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ABSTRACTS AND POSTER PRESENTATIONS AND PARTICIPATION IN WORKSHOPS AND SYMPOSIA

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Effects of vanadate, insulin and fenugreek (*Trigonella foenum graecum*) on creatine kinase levels in tissues of diabetic rat

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The in vivo effects of insulin, and other insulin mimetic agents like vanadate and fenugreek (*T. foenum graecum*) were followed on the changes in the activities of creatine kinase in heart, skeletal muscle and liver of experimental diabetic rats. As compared to control rats, creatine kinase activities were found to decrease significantly in the tissues during experimental diabetes. All the antidiabetic compounds used namely, insulin, vanadate and Fenugreek seed powder normalized the decreased activities to almost control values. The effects of insulin and vanadate were comparable in restoring normoglycemia and the creatine kinase activities.

Creatine kinase (CK:ATP: creatine N-phosphotransferase, EC 2.7.3.2) is a member of the guanidinophosphotransferase family which catalyses the reversible transfer of the n-phosphoryl group of phosphocreatine (PCr) to ADP in order to generate ATP.

In vertebrates, three distinct cytosolic isoenzymes and two mitochondrial isoforms are known to exist, each showing its characteristic developmental regulation and tissue-specific distribution, represented by muscle type dimer (MM), brain type dimer (BB) and a hybrid dimeric form (MB) which is found in heart and other tissues. In most tissues, cytosolic and mitochondrial CK isoenzymes, are co-expressed. The three cytosolic isoenzymes always exist as dimeric molecules composed of the muscle (M) and brain (B) subunits. In contrast, the mitochondrial isoforms form either octameric or dimeric molecules.

The CK isoenzymes play a pivotal role in energy transduction in tissues, with large fluctuating energy demands, such as skeletal muscle, heart, brain and spermatozoa. The CK/PCr system represents an intricate energy distribution network, connecting intracellular sites of ATP production (mitochondria and glycolysis) with sites of ATP consumption (ATPases). The PCr circuit model has been proposed for tissues with high and fluctuating energy requirements. All the CK isoenzymes can be separated by cellulose-acetate electrophoresis.

Measurement of CK levels in serum has been clinically used for the diagnosis of myocardial infarction and muscular dystrophy. In this communication, we report the alterations in the levels of total CK isoenzymes in the different tissues of the diabetic rat and their modulation by insulin, vanadate, and fenugreek seed powder.

Animals—Female albino rats of the Wistar strain weighing around 180-200g were used in the study. Animals were maintained at constant temperature of about 26-28°C and given food ad libitum.

Induction of diabetes—A group of 40-50 rats were starved for 24 hr and diabetes was induced by a single subcutaneous injection of alloxan monohydrate (20 mg/100g body weight) dissolved in freshly prepared 0.15M acetate buffer (pH 4.5). Glycosuria was confirmed by using glucose test kit. Age matched control animals were treated with the same volume of acetate buffer. The alloxan treated animals were then injected ip with 2 IU of protamine zinc insulin for 7 days. This procedure reduces the mortality of the animals. Insulin was withdrawn and diabetic rats were divided into four groups: diabetic (D), diabetic treated with insulin (D+I), diabetic treated with vanadate (D+V), and diabetic treated with methi (*Trigonella foenum graecum*) seed powder (D+M).

The vanadate treated animals received 0.2 mg/ml vanadate in their drinking water for the first two days which was increased to 0.6mg/ml dissolved in 0.5% NaCl solution for the following days. NaCl is added with vanadate as it is known to decrease vanadate toxicity.

The D+M group were given 5% (w/w) methi powder in their normal powdered diet. The D+I were given 2 IU of ip injection of insulin. The standard laboratory chow was given for all groups ad libitum and treatment continued for three weeks.

Preparation of homogenate—Animals from each group were sacrificed by cervical dislocation, tissue taken out and kept on ice. Homogenates (1:10) (w/v) were prepared using a Potter Elvehjem homogenizer fitted with a teflon plunger in 0.25M sucrose, 0.02M triethanolamine buffer pH 7.4 containing 0.12mM dithiothreitol (DTT). The supernatant fraction was separated by centrifugation at 12,000 rpm for 40 min.

Enzyme assay—The activity of the enzyme creatine kinase was measured spectrophotometrically at 340nm by the method described by Bergmeyer. One unit of enzyme activity is defined as one micromole of NADPH oxidized per min at 25°C. A coupled enzyme assay was used in...
Significance of difference was assessed by students t-test. P values: ** < 0.001, * < 0.01.

Table 2—Changes in activity of creatine kinase in tissues of diabetic rats (21 days).

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Muscle %</th>
<th>Heart %</th>
<th>Liver %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.30 ± 0.42</td>
<td>100</td>
<td>3.97 ± 0.31</td>
</tr>
<tr>
<td>Diabetic (D)</td>
<td>3.23 ± 0.27***</td>
<td>75</td>
<td>2.86 ± 0.34**</td>
</tr>
<tr>
<td>D + insulin</td>
<td>4.21 ± 0.46</td>
<td>98</td>
<td>3.57 ± 0.41*</td>
</tr>
<tr>
<td>D + vanadate</td>
<td>3.87 ± 0.32</td>
<td>90</td>
<td>3.30 ± 0.36</td>
</tr>
<tr>
<td>D + fenugreek</td>
<td>3.44 ± 0.31</td>
<td>80</td>
<td>3.10 ± 0.24*</td>
</tr>
</tbody>
</table>

Significance of difference was assessed by students t-test. P values: *** < 0.001, ** < 0.005, * < 0.05.

which the formation of ATP was linked with hexokinase and glucose-6-phosphate dehydrogenase by following the reduction of NADP at 340 nm using a Beckman DU-68 recording spectrophotometer at 25°C.

Protein and blood glucose estimation—Cytosolic protein was estimated by the method of Lowry et al. Enzymes and chemicals used in the assay HK, G6PDH, ADP, AMP, NADP, alloxan, sodium orthovanadate, DTT, and phosphocreatine (PCr) were purchased from Sigma Chemical Company, USA. Protamine zinc insulin was purchased from Boots India Ltd., India. Fenugreek was purchased from Agmark Company, India. The rest of the chemicals were of analytical grade.

The changes in the general parameters are presented in Table 1. There was a loss in the body weight of the alloxan diabetic rats by about 30%, which was partially restored by insulin, vanadate, and fenugreek seed powder administration. There was a 39% decrease in the liver weight of alloxan diabetic rats as compared to the controls. A significant restoration of liver weight was observed in the insulin, vanadate, and fenugreek administered diabetic rats. Alloxan-induced diabetes showed nearly a four-fold increase in the blood glucose level which was restored to control levels after one week of treatment with vanadate consistent with previous reports. Feeding diabetic rats with 5% fenugreek powder in their diet also significantly decreased the blood glucose level and showed an increase in the body weight of diabetic rats.

The changes in the activities of creatine kinase are shown in Table 2. Vanadate treatment in the form of 0.6 mg/ml sodium orthovanadate in the drinking water of experimental diabetic rats normalised the decreased levels of CK isoenzymes in the muscle, heart, and liver. The CK activities during experimental diabetes were decreased by 25%, 28%, and 44% in the muscle, heart, and liver, respectively. A reversal to complete normalcy was observed by insulin in the muscle, heart, and liver with a significant improvement by vanadate and fenugreek powder. In the heart the reversal was from 28% to 10% by insulin and from 28% to 11% by vanadate. In the liver, the decrease was by 39% with insulin, 24% with vanadate, and 14% by fenugreek.

The levels of CK isoenzymes when compared in the different tissues were in the order: muscle > heart > liver which is in agreement with previous findings. The reversal in the activities of CK by insulin and vanadate was effective as compared to control rats especially in tissues like muscle and liver which are insulin dependent for the metabolism of glucose.

Alloxan diabetes is believed to result from production of free radicals which damage the β-cells of the pancreas. This production of free radicals can also cause peroxidation of membranes. Vanadate, known to have insulin-mimetic
effect, normalises blood glucose levels and the disturbed carbohydrate, and lipid metabolism\textsuperscript{13}.

CK isoenzymes play a key role in the energy metabolism of cells\textsuperscript{6}. In addition to its role as an energy buffer, the CK-PCr system is believed to function in energy transport. Since on the onset of diabetes the rate of glycolysis is decreased in insulin dependent tissue because of decreased uptake of glucose, due to lack of insulin, there is a perturbation of ATP production and ATP/ADP ratio. Normally, the [ATP] and the ratio of ATP/ADP is maintained at a constant level in the cells and this is brought about by the shuttling of high energy phosphates in the form of PCr which in the cytosol replenishes ATP by a reaction catalysed by the cytosolic CK isoenzymes. PCr may be produced in two ways: the first of which is catalysed by cytosolic CK which is functionally coupled to glycolysis and the second route is catalysed by the mitochondrial CK found on the outer face of the inner mitochondrial membrane which is functionally coupled to oxidative phosphorylation\textsuperscript{14}. It seems probable that, during diabetes in insulin dependent tissues the rate of glycolysis is decreased, on the other hand the ATP/ADP ratio is usually constant and therefore the increased CK activity may compensate for the need to keep the ATP/ADP constant, decrease the [ADP] and maintain the [ATP] at the normal physiological range. Administration of antidiabetic compounds like insulin and vanadate brings back the energy levels to control values again by regulating the concentration of ATPase, it could increase the levels of ATP and thereby also controlling the levels of phosphorylation-dephosphorylation of regulatory cytosolic and mitochondrial enzymes involved in the utilisation and generation of ATP like phosphofructokinase and pyruvate dehydrogenase. In this way the energy demand of especially fast twitch muscles like the heart and muscle is constantly met.

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