# Biochemical and pathological study of protective effect of Vitamin A in Azathioprine - induced pancreas toxicity in Rat

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**Abstract:** Azathioprine (AZA) is the most widely used immunosuppressive drug for preventing graft rejection and autoimmune disease. However, the therapeutic treatment induces several side effects such as toxicity to bone marrow, pancrease, liver and gastrointestinal tract. One of the major functions of Vitamin A is to act as a natural antioxidant by scavenging free radicals. Considering the kind of Azathioprine-induced damage in Pancrease tissue, we decided to study the protective effect of Vitamin A against Azathiprine-induced toxicity. Forty Male Wistar rats were divided into 4 groups (each group contains 10 rats). Group 1 was control group and only took normal saline. Groups 2 & 3 were administrated daily use of Vitamin A for 7 days I.M. and Group 4 was administrated with normal saline instead of Vitamin A in same condition as groups 2&3. In the last day groups 3 & 4 were administrated with single dose of AZA, 15 mg/kg (IP). After 24 hours, we took the animals blood and tissue samples and studied them for biochemical and pathological examinations. This study showed that Azathioprine-induced damage on pancrease in group 3 is less than that in group 4 while the function of organ in group 3 is nearly the same as control group. Also vitamin A decreases Azathioprine-induced toxicity on pancrease in rats. With Regard to importance of Azathioprine-induced damage, the usage rate of this drug in medicine, and the results of this study, we suggest that co-administration of Azathioprine and vitamin A decreases the toxicity of this drug.
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## 1. Introduction

AZA is one of the immunosuppressive drugs that use in medicine and Veterinary in different remedies that derived from 6 mercaptopurine. Use of AZA in autoimmune disease is routine and co-administration of this drug with corticosteroids is choice for prevention of rejection of graft (3). Also this drug Uses in treatment of different disease like as Inflammatory Bowel Disease (IBD)(10,13), Acute Lymphoblas Leukemia , Rheumatoid Arthritis (8), Ulcerative Colitis (6,14) , etc.

Mechanism of this drug is depended to prevention of synthesis of purines and followed prevention of RNA and DNA synthesis. Although , Azathioprine is mostly use but despite of its lymphocytic suppressive effect in patient, there is evidence that it cause bone marrow toxicity , digestive system toxicity and other organ toxicities. That is demonstrated that this druge induced oxidative stress and damage on organs (9,11,15) that it is related to free radicals production like as superoxide onions (7,15).

Broe PJ ad Cameron, J.L. demonstrated that no change induced by administration of AZA on amylase levels but reduction of bicarbonate and trypsine levels in compprasion to control group was observed (1,2).

Foitzik T. in 1998 showed that cell necrosis is one of other damages that induced by AZA administration (4).

Watanabe et al. (1979) confirmed that Azathioprine oral consumption increases alkaline phosphatase (ALP), Gama Glutamyl Transferase (GGT) in liver. In addition, from a pathological respect this drug cause necrosis in lobular center , proliferation of mitochondria and endoplasmic reticle (15).

Vitamin A is one of the fat soluble vitamins that have more functions that are related to skin, auditory and … . this vitamin is an antioxidant and prevents against oxidative stress that demonstrated by findings of other researches (5,12).

Spain scientists (2006) showed that vitamin A is one of the important antioxidants (5). Noyan S. and et al. (2006) demonstrated hepatoprotective effect of vitamin A against CCl4 induced liver toxicity (12).

As the rat's pancrease toxicity is because of oxidative stress of Azathioprine , and producing free radicals, the objective of this study was to evaluate the effects of vitamin A against pancrease damage due to Azathioprine prescription.

## 2. Material and Methods

Investigation using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the united states guidelines (United States National institute for health publication No.85-23,revised in 1985) and the ethical Committee on animal care approved the protocol. Forty male wistar rats that were apparently healthy have selected and divided into 4 equal groups. After body-weighting of each group by digital balance, all animals were kept in individual cages during the whole experimental period, under strict hygienic conditions and fed with standard ration for rat ad libitum.

In lightning for 12 h and darkness for the same houres in 25°C to get used to the environment. Then 1.5 ml of blood sample was taken via the tail vein from the members of all groups and the samples were studied in a way that will describe as following.

For the first group, as the control one,normal saline was given.the second and third groups received 100 mg/kg of vitamin A daily and for 7 days by IM injection. The forth group, that had similar state with three others, normal saline was injected for 7 days. On the 7$^{th}$ day , both group 3 and 4 treated by 15 mg/kg Azathioprine ( Ramopharmin Pharmaceutical lab-50 mg per tablet) as a single dose and IP form. Two other groups only received the dissolvent of Azathioprine in the same dose and manner. Twenty four hours after Azathioprine injection, the animals after being weighted were anesthetized by ether and blood sample were taken via the tail vein and pathological sample was got from pancrease. The samples were allowed to clot and then their serum was separated by centrifuge machine of 2500 rpm for 10 min.

Biochemical parameters including amylase and lipase and glucose were measured by identification kit of biochemistry and biowave spectrophotometer apparatus. Tissue samples were fixed in formalin 10% and then pathology cope was produced of them. After Hem toxin and Eosin staining , they were compared in terms of cellular damages such as degenerative changes, cellular death and inflammation changes.

### Statistical analysis

All biochemical results were expressed as mean+/- SD. Significant differences among groups were determined by one-way analysis of variance (ANOVA) followed by student t-test using the statistical analysis software (SPSS) Ver.13, significance was considered at $p<0.05$.

## 3. Results

Differences of weight values between groups:

Mean of weight parameters between groups are observed in table 1. parameters showed that final mean of weight in control group compared to initiate mean of weight increased. Group vitamin A and co-administration of vitamin A and AZA were same with control group and showed significant increase between first and last mean of weight values ($p<0.05$).

AZA group showed significant decrease of final values of weight compared to first values ($p<0.05$).

Table 1: Initial and final weight averages in several groups of animals

| Groups | Initial weight averages | Final weight averages |
|---|---|---|
| Control | 153+/- 5 a | 161+/- 4 b |
| AZA | 158 +/- 3 a | 144+/- 5 b |
| Vit A | 150 +/- 9 a | 159 +/- 1 b |
| AZA + Vit A | 153 +/- 3 a | 157 +/- 5 b |

Dissimilar letter(s) in each row shows that there is a meaningful difference($p<0.05$).

Table 2 shows the average of chemical parameters such as insulin, glucose, amylase , lipase in both control and Azathioprine receiving group and the group got vitamin A and the group got vitamin A co-administrated with Azathioprine.

Glucose values between groups show no differences between Control and Vitamin A and co-administration of vitamin A and AZA but there are significant increases observed between AZA and control groups. Amylase and Lypase values were same with glucose and had no difference between Control and AZA+vitamin A and Vitamin A , but there are significant increases between AZA and control groups ($p<0.05$).

Values of insulin measurement were compared between groups and showed significant decrease in AZA group to others ($p<0.05$).

Table 2: Biochemical parameters

| | Amylase(u/l) | Lypase(u/l) | Insulin(iu/l) | Glucose(mg/dl) |
|---|---|---|---|---|
| Control | 703.75+/-3.59 a | 1.8 +/- 0.2 a | 0.19 +/- 0.05 a | 74.97 +/- 9.76 a |
| AZA | 1020.75+/- 0.96 b | 2.5 +/- 0.01 b | 0.11 +/- 0.06 b | 160.67 +/- 15.71 b |
| Vit A | 785+/- 3.22 a | 1.81 +/- 0.5 a | 0.18 +/- 0.1 a | 78.95 +/- 2.65 a |
| AZA+Vit A | 899.75+/-8.62 a | 1.95+/-0.17 a | 0.16+/- 0.05 a | 102.17+/- 15.78 a |

Data are expressed as Mean+/-SEM, N=10.Dissimilar letter in each row shows that there is a meaningful difference ($p<0.05$).

**Pathological Study**

Histological studies of the pancreas were carried out on experimental groups. Histological parameters of the pancreas were normal in control rats. Histopathologic changes in the pancreas of Azatioprin treated rats include: Acute necrosis of the exocrine pancreas and loss of cell polarity and zymogen granules, chronic post necrotic interstitial pancreatitis, dilation of ductus and hydropic degeneration of ductal epithelium and periductal inflammation and fibrosis, severe atrophy of acinar parenchyma, necrosis of pancreatic fat, evidence of chronic post necrotic interstitial scarring, accumulation of fibrinous exudate and edema within the interlobular septa and inflammatory cell infiltrate, large areas of hemorrhage, severe destruction of the islet of Langerhans with reduced number of islet cells (Figures 1-2).

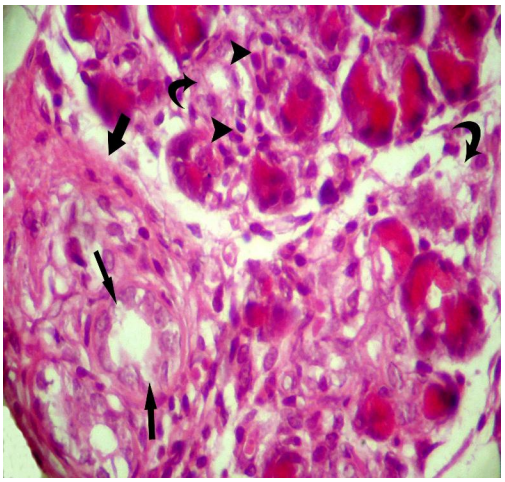

FIG 1- In Azatioprin treated animals, acute necrosis of the exocrine pancreas (curved arrows) and chronic post necrotic interstitial pancreatitis (arrowheads) is prominent. Dilation of ductus and hydropic degeneration of ductal epithelium (thin arrows) and periductal inflammation and fibrosis (thick arrow) are also seen.

After administration of Azatioprin and Vitamin A respectively, there were any significant recovery in pancreatic damages caused by Azatioprin. However, histopathology of the pancreas in these groups showed moderate to severe tissue damages, almost similar to group AZA, which treated merely with Azatioprin (Figure 3).

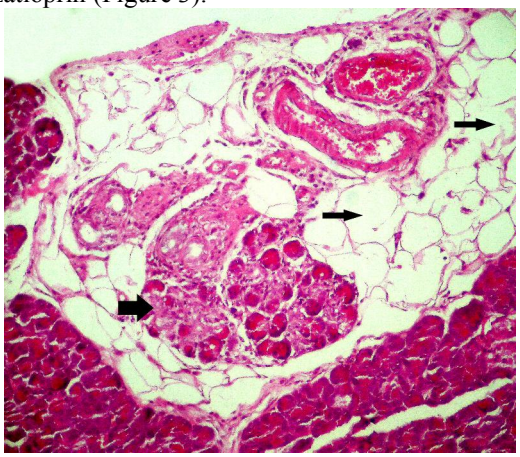

FIG 2- Note the severe atrophic lobule of acinar parenchyma, centered on necrotic pancreatic fat (thin arrows) with evidence of chronic post necrotic interstitial scarring (thick arrow) in Azatioprin treated animals.

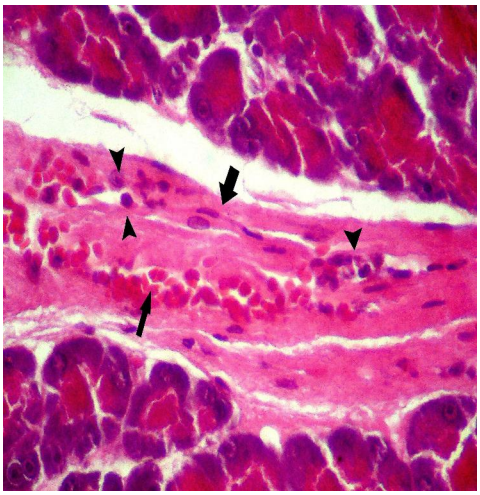

FIG 3- Microscopic appearance of Azatioprin& Vitamin A treated pancreas shows abundant fibrous connective tissue (thick arrow) which contains chronic inflammatory cells (arrowheads) and hemorrhage within the interlobular septa.

**4. Discussion**

Now a days, disease such as cancers and autoimmune problems are common throughout the world and increase continuously. In order to encounter against this troubles, it involves using drugs that not only prevent disease progression, but has also lessen side effects on natural function of cells and organs patient's body.

Among this drugs, azathioprine is one of the widely used of them in these days (9).

This drug is prescript in disease such as IBD, acute lymphoblastic leukemia , rheumatoid arthritis , ulcerative colitis, auto immune hepatitis, IgA nephropathy, dermatologic disease and etc.

It can prevent resynthesis of purine bases and so preventing cellular replication by inhibiting the RNA and DNA synthesis. The toxicity effect of this drug in different organs , such as bone marrow, liver, digestive system and pancrease ,following its usage is distinct ( 9,11,15). The toxicity of this drug is because of producing free radicals in body. In current study we decided to investigate protective effects of vitamin A against toxicity of AZA in pancrease tissue.

Weight results show that following toxicity and damages of Azathioprine in AZA group, growth of animals had defects and final weights are lower than initiate weights. In co-administration of AZA and vitamin A , results aren't same with control group but shows that vitamin A could protected toxicity induced by Azahioprine in pancrease tissue. Vit A group had same results with control group.

Results showed that Insulin values in AZA group in compare with control group had significant decrease that this is another cause of toxicity of Azathioprine in AZA group. This could related to production of free radicals and induction of defects in function of antioxidant systems of tissue following to administration of Azathioprine drug. Results in AZA+Vit A group and Vit A group show no significant differences to control group and showed that vitamin A could protect against Azathioprine drug.

Serum values of Amylase and Lypase in AZA group showed significant decrease in compare to control group that it could be related to toxicity of drug in exocrine site of pancreas but values in Vit A group are similar to control group and in AZA + Vit A group results are close to control and had significant differences with AZA group and showed protective effects of Vit A against Azathioprine. This is important that our results don't demonstrated with Broe PJ. و Cameron, J.L. about serum values of Amylase(2).

Pathological results showed acute toxicity induced by Azathioprine in endocrine and exocrine sites of pancreas tissue. Results in co administration of vitamin A and Azathioprine group showed low improvement in this group in compare to AZA group.

In current study the toxicity of Azathioprine in different tissues has been approved, so we should try to use this drug as less as possible except in emergency occasions. Then , as this drug can affect body's antioxidant system, it's better to use vitamins and antioxidant drugs together with it.

Our results showed that Vitamin A as a weak antioxidant have protective effects against toxicity induced by Azathioprine drug in pancreas tissue but that is suggested to investigate other antioxidant vitamins and agents to identifying the best protective agent against toxicity induced by Azathioprine.

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