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Influence of Aloxanes on the Apoptosis of Pancreas B-Cells of Rat

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Abstract: inducing the production if free radicals of oxygen (ROS), aloxan mediate in toxicity of B-cells. Radicals also result in apoptotic death of cells. This study aimed to examine the role of aloxan in apoptosis. In this experimental study, some rats, were divided divided into four groups of 5. In treatment group, they received 135, 90 or 45 mg/kg body weigh, intra peritoneum injection, of aloxan and control groups received only normal saline. 48 h after injection, pancreas tissue of controls and treatments were sampled and sent for sectioning in 5-6 microns and applying Tunel method. before sacrificing rats, their blood were sampled in intervals of 12, 24, 36 and 48 after injection, to measure blood sugar. Laboratory studies of treatments indicate various forms of apoptotic cells and their blood sugar was higher than controls such that the mean of apoptotic cells in five microscopic field of samples treated by aloxan with three dosages was significantly more than control groups ($p<0.001$). The role of aloxan induction in apoptosis is unknown but it may be due to free radicals of oxygen resulted from its injection.

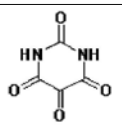
Key words: Aloxan · Apoptosis · Pancreas B-cells

INTRODUCTION

Aloxan is obtained from uric acid in a powder shape form pink color and can be easily solved in water. This drug mainly used in cancers of pancreas tissue and in studies as diabetic drug and specifically influence on pancreas B-cells [1]. Table 1 reveals some brief data of chemical structure of this drug. Aloxan induces diabetes in animals; however it is not known how it can affect these cells. There are so many interpretations for this; results of some studies indicate the main role of superoxide radicals and hydrogen peroxide in the mechanism of aloxan toxicity, but the influence of hydroxyl radical has not been yet verified. Due to structural similarities with glucose, aloxan and streptosotocin can link to B-cells or enter such cells using by-passes of glucose receptors. These are drugs which demolishing B-cells selectively; thus they are used as a suitable device to make experimental diabetes. Aloxan also produces activated species of oxygen only in the pancreas islands [2-5].

Table 1: Chemical properties of aloxan

* 2,4,5,6(1H,3H)-Pyrimidinetetrone monohydrate	Chemical name
$C_4H_4N_2O_2$	Chemical formula
160.09g/mol	Molecular weight
253°C	Melting point
o°C	Boiling point



Previous studies indicated that treating lab rats with calcium antagonist drugs like Lanthanum and verapamil can inhibit hyperglycemia resulting from injecting aloxans. Aloxan increases free calcium in cytosol of pancreas B-cells and its diabetic role may be related to intra cytosol calcium. Free radicals of oxygen result in DNA fragmentation and damage DNA and divide poly adenosine di phosphate ribose polymerase (PARP) into two parts 89 and 24 kdal by which cell can not

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enter the DNA repairing pathway and thus follow the apoptosis pathway. On the other side, increased cytosol calcium can affect the potential for mitochondrion membrane permeability and leaking cytochrom C and induce apoptosis. This study aimed to determine the type of cell death following the effect of aloxan on the pancreas B-cells [1,6,10].

MATERIALS AND METHODS

In this experimental study, 3 treatment groups and one control group were used such that 5 rats were used by names of Rat-A/45mgk, Rat-B/90mgk, Rat-D/135mgk and control. Three treatment groups received different doses of aloxan drug and control group injected by normal saline intra-peritoneum; before sacrificing them, their blood (both groups) were sampled in intervals of 12, 24, 36 and 48 h after injection, to measure blood sugar. After 48 h, rats were anesthetized using chloroform and their pancreas were collected., kept in the formalin 10% and histopathologically processed. After and stained and subjected to with Tunel, technique, pathologically examined and apoptotic cells were counted in five microscopic fields. Statistical analysis were carried out using one way ANOVA and t-Test (with significance of $p<0.05$).

Diagnostic Tunel Technique: Initially, Proteinase K was added to section, before hydration and incubated for about 30 min in 37°C and then washed by PBS. Tunel reaction mixture (50il) was added to section and left about 60 min in 37°C and washed by PBS. After incubation, sections were washed with Converter-POD (50il), 30min in 37°C and then washed with PBS and incubated in 25°C again. Finally, washed with PBS and stained with Tuloidin- blue [11,12].

RESULTS

Microscopic studies of langerhance B-cells indicated that increasing dosage of drug will increase the apoptotic cells. Table 2 indicates the changes in the numbers of apoptotic cells in pancreas B-cells and blood glucose in different times.

As shown in the table, intra- peritoneum injection of aloxan (by different dosages) during 12h results in increased blood glucose in rats. On the other hand, after 48h, aloxan has induced more effects on diabetes; such that injecting intra-peritoneum aloxan beginning of 45mg/kg will increase the concentration of blood glucose more than 86±6 mg/dl.

Table 2: Blood glucose 12, 24, 36 and 48h after intra- perineum injection by different dosages of aloxan in rats, Wistar strain ($p<0.001$)

	Blood glucose after injecting Aloxan (h)			
Groups	12	24	36	48
Control (Saline)	86±6	89±6	88±5	85±6
Aloxan (45mg/kg)	314±52	372±47	466±53	425±84
Aloxan (90mg/kg)	425±81	429±46	460±59	494±67
Aloxan (135mg/kg)	505±14	511±25	514±41	590±45

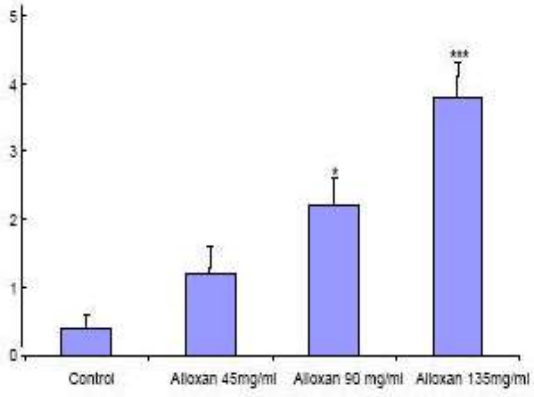

Fig. 1: Mean of apoptotic cells in 5 microscopic fields obtained from langerhance islands by Tunel technique in rats receiving saline (controls) and Aloxan with different doses ($p<0.001$, $p<0.05$, comparing control group)

The number of apoptotic cells in langerhanse islands in control groups that received saline and treatment groups received Aloxan, has indicated in Figure 1. Aloxan is fully dose-dependent and has significantly increased the number of apoptotic cells such that dosage of 135mg/kg has increased the number of such cells from 0.4±0.2 to 3.8±0.5 in control groups ($p<0.001$).

DISCUSSION

Results indicated that IP injection of Aloxan in rats with different doses will increase differences in pancreas B-cell death. This drug was known based on findings of researches like Conen and Heikkila [1974] and Takasa [1991] as a target drug for B-cells DNA, such that it assumes that Aloxan applies its demolishing role on B-cells by inhibition of Glucokinase (GK). This is done using oxidation of two tihol groups that is the place of opening the glucose on the enzyme. Inhibiting GK, besides reducing the expression of generating genes of that enzyme and Glucose Transporter-2 (Glutz) in B-cells and so by producing free radicals, the pathway of apoptosis

of B-cells will be induced. Free radicals increase permeability potential of mitochondria membrane, by which initiates exiting of cytocrome C and caspase cascade and this makes apoptosis in B-cells [13].

Based on Kim *et al.* [1994] this drug also induces apoptosis in B-cells by increasing the cytosol calcium. When cytosol calcium increases, PT canals of mitochondrion can be opened and inner calcium will be absorbed by mitochondrion. By opening the mitochondrion chanales, cytocrom C will go out of mitochondrium and apoptosis will initiate [1,3,6]. On the other hand, injecting Aloxan and reducing inner cell glucose various pathways will be activated with oxygen free radical as their final product. These pathways include:

- Pathway of sorbitol metabolism by which sorbitol oxidation by NAD^+ will increase NADH:NAD^+ ratio. This factor inhibits GAPDH activity and reduces Trios Phosphates, Methylglyoxal and Di acyl glycerol. These events relates to consuming NAD^+ by activating poly adenosine di phosphate ribos polymerase which is the initial factor for hyperglycemia due to Aloxan injection and can induce apoptosis related to PARP enzyme.
- Injecting Aloxan inhibits protein kinase C of pancreas B-cells because of reducing di acyl glycerol. Reducing di acyl glycerol will activate protein kinase A and reduce tolerance of cell than increased calcium in the cell. Thus, it favors the conditions for apoptosis induction.
- Another pathway may justify the apoptosis following Aloxan injection is activating the pathway of oxidative stresses which produces more free radicals as the final product [14,3,16]. Increased free radicals in the cell will increase permeability potential of mitochondria membranes and cytochrom C leakage and this has a determiant role in causing apoptosis pathways.

One can interpret results of injecting various doses of Aloxan and the rate of apoptotic cells by ascending changes of drug dosage due to changes in the calcium concentration of cytosol and the role free radicals play in apoptosis induction, as described above. The maximum and minimum changes of apoptosis were in 135mg/kg and 45mg/kg Aloxan respectively. Aloxan plays its role by inhibiting the function of glucokinase and GT-2, overgereration of free radicals and increased cytosolic Ca^{2+} which can be more informative for apoptotic pancreas cell death. Absolutely, diabetogenic effects of this drug is due to inducing apoptosis in pancreas B-cells such that results among treatment group with different dosages and between treatments and controls were statistically significant. It noteworthy telling that Aloxan may take effects related to its dosage as well [3].

CONCLUSION

The role of aloxan induction in apoptosis is unknown but it may be due to free radicals of oxygen resulted from its injection.

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