EFFECT OF VATHARASAVANGAM ON U^{14}C-GLUCOSE ABSORPTION AND UTILIZATION IN HYPERGLYCEMIC RABBITS

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Summary: Vatharasavangam (VRV) is a hypoglycemic drug mentioned in the Siddha Literature. Previous studies showed that VRV therapy brings down the blood glucose levels in the hyperglycemic rabbits. The present intestinal perfusion studies with labelled glucose indicate that the blood glucose homeostasis in VRV treated animals is brought about by a significant reduction in the rate of glucose absorption in the intestine. Further an enhanced incorporation of U^{14}C glucose into tissue glycogen is observed in the VRV treated rabbits when compared to the hyperglycemic rabbits.

Key words: VRV incorporation hyperglycemia perfusion U^{14}C glucose glycogen

INTRODUCTION

Glucose transported to liver via portal circulation after absorption in the intestine is either stored as glycogen or channeled to the catabolic pathway. Hyperglycemia results due to increased glucose absorption through intestine and reduced utilization or increased production of glucose. Insulin deficiency is reported to stimulate the functional activity of the brush border membrane to help increased glucose absorption and also a number of transport systems (8, 7). These observations indicate that hyperglycemia stimulates the absorption of glucose through the intestinal wall (3). Vatharasavangam (VRV) is a siddha medicinal preparation prescribed for hyperglycemic patients. Experimental observations in this laboratory showed the function of VRV as a hypoglycemic agent in rabbits. Hence it was planned to study the effect of VRV treatment on the intestinal absorption of glucose in hyperglycemic rabbits.

The intestinal glucose absorption in the control and experimental animals are monitored through perfusion studies. U^{14}C labelled glucose was employed in the studies as the
metabolism of radioactive glucose provides a reflection of the metabolism of non-radioactive glucose (2).

MATERIAL AND METHODS

Male albino rabbits weighing 1 to 1.5 kg were obtained from inbred stock of animals from the King's Institute of Preventive Medicine, Madras. They were maintained on Lipton rabbit feed and water given ad libitum. VRV was prepared according to the instructions prescribed in the Agathiar Vaithia Kaviam (I) with the help of a siddha medical practitioner.

Experimental design: Hyperglycemia was induced in fasting rabbits by injecting (iv) 80 mg/kg of alloxan monohydrate (Sigma). Animals with a fasting blood sugar of 200-250 mg/dl were included in the group of hyperglycemia for the experiment. They were maintained in hyperglycemic state for four weeks. VRV (dose 25 mg/kg body weight) was mixed with sufficient quantity of pure honey and orally administrated to rabbits once a day. The animals were grouped as follows: control rabbits (group I), control treated rabbits fed with 25 mg of VRV/kg body weight (group II), hyperglycemic rabbits (group III) and hyperglycemic rabbits treated with 25 mg of VRV/kg body weight (group IV). Animals from the group II and IV were treated with the drug for a period of 8 weeks.

In vivo absorption of $^{14}$C glucose by the intestine: The rate of absorption of glucose in the small intestine was determined by the perfusion technique of Younozai and Schedl (12). The cannulated small intestine was perfused at a constant rate of 0.5 ml/min with the perfusion fluid containing 159 mM NaCl, phenol red (20 mg/litre), 5 mM of unlabelled glucose and 40 $\mu$Ci/litre of $^{14}$C labelled glucose in 11 volume. After and initial period of 20 min, the perfusate was collected at the end of every 10 min for 1 hr. The radioactivity of individual effluent samples was measured in Beckman Liquid Scintillation counter. The absorption of glucose is calculated as follows:

\[
\text{Glucose absorption cpm} = V[(\text{cpm/ml})_i - (\text{cpm/ml})_f \frac{\text{PR}_i}{\text{PR}_f}]
\]

where cpm is counts per min; $V$ = volume; PR = Phenol red concentrations; the subscripts i and f refer to initial and final values.

Measurement of glucose utilization by the tissue: After perfusion, the animals were sacrificed and the blood was collected and serum separated. The urine collected in the bladder was removed with the help of a syringe. The liver, kidney and muscle tissues were speedily removed to estimate the incorporation of $^{14}$C glucose in the tissues. Weighed amount of the tissues was homogenised in water and 0.1 ml of the homogenate was added to the scintillating fluid for counting. A weighed portion of the tissue was treated with 30% potassium hydroxide
to separate the glycogen. The incorporation of $^{14}$C glucose into glycogen in the tissue was determined separately.

### RESULTS

The *in vivo* $^{14}$C-glucose absorption in control and experimental animals is presented in Fig. 1. The hyperglycemic animals when compared to the controls show 35% increased glucose absorption in 20 min and 48% absorption at the end of 60 min perfusion. In the VRV treated animals the absorption after 20 min is only 12% and 10% at the end of 60 min. There is a significant decrease in the glucose absorption in VRV treated animals after perfusion compared to that of hyperglycemic animals. However, the glucose absorption is about 12% higher than that observed in the control animals.

Table I gives the uptake of $^{14}$C labelled glucose from the intestinal perfusion fluid into serum, urine and tissues of control and experimental animals. The hyperglycemic animals show significant ($P<0.001$) increase in the serum glucose and glucose excretion in the urine. In the VRV treated animals there is a significant ($P<0.001$) reduction in the glucose in circulation and that excreted in urine. The values are close to those of control animals.
TABLE I: Uptake of U\(^{14}\)C labelled glucose from the intestinal perfusion fluid into serum, urine and tissues of control and experimental animals at the end of 60 min perfusion. The values are expressed as cpm/ml in serum and urine and cpm/g in tissues, mean±SD for n number of samples. Values obtained in group II and III are compared with group I and in group IV with group III. n =8.

<table>
<thead>
<tr>
<th></th>
<th>Control Group I</th>
<th>Control treated Group II</th>
<th>Hyperglycemic Group III</th>
<th>Hyperglycemic Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1280±82</td>
<td>1312±96</td>
<td>2656±87(^{a})</td>
<td>1421±107(^{a})</td>
</tr>
<tr>
<td>Urine</td>
<td>821±47</td>
<td>846±58</td>
<td>2370±121(^{a})</td>
<td>1224±112(^{a})</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6410±218</td>
<td>6620±240</td>
<td>4320±292(^{a})</td>
<td>5952±198(^{a})</td>
</tr>
<tr>
<td>Glycogen</td>
<td>1710±108</td>
<td>1762±98</td>
<td>745±124(^{a})</td>
<td>1440±121(^{a})</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2456±162</td>
<td>2317±121</td>
<td>1642±98(^{a})</td>
<td>2214±104(^{a})</td>
</tr>
<tr>
<td>Glycogen</td>
<td>613±47</td>
<td>636±52</td>
<td>322±81(^{a})</td>
<td>570±69(^{a})</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4216±182</td>
<td>4121±176</td>
<td>2819±151(^{a})</td>
<td>3741±146(^{a})</td>
</tr>
<tr>
<td>Glycogen</td>
<td>847±56</td>
<td>820±41</td>
<td>601±62(^{b})</td>
<td>756±58(^{b})</td>
</tr>
</tbody>
</table>

\(^{a}=P<0.001\) \(^{b}=P<0.01\)

\(^{14}\)C-glucose uptake in liver, muscle and kidney of the hyperglycemic animals is significantly \((P<0.001)\) reduced whereas in VRV treated animals the \(^{14}\)C glucose uptake is increased \((P<0.001)\) and the percentage of glycogen formation is nearly same as that of control animals. In the kidneys there is no change in the percentage of glycogen formation in the control and experimental animals though there is a significant \((P<0.001)\) increase in the \(^{14}\)C-glucose uptake by VRV treated animals.

DISCUSSION

The intestinal glucose absorption in the hyperglycemic animals is significantly elevated in the present studies. Several authors have reported elevated glucose uptake in hyperglycemia \((10, 11)\). The loss of about 60-80% of injected carbohydrates as glucose in urine of the diabetic animals leads to metabolic stress \((12)\). Hence for the survival, the diabetic animal adapts itself by increasing intestinal absorption through recruitment of additional brush border membrane carriers of sugar \((5)\). Moreover, insulin deficiency was reported to stimulate the functional activity of brush border membrane \((9)\) and a number of transport systems which may result in increased glucose absorption in hyperglycemic animals. The VRV drug therapy influences the reduction in the intestinal absorption of glucose. There is 48% increase in glucose absorption at the end of 60 min perfusion in hyperglycemic rabbits but the increase is only 10% in VRV treated animals. The urinary excretion of glucose is significantly reduced by VRV treatment indicating that the metabolic stress caused by the loss of glucose in urine is
reduced. This reduced strain may influence the reduction in intestinal glucose absorption after VRV treatment.

The VRV treated hyperglycemic rabbits exhibit significant increase in glucose utilization by the tissues as indicated by increased $^{14}$C glucose uptake and incorporation into glycogen, whereas in the hyperglycemic animals the glucose utilization by the tissues is low. Since insulin is a factor which stimulates the transport of glucose into the tissues and its utilization, its insufficiency in hyperglycemic animals may be the main reason for the observed changes. Increased accumulation of $^{14}$C glucose in the blood of diabetic rats (4) and reduced utilization of glucose by diabetic liver have been reported (6) similar to the present observations. The results of the present study indicate that the increased glucose absorption in hyperglycemic rabbits is reversed after VRV therapy.

REFERENCES