α-Lipoic acid ameliorates altered colonic contractility and intestinal transit in STZ-diabetic rats

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α-Lipoic acid treatment (100 mg/kg/day for 2 weeks after 6 weeks of untreated diabetes) of streptozotocin diabetic rats partially but significantly reversed both reduced contractile response of distal colon to acetylcholine and delayed transit of charcoal meal in small intestine compared to diabetic control. These effects of α-Lipoic acid were associated with complete reversal of diabetes induced increased plasma lipid peroxidation level. α-Lipoic acid had no effect on any of the parameters measured in non-diabetic rats. These findings demonstrate contribution of oxidative stress in the development of physiological changes of gut in diabetes.

Keywords: Lipoic acid (α), Colonic contractility, Intestinal transit, STZ-diabetes, Rat

The gastrointestinal tract is frequently affected in diabetes mellitus. The changes include reduced peristalsis and dilation of esophagus, delayed gastric emptying, disordered small intestinal movement, and colonic atony or mega colon and these may be manifested as constipation, diarrhoea or fecal incontinence. Reduced contractile response of colon to acetylcholine, delayed small intestinal transit together with a deficit of cholinergic innervation of the colon and small intestine have been demonstrated in experimental diabetes.

The diabetic state, in both human and experimental animals, is associated with oxidative stress and oxidative damage has been suggested to be contributory factor in the development and complications of diabetes. General increase in the antioxidant state achieved by dietary supplementation can help to diminish oxidative stress associated with diabetes mellitus. α-Lipoic acid (ALA) is a powerful lipophilic free radical scavenger in vitro and in vivo. ALA has potential preventive or ameliorative effect in both type 1 and type 2 diabetic complications. ALA supplementation has shown to prevent cataract formation, reduce the symptoms of diabetic peripheral neuropathy and improve cardiac dysfunction. Further, ALA administration prevented rat intestinal short term ischemia-reperfusion. However, the involvement of oxidative stress in the development of functional changes in gastrointestinal tract and effect of ALA on such changes are less documented.

The aim of the present study is to examine the effects of ALA treatment on altered response of distal colon to exogenous acetylcholine and small intestinal transit of charcoal meal in streptozotocin induced diabetic rats.

Materials and Methods

Animals and in vivo treatment—Animal experiments were approved by the Institutional Animal Ethical Committee, Mysore, India. Wistar rats (150-180 g) of either sex were fasted overnight and then injected, ip, with 45 mg/kg streptozotocin (Himedia Ltd., Mumbai, India) in 0.5 ml of 0.05 M citrate buffer (pH 4.5). Diabetes was defined as a non fasting plasma glucose level more than 200 mg/dl in tail vein blood (Pulsatum Health Care Pvt. Ltd., Calibration Code 1000, Bangalore, India) 48 hr after streptozotocin injection and again on the day before death. Uninjected non-diabetic (ND) and streptozotocin injected diabetic (STZ-D) rats were randomly assigned after 6 weeks to receive no treatment or to receive, ip, 100 mg/kg day 1 ALA (NDIL) or 4 IU of NPH insulin subcutaneous
Charcoal meal transit in small intestine—At the end of the treatment period, overnight fasted animals of different groups were administered, po, 2 ml/rat with charcoal meal (10% charcoal in 5% gum acacia) and 20 min later the rats were killed by cervical dislocation. The abdomen was opened and the intestine was removed from pyloric junction to caecal end. Then colon was separated and kept in continuously aerated Tyrode’s solution. The farthest distance travelled by the charcoal meal through the small intestine and total length of the intestine were measured. Gastrointestinal transit was expressed as the percentage of the distance travelled by the charcoal meal relative to the total length of small intestine.

Contractile response of colonic smooth muscle—Immediately after cleansing the colon, 1 cm of distal colon was mounted under a resting tension of 0.5 g in an organ bath (40 ml) containing continuously aerated Tyrode’s solution with the composition (mmol/l) NaCl: 136.9, KCl: 2.7, CaCl2: 1.8; MgCl2: 10, NaHPO4: 0.4, NaHCO3: 11.9, and dextrose: 5.6 (pH 7.45). The temperature was maintained at 37±1°C throughout the experiment and the tissue was allowed to equilibrate for 30 min before exposing to acetylcholine (Hi Media Ltd., Mumbai, India). A primary dose 100 ng of acetylcholine was tested before starting the actual concentration response curve. The contractile responses were recorded isotonically on a Smoked Kymograph drum. At the end of the initial equilibration period dose response curves were obtained for ascending dose of acetylcholine. ED50 values of acetylcholine were calculated from the graph plotted using percent response against log dose.

Lipid peroxidation in plasma—Lipid peroxidation in plasma was estimated by measuring malondialdehyde (MDA) level in plasma. Amount of malondialdehyde formed was quantitated by reaction with thiobarbituric acid as reported previously.

Statistical analysis—Data are presented as the mean±SE from 7 rats per group. Comparison of mean values among the various groups was performed by one way ANOVA. For the single comparison between the groups unpaired Student’s t-test was used. P values less than 0.05 were considered significant.

Results and Discussion

Baseline body weights were similar in all groups. Table 1 shows the mean weights for the animals and the mean blood glucose concentration at death. Eight weeks after injection of streptozotocin, diabetic rats had significantly lower body weights and increased plasma glucose levels when compared with their age matched non-diabetic controls. Treatment of streptozotocin diabetic rats with ALA or insulin had no significant effect on body weights and blood glucose level when compared with diabetic controls. Similarly, treatment of non-diabetic rats with ALA had no significant effect on body weight and blood glucose level compared to non-diabetic controls.

The ED50 of acetylcholine, percent transit of charcoal meal in small intestine and plasma MDA levels all showed significant difference among groups. [F(4,30) = P<0.01, ANOVA; Table 2]. Streptozotocin induced diabetic rats resulted in significant increase in ED50 of acetylcholine (P<0.001), plasma MDA level (P<0.001) and significant reduction of transit of charcoal meal (P<0.001) compared to normal controls.

ALA treatment of diabetic rats significantly reduced ED50 of acetylcholine (P<0.01) and increased the percent distance travelled by charcoal meal (P<0.001) compared to streptozotocin diabetic controls. But these parameters were still higher than those of non diabetic control rats. Effect of ALA in diabetic rats were associated with reduction in plasma lipid peroxidation level which was significantly less (P<0.001) compared to diabetic controls but was not significantly different compared to non-diabetic control rats.

Insulin treatment of diabetic rats significantly reduced ED50 of acetylcholine (P<0.05), increased percent distance travelled by charcoal meal (P<0.001) and reduced the plasma MDA level (P<0.05) compared to diabetic control. But these parameters were still higher compared to non-diabetic control rats.

Table 1—Characteristics of experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>End weight (g)</th>
<th>End blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>255±6</td>
<td>96±3</td>
</tr>
<tr>
<td>ND/L</td>
<td>260±5</td>
<td>90±5</td>
</tr>
<tr>
<td>STZ-D</td>
<td>157±7 *</td>
<td>297±16 *</td>
</tr>
<tr>
<td>STZ-D/L</td>
<td>159±5</td>
<td>286±24</td>
</tr>
<tr>
<td>STZ-D/I</td>
<td>165±7</td>
<td>272±20</td>
</tr>
</tbody>
</table>

*P<0.001 when compared with ND. Baseline body weight is 231±12g.

ND= uninjected non-diabetic, STZ-D= streptozotocin injected diabetic, L= α-Lipoic acid treated, and I= Insulin treated
Table 2—Effect of ALA or Insulin on lipid peroxidation, contractile response of distal colon to exogenous acetylcholine and small intestinal transit of charcoal meal in non-diabetic and STZ-diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (n mol/100 ml of plasma)</th>
<th>Contractile response of distal colon to exogenous acetylcholine</th>
<th>Charcoal meal transit in small intestine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>409.75±12.98</td>
<td>1.21±0.02</td>
<td>84.12±1.76</td>
</tr>
<tr>
<td>NO/L</td>
<td>417.81±31.96</td>
<td>0.63±0.23</td>
<td>85.21±0.41</td>
</tr>
<tr>
<td>STZ-D</td>
<td>801.24±31.44</td>
<td>13.61±2.91</td>
<td>62.58±1.18</td>
</tr>
<tr>
<td>STZ-D/L</td>
<td>499.74±45.09</td>
<td>3.35±0.42</td>
<td>71.86±1.03</td>
</tr>
<tr>
<td>STZ-D/I</td>
<td>574.04±17.36</td>
<td>5.75±0.63</td>
<td>68.48±1.98</td>
</tr>
</tbody>
</table>

Values are mean±SE

P values: *P<0.001 when compared to NO rats, **P<0.01, ***P<0.05 when compared to STZ-D rats.

ND= uninjected non-diabetic, STZ-D= streptozotocin injected diabetic, L= α-Lipoic acid treated, and I= Insulin treated

significant change in any of the parameters measured was observed after treating non diabetic rats with ALA compared with non-diabetic controls.

Distal colon from untreated diabetic rats were found to be less sensitive to acetylcholine, further, delay in transit of intestinal content was also observed. These observations are in agreement with previous reports. There was parallel increase in lipid peroxidation level in diabetic rats. This observation confirms the fact that hyperglycemia per se results in lipid peroxidation. Treatment of diabetic rats with ALA significantly reversed all the parameters measured, suggesting oxidative stress may be contributory factor causing changes in these parameters in diabetic rats. This theory is supported by the present observations in non-diabetic rats treated with ALA and diabetic rats treated with insulin. ALA treatment of non-diabetic rats had no significant effect on plasma lipid peroxidation level, contractile response of colon to acetylcholine and transit of charcoal meal in intestine compared to normal controls, which suggest that DL-α-lipoic acid effects are diabetes dependent. Further, improvement of contractile response of distal colon to acetylcholine and propulsive movement of small intestine along with reduction of lipid peroxidation after treatment of diabetic rats with insulin indicate that hyperglycemia stimulated oxidative stress may contribute physiological changes in colon and small intestine.

The reduced contractile response of colonic smooth muscle to exogenous acetylcholine may be the result of excessive degradation of acetylcholine by tissue acetylcholine esterase, diminished muscaranic receptor sensitivity or density or defective interaction between muscaranic receptor and intracellular contrac-
tile process. The myogenic-phenomenon in distal small intestine of diabetic rats is not affected. Studies of the responsiveness of diabetes colonic smooth muscle to acetylcholine are limited, whereas vascular and cardiac muscle in experimental diabetes shows altered sensitivity to acetylcholine. Decrease in muscaranic receptor density in atrial muscle has been suggested for reduced cardiac sensitivity to cholinergic agents. Therefore, it appears that oxidative stress may induce changes in muscaranic receptor density and binding affinity leading to reduced cholinergic response and ALA treatment may abolishes such changes.

Impaired cholinergic response of distal small intestinal smooth muscle has been reported. Further, increase in noradrenaline level and in its turnover have also been observed in ilea form 6-week streptozotocin diabetic rats. ALA therapy improved the cholinergic response of colonic smooth muscle in diabetes rats in the present study. If this would also happen in small intestinal smooth muscle, it may provide one mechanism to explain enhanced intestinal transit of charcoal meal after ALA therapy. Other possible mechanisms include decrease in noradrenaline concentration and its turnover.

In conclusion, this study suggests that the role of oxidative stress in the development of gastrointestinal functional changes associated with experimental diabetes. Beneficial effects of ALA on gastrointestinal functional changes in diabetes of long duration needs to be investigated.

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References