in the liver. The impact of drug-induced damage to the liver may be a natural cycle and its effects on some materials just does not
happen all come together. If amino acids are produced in the liver converts ammonia to urea in the blood that cannot be moved. Ammonia is highly toxic and can cause damage to the kidney tissue [14], MDA as a specialized test known to show cause oxidative damage [21] and the studies, blood glucose and lipid peroxidation Glicolization due to changes in the structure of proteins and lipids, and oxidative stress would result in the creation of this lesion and subsequent diabetes increased levels of malondialdehyde [17]. The short duration of diabetes research opportunities for Oxidative Damage in mice did not develop type II diabetes, especially the long-term effect, On the other hand, according to some studies, after taking some poison [7], or drugs such as azathioprine [3], MDA in blood or tissue, is increased [18]. Diabetes is an indicator of kidney damage. Azathioprine metabolism purine antagonist and perhaps create free radicals, which inhibit the synthesis of nucleic acids and proteins, and lipids [4]. Changing some parameters, such as increased total bilirubin and direct, cholesterol, LDL and HDL in the treatment of diabetic control, consistent with the findings of others [3,2], mostly due to complications from diabetes due to insulin resistance, cholesterol metabolism and lipid is disrupted [19]. Increase in fasting glucose in diabetic treatments induced resistance in skeletal muscle, liver and adipose tissue to insulin leading to reduced glucose uptake (increased glucose), and increased hepatic glucose production is Lipogensis [19]. Also the influence of drugs on the synthesis of insulin receptors in cells can be elevated fasting glucose [4].

Conclusions:

According to the biochemical results, azathioprine at doses used in this study did not cause significant adverse effects except in diabetic patients due to synergistic effects aggravate the damage. Therefore, in such cases the drug has limitations.

References