BIOCHEMICAL STUDIES ON THE EFFECT OF S-1,3-BUTANEDIOL OF DIABETES INDUCED RATS

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Abstract: The biochemical effect of S-1,3-butanediol on streptozotocin induced diabetic rats was studied. Rats were made diabetic by the intraperitoneal injection of 40 mg/kg body weight streptozotocin in sodium citrate buffer. A dosage of 25 mmol/kg body weight of S-1,3-butanediol was injected intraperitoneally for treatment. The streptozotocin induced diabetic rats showed a marked increase in blood glucose level, and significant increase in the level of cholesterol, triglycerides and free fatty acids. The glycogen levels in liver and kidney were greatly decreased in diabetic rats. Treatment with butanediol normalised the glucose and glycogen level but had no significant effect on protein and lipid levels.

Key words: butanediol hypoglycemic agent streptozotocin diabetes glucose glycogen

INTRODUCTION

Synthetic sources of dietary calories and proteins are of interest as potential food reservoirs for an expanding world population and in the control of etiology of numerous nutritionally related diseases such as atherosclerotic vascular disease, obesity and diabetes. Dymiszka and Miller (1) showed that 1, 3-butanediol contains approximately 6 K.Cal/g of metabolizable energy and can be utilized by the rat when fed at levels up to 20% of the diet. The role of 1, 3-butanediol as an energy source for human nutrition has been studied (2).

Considerable work has been focussed on the metabolic fate of this relatively well utilized polyhydric alcohol (3, 4). Butanediol is very rapidly metabolized through well established pathways by alcohol dehydrogenase and fatty acid oxidation (5), caloric content and low acute and chronic toxicity of butanediol.

In the present study, we proposed to observe the hypoglycemic effect of 1, 3-butanediol on experimentally induced diabetic rats. Nakagawa et al (6) reported a marked decrease (from 25 mM to 8 mM) in glucose level in alloxan diabetic rats fed with a diet containing 6% of the energy as S-1,3-butanediol which was not observed with a control diet or a diet containing 6% of the energy as R-1,3-butanediol. So in the present study particularly S-enantiomer was used to study the biochemical effect of 1,3-butanediol. Moreover, other group (7) has used alloxan induced diabetic rats fed with diet mixed with 1,3-butanediol. We have used streptozotocin to induce diabetes in rats and 1,3-butanediol was administered intraperitoneally for the first time and biochemical parameters were studied in tissues and in blood.

METHODS

Adult male albino rats weighing 150 to 200 g were obtained from Fredrick Institute of Plant Protection and Toxicology, Padappai, Madras, India. The animals were housed in spacious polyurethane cages and maintained in well-ventilated room. They were fed with commercial rat feed obtained from M/s Hindustan Lever Limited. Food and water were provided ad libitum.

The reported dosage of S-1,3-butanediol to cause
a significant decrease in glucose level was 20-40 mmol/kg body weight administered intraperitoneally (8).

The animals were divided into four groups based on the administration of streptozotocin and 1,3-butenediol. The number of rats in each group = 3.

Group I - Normal rats which formed the control group.

Group II - Normal rats treated with S-1,3-butenediol and sacrificed after 15 hr from the time of injection.

Group III - Diabetic rats which were maintained for a month.

Group IV - Diabetic rats treated with S-1,3-butenediol and sacrificed after 15 hr from the time of injection.

Group III and Group IV rats were made diabetic by the intraperitoneal injection of 40 mg/kg body weight streptozotocin in 0.2-0.5 ml of 50 mM sodium citrate buffer pH 4.5-5.0 and the solutions were prepared fresh just before use. An equal volume of citrate buffer was injected to Group I rats. The Group II rats were treated with 25 mmol/kg body weight S-1,3-butenediol and maintained for 15 hours. Among the Group IV rats, only those rats showing blood glucose levels more than 250 mg/dl were administered with S-1,3-butenediol (25 mmol/kg body weight intraperitoneally) and maintained for 15 hours.

At the end of each experimental period, the animals were sacrificed by cervical dislocation. Blood was collected and the tissues like liver and kidney were dissected, washed in ice-cold saline and chilled on ice immediately before processing.

Statistical analysis were performed using the students ' t' test.

Blood glucose (9), proteins (10), cholesterol (11), triglycerides (12) and free fatty acids (13) were estimated. Tissue glycogen (14) was extracted and estimated.

RESULTS

Table I shows the levels of blood glucose, plasma lipids and protein. It is shown the Group III rats show a significant increase in glucose level in Group IV, rats were observed to be near normal when treated with butenediol. The Group II rats showed a decrease in glucose level when compared with Group I rats.

The Group II rats showed an increase in cholesterol levels and free fatty acids levels and there is not much significant change in tryglyceride levels. The level of cholesterol and free fatty acids were increased in Group III rats but on treatment with butenediol, no significant changes in lipid levels were observed in Group IV rats.

The Group III diabetic rats showed a decrease in levels of plasma protein and on treatment with butenediol, no significant alterations were observed.

Table II shows a marked decrease of liver and kidney glycogen and total protein levels in Group III rats when compared with Group I rats. The glycogen levels in liver and kidney of Group II and Group IV

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>90.3 ± 8.6</td>
<td>66.5 ± 5.9*</td>
<td>340.9 ± 20.4**</td>
<td>120.8 ± 10.7***</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>98.2 ± 7.3</td>
<td>113.3 ± 8.6*</td>
<td>167.8 ± 12.4**</td>
<td>165.0 ± 9.2NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>99.3 ± 9.4</td>
<td>90.6 ± 9.7**</td>
<td>149.7 ± 11.2**</td>
<td>143.4 ± 8.7**</td>
</tr>
<tr>
<td>Free fatty acids (mg/dl)</td>
<td>22.9 ± 2.6</td>
<td>27.3 ± 2.8*</td>
<td>62.7 ± 5.1**</td>
<td>64.8 ± 4.3**</td>
</tr>
<tr>
<td>Proteins (g/dl)</td>
<td>9.8 ± 0.07</td>
<td>9.69 ± 0.08**</td>
<td>6.98 ± 0.072**</td>
<td>6.90 ± 0.11NS</td>
</tr>
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Group II and Group III were compared with Group I rats.
Group IV was compared with Group III rats.

**P < 0.001; *P < 0.01; P < 0.05; NS - Non-significant
TABLE II: Glycogen and protein levels in liver and kidney of control and experimental rats.

Values are expressed as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Glycogen (mg/g tissue)</th>
<th>Protein (mg/g tissue)</th>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Group I</td>
<td>42.13 ± 2.89</td>
<td>8.29 ± 0.59</td>
</tr>
<tr>
<td>Group II</td>
<td>46.38 ± 3.23</td>
<td>8.62 ± 0.39NS</td>
</tr>
<tr>
<td>Group III</td>
<td>32.67 ± 2.60***</td>
<td>5.33 ± 0.31***</td>
</tr>
<tr>
<td>Group IV</td>
<td>41.30 ± 2.89**</td>
<td>7.35 ± 0.29***</td>
</tr>
</tbody>
</table>

Group II and Group III were compared with Group I rats.
Group IV was compared with Group III rats.

***P < 0.001; **P < 0.01; *P < 0.05; NS - Nonsignificant

rats were normalised on treatment with butanediol. No significant changes were noticed in total protein levels on treatment with the drug.

DISCUSSION

The rats were made diabetic by using 40 mg/kg bodyweight streptozotocin and these diabetic rats on treatment with 25 mM/kg body weight butanediol showed a marked decrease in glucose level. The results obtained in the present investigation is in coincidence with the earlier report showing that the repeated injection of 25 mmol/kg, (ip), butanediol for every 3 hr led to a significant and sustained increase in the plasma level of beta-hydroxy butyrate, which was associated with a 20% decrease in the plasma level of glucose. These observations confirm the hypoglycemic effect of S-1,3-butanediol.

The effect of butanediol on tissue glycogen level are in agreement with the work of Miller and Dymszia (4) who reported that as the level of butanediol in the diet increases, there was a concomitant and significant increase in liver glycogen levels. A progressive increase in glycogen level with time in the brain of non-embolized rats was reported by Gueldry et al (15). One of the reasons for the hypoglycemic effect of butanediol may be attributed to its effect on glycogen metabolism. Probably butanediol may reduce the activity of glycogenolytic enzymes and increase the activity of enzymes involved in glycogenesis. Further work is needed to study the effect of butanediol on glycogen metabolism.

Since Mehlman et al (16) reported that animals fed diets containing butanediol significantly decreased the adipose tissue lipid levels and increased the plasma triglyceride levels, we also proposed to study the experimental groups. By comparing the results obtained in our study with the results of previous studies, it may be explained that the acetoacetyl-CoA, acetyl-CoA and HMG-CoA formed during the metabolism of S-1,3-butanediol may be channeled to fatty acids and sterol synthesis. These results also confirmed the reports of Gessner et al (17) who proposed that butanediol was completely oxidised through beta-hydroxy butyric acids to CO₂ and H₂O. The increase in body weight on butanediol treatment as shown by Tobin et al (18) could be due to an increase in cholesterol and fatty acid synthesis. The increase in free fatty acid levels on butanediol treatment may have inhibitory effect on glycolysis and enhancing effect on gluconeogenesis. Mehlman et al (19) have shown that phosphoenol pyruvate carboxy kinase (a rate limiting gluconeogenic enzyme) increased in normal rats treated with butanediol.

The treatment of butanediol did not make significant changes in protein levels. Kies et al (2) have also shown that there was no significant alterations detected in serum proteins of human subjects receiving butanediol had a significantly higher nitrogen balance (ie) lower urinary nitrogen excretion. Some earlier in vitro studies on metabolism of 1,3-butanediol by Dymszia et al (1) showed that the labelled dietary 1-14C butanediol was incorporated in the liver preferentially into glycogen rather than into proteins. These observations may lead to the conclusion that butanediol may not have significant effect on protein metabolism.
The potential value of butanediol is very much clear in human nutrition (2). The lowering of blood glucose level may show a promising way to evaluate more about S-1,3-butanediol as a hypoglycemic agent in diabetic. However, additional research incorporating more dosage of this agent with a longer standing time is needed to evaluate the ultimate role of butanediol. Since this study is open, the enzymes, metabolites and intermediates involved in different biochemical pathways may be studied. May be in future this agent will unravel the mystery of diabetes.

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REFERENCES