Role of Antioxidant on Nephropathy in Alloxan Induced Diabetes in Rabbits

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ABSTRACT:
BACKGROUND: The development and progression of diabetic nephropathy is dependent on glucose homeostasis and many other contributing factors. Diabetic nephropathy is a leading cause of end-stage renal failure, accounting for 35 to 40% of all new cases that require dialysis therapy worldwide. Recent clinical studies clearly demonstrated that hyperglycemia and oxidative stress is an important causal factor in mediating the development and progression of diabetic kidney disease.

OBJECTIVE: To evaluate the therapeutic effect of levamesole in diabetic nephropathy.

METHODS: The study included 10 rabbits Weight (1kg ±20 gm); they were followed up for 7 months. Blood was aspirated from marginal ear vein after agitation with xylol for estimation of fasting blood glucose and malondialdehyde (MDA) which is used as marker of oxidative stress. Rabbits were given 110 mg / kg alloxan to induce diabetes. In the second month rabbits became diabetic without development of nephropathy. After 1 month from being diabetic a boosted dose of alloxan was given (125 mg/kg). After 1 month of the boosted dose blood glucose level further increased and rabbits developed albumin urea. Once rabbits developed albumin urea they received levamesole 2 mg/kg EOD for 6 weeks.

RESULTS: The study results showed that diabetic nephropathy is associated with high blood glucose level (300-400 mg/dl) and oxidative stress (significant increase in MDA level). The nephropathy (albumin urea) and oxidative stress can be reversed by levamesole.

CONCLUSION: The antioxidant effect and immune modulating properties of levamesole provided a protective therapy against the development of diabetic nephropathy.

KEY WORDS: diabetic nephropathy, levamesole, MDA.

INTRODUCTION: Diabetic nephropathy is a leading cause of end-stage renal failure, accounting for 35 to 40% of all new cases that require dialysis therapy worldwide. Clinical studies clearly demonstrated that hyperglycemia is an important causal factor in mediating the development and progression of diabetic kidney disease. However, a growing number of patients still have diabetic kidney disease, although diabetic patients with renal complications have been treated with intensive insulin treatment and antihypertensive therapy with angiotensin-converting enzyme inhibitors and/or angiotensin II receptor antagonists.

Therefore, an understanding of the mechanisms by which prolonged exposure to hyperglycemia induces nephropathy has become essential to provide new insights into therapeutic strategies for diabetic kidney disease. Multiple biochemical mechanisms have emerged to explain the adverse effect of hyperglycemia, including protein kinase C (PKC), mitogen-activated protein kinase (MAPK), polyol pathway, advanced glycation end products (AGE), and oxidative stress. Among these, oxidative stress has been suggested extensively as a potential mechanism for diabetic kidney disease because oxidative stress promotes the formation of AGE as well as PKC-MAPK activation. Indeed, involvement of oxidative stress has been indicated by the presence of lipid peroxidation products like malondialdehyde. In Alloxan induced diabetic rabbits, the defense system against oxidative stress, like antioxidant enzymes such as catalase and Cu/Zn-superoxide...
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Dismutase (Cu/Zn-SOD) were found to be enhanced in kidneys of diabetic rabbits. However, it remains unknown whether these findings reflect a common consequence of the tissue damage in diabetic kidney or oxidative stress has a primary role in the pathogenesis of diabetic kidney disease.

Levamesole is an antihelmenthctic drug used in different tissues, in animals and human. Recently its used as immune modulator since it can modulates lymphocytic function that assist immune function, it affects phagocytic cells that surround and digest microorganisms and cellular debris. Alloxan is one of synthetic products of uric acid; its mechanism of action is by oxidizing 2 adjacent SH groups with formation of disulfide bond and so inactivation of the enzyme. The most reactive keto group of alloxan in position c-5, which acts as hydrogen acceptor. Alloxan inhibits glucose kinase enzyme (hexokinase), This enzyme is present in pancreas B-cell and in the liver which is sensitive to inhibition by alloxan, and may be the primary target for alloxan in pancreas B-cell which is responsible for inhibition of glucose induced insulin secretion from pancreatic B-cell. The affection of B-cell is through interaction with SH groups in sugar binding site of glucose kinas. Alloxan may be toxic to B-cell by its ability to generate reactive oxygen containing radicals, only dithiols can reverse the effect of alloxan on pancreatic islet function.

DNA islet is susceptible to damage by free radical and cytokine thus alloxan can produce damage to insulin producing cells (Islet of Langerhans) and hence produce diabetes mellitus.

MATERIALS AND METHODS:

This study was carried out on laboratory animal for 7 months. It included 10 rabbits Weight (1kg ±20 gm), they were fed a balanced diet that contain vitamins minerals protein and carbohydrates. They were given 110 mg / kg alloxan intravenously to induce diabetes mellitus plus glucose (5%) 10 ml subcutaneous, 5 ml IP immediately. Fasting blood glucose was checked every 3 days. Blood was aspirated from marginal ear vein after agitation with xylol. Blood samples were also processed for estimation of lipid peroxidation; using thiobarbituric acid reactive species malondialdehyde (MDA) according to (Satoh). Malondialdehyde was used as a marker of oxidative stress. Then rabbits became diabetics. After 1 month from being diabetics a bouture dose of alloxan was given (125 mg/kg iv) plus glucose 5% 10 ml given subcutaneous and 5% glucose for 5 days in drinking water. The development of nephropathy was assessed by ruin strips (Cybow Health Mate) every week. Later on when rabbits developed nephropathy albumin urea were checked every 3 days. When rabbits developed nephropathy they received levamesole 2 mg/kg EOD for 6 weeks.

RESULTS:

Rabbit weight (1kg ±20 gm) at the start of study. At the 6th month their weight decreased to 900mg±12gm. Initial fasting blood glucose level was (80±5mg/dL).In the second month of induction by alloxan, blood glucose level was (190±5mg/dL), without nephropathy assessed by albumin in urine. Then in the third month blood glucose became (300±15mg/dL), then after 1 month of the bouture dose of alloxan (fourth month) the blood glucose level increased to (400±5mg/dL) Fig (1), and the rabbits developed albumin urea (150±25 mg/dL). Immediately after development of albumin urea rabbits received levamesole 2 mg/kg EOD for 6 weeks .Then after 6 weeks albumin urea decreased to (30 mg/dL) Fig (2). MDA level increased gradually and significantly with the increment in blood glucose level (Table.1) the mean MDA level in the first month was (2.34±0.11nmol/ml), while in the fourth month mean value reached to (5.87±0.31nmol/ml), after treatment with levamesole MDA level decreased to 3.84±0.02 but it's still more than MDA level of the first month. Fig (3).

Table1: level of FBS, Albumin in urine & MDA level in experimental rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st mon.</th>
<th>2nd mon.</th>
<th>3rd mon.</th>
<th>4th mon.</th>
<th>5th mon.</th>
<th>6th mon.</th>
<th>7th mon.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS mg/dL</td>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.01</td>
<td>±0.31</td>
<td>±0.11</td>
<td>±0.11</td>
<td>±0.02</td>
<td>*</td>
</tr>
<tr>
<td>Urine albumin</td>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.01</td>
<td>±0.31</td>
<td>±0.11</td>
<td>±0.11</td>
<td>±0.02</td>
<td>*</td>
</tr>
<tr>
<td>MDA nmol/ml</td>
<td>2.34</td>
<td>4.03</td>
<td>5.87</td>
<td>3.34</td>
<td>3.84</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at P<0.05
Fig1: changes in FBS during the period of the study.

Fig2: Albumin in urine during the study.

DISCUSSION:
This study showed that the increment in blood glucose level is associated with the development of oxidative stress indicated by high MDA level. Also high blood glucose level is associated with albumin in urine as shown in the table; this albumin urea can be reversed by administration of the anti oxidant levamesole to the rabbits. Numerous reports have demonstrated that oxidative stress induced by diabetes plays an important role in the development and progression of diabetic vascular complications including nephropathy. Indeed, there is emerging evidence that the formation of reactive oxygen species (ROS) is a direct consequence of hyperglycemia.

Biomarkers for oxidative damage to DNA, lipids (MDA), and proteins are also supporting the concept of increased oxidative stress in diabetes and diabetic nephropathy. A study by Montonen supports the hypothesis that development of diabetes and its systemic effects may be reduced by the intake of antioxidants in the diet. Levamesole is used for the first time in diabetic nephropathy. Levamesole is an inhibitor of protein kinase C. High blood glucose level induces cellular reactive oxygen species through protein kinase C (PKC)-dependent activation of NADPH oxidase, and through mitochondrial metabolism.

Fig3: changes in MDA level during the period of the study.
Activation of protein kinase C (PKC), increased expression of transforming growth factor-beta, GTP-binding proteins, and generation of reactive oxygen species (ROS). The ROS seem to be the common denominator in various pathways and are central to the pathogenesis of hyperglycemic injury. Hence levamesole is protein kinase c inhibitor and an antioxidant so it reduces the diabetic nephropathy as seen in the results of our study. Levamesole is an immune modulator since it can affect lymphocyte function and lymphocyte apoptosis, and hence diabetes is regarded as an immunological disease since it's associated with development of antibodies against Islet of Langerhans and apoptosis of these cells, so levamesole action as immune modulator can ameliorate the course of diabetes and diabetic nephropathy.

CONCLUSION:
The antioxidant and immune modulating effect of levamesole provide protection against the development of diabetic nephropathy.

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