THE EFFECT OF GLUCOCORTICOID ON GLUCONEOGENESIS IN RATS WITH LIVER DAMAGE

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It has been noted that steroid diabetes occurs more frequently in patients with liver disease and patients with blood disease than in those without such diseases during treatment of glucocorticoid (Yamagata and Goto, 1963). We have also reported that glucosuria during administration of glucocorticoids was often noted in patients with liver disease, especially in the acute stage of liver damage (Oji, 1963b; Oji and Wada, 1963).

It is known that the diabetes produced by glucocorticoids in characterized by a marked increase in gluconeogenesis. However, a particular effect on gluconeogenesis in the damaged liver has not been recognized.

Therefore, in the present study, the influence of cortisone upon gluconeogenesis was investigated with DL-alanine-1-C\textsuperscript{14} in rat with acute or chronic carbon tetrachloride poisoning.

MATERIALS AND METHODS

Male rats of Wistar strain weighing 120–150 g were used for the experiment. Acute CCl\textsubscript{4} poisoning was induced by a single intramuscular injection of 3.0 ml of 20\% CCl\textsubscript{4} in olive oil per kg of body weight. Chronic CCl\textsubscript{4} liver damage was produced by serial injection of 1.0 ml of 20\% CCl\textsubscript{4} in olive oil per kg once a week for 2 months. For 7 days 2.5 mg of cortisone was administered intramuscularly in chronic CCl\textsubscript{4} poisoning and for 3 days in acute CCl\textsubscript{4} poisoning respectively.

Solutions containing 20 \mu\text{C} DL-alanine-1-C\textsuperscript{14} and 300 mg of DL-alanine per 100 g body weight in 0.4 ml was given intravenously. The blood was collected by heart puncture and deproteinized by adding 2 vol. of 2\% ZnSO\textsubscript{4} and 1.8\% Ba(OH)\textsubscript{2} solution per vol. of blood. The liver was removed without delay and digested in 30\% KOH solution at 80\°C for 1 hr.

The C\textsuperscript{14}-incorporation into the blood glucose and liver glycogen was measured using Segal’s method and Weinhouse’s method respectively. (Weinhouse and Briedmann, 1951; Segal and Blair, 1960). Specific activity of glycogen was determined by Armstrong’s method (Armstrong and Schubert, 1948). Blood glucose was measured using Somogyi’s method (1952).

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RESULTS

Studies on the incorporation of C¹⁴ from DL-alanine-¹-C¹⁴ into blood glucose and liver glycogen in normal rats and rats with CCl₄ liver damage are summarized in Table 1.

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Mean of fasting blood glucose (mg/dl)</th>
<th>Blood glucose</th>
<th>Liver glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mµc/ml of blood glucose</td>
<td>mµc/mg C of glucose</td>
</tr>
<tr>
<td>Normal control</td>
<td>None</td>
<td>5</td>
<td>114</td>
<td>2.29±0.15</td>
<td>5.03±0.48</td>
</tr>
<tr>
<td>Allotaxan diabetes</td>
<td>None</td>
<td>4</td>
<td>302</td>
<td>8.26±0.57</td>
<td>6.85±1.23</td>
</tr>
<tr>
<td></td>
<td>Insulin 1 u inj, daily for 7 days</td>
<td>3</td>
<td>85</td>
<td>2.18±0.23</td>
<td>6.42±1.42</td>
</tr>
<tr>
<td>Steroid diabetes</td>
<td>None</td>
<td>3</td>
<td>219</td>
<td>7.32±1.53</td>
<td>8.39±1.80</td>
</tr>
<tr>
<td></td>
<td>Insulin 1 u inj, daily for 7 days</td>
<td>3</td>
<td>63</td>
<td>1.64±0.29</td>
<td>6.51±1.51</td>
</tr>
<tr>
<td>Acute CCl₄ liver poisoning</td>
<td>None</td>
<td>3</td>
<td>108</td>
<td>0.27±0.04</td>
<td>0.63±0.13</td>
</tr>
<tr>
<td></td>
<td>Cortisone 2.5 mg inj, daily for 3 days</td>
<td>4</td>
<td>125</td>
<td>3.76±1.78</td>
<td>7.53±2.31</td>
</tr>
<tr>
<td>Chronic CCl₄ liver poisoning</td>
<td>None</td>
<td>3</td>
<td>92</td>
<td>0.80±0.04</td>
<td>2.18±0.35</td>
</tr>
<tr>
<td></td>
<td>Cortisone 2.5 mg inj, daily for 7 days</td>
<td>3</td>
<td>113</td>
<td>2.33±0.79</td>
<td>5.21±1.58</td>
</tr>
<tr>
<td></td>
<td>Cortisone 2.5 mg and insulin 1 u inj, daily for 7 days</td>
<td>87</td>
<td>1.35±0.24</td>
<td>3.89±0.88</td>
<td>625±74.3</td>
</tr>
</tbody>
</table>

* ±: standard deviation

The incorporation of labelled carbon from DL-alanine-¹-C¹⁴ into blood glucose and liver glycogen was found to be decreased in rats with acute and chronic liver damage. The decrease was much more marked in rats with acute CCl₄ liver damage than in rats with chronic CCl₄ liver damage.
Previously we recognized that the C14-incorporation into blood glucose and liver glycogen was increased diabetic rats (Oji, 1963a). However, the administration of 2.5 mg of cortisone for 3 days in acute CCl4 poisoning in rats caused more marked increase in C14-incorporation into blood glucose than in chronic CCl4 liver damage. Marked decrease in C14-incorporation into the liver glycogen in acute CCl4 poisoning was not improved by the administration of cortisone, although the C14-incorporation into liver glycogen was found to be increased by cortisone in livers damaged by chronic CCl4 poisoning more than in rats with acute CCl4 liver damage.

As shown in Table 1, insulin caused further increase in C14-incorporation into liver glycogen in alloxan diabetic rats, although insulin decreases the total C14-incorporation into blood glucose in these rats. Also simultaneous administration of cortisone and insulin caused more marked increase in C14-incorporation into the liver glycogen than did cortisone alone in chronic CCl4 poisoning.

DISCUSSION

It has been reported that glucosuria was found more frequently in patients with liver disease than in those without disturbance of liver function during administration of glucocorticoid. A disturbance of carbohydrate oxidation or decreased deposition of glycogen in the liver could be factors which cause glucosuria more frequently in patients with liver damage.

An increase in gluconeogenesis might also play an important role in the formation of both glucose and glycogen in the liver after the long-term administration of glucocorticoid, as indicated by many investigators. Haynes (1962) and Eisenstein et al. (1964) suggested that glutamic-pyruvic transaminase in the liver may be a rate-limiting factor in carbohydrate synthesis from protein and glycogenic amino acids. Glucocorticoid increases activity in hepatic transaminase and stimulates the synthesis of glutamic-pyruvic transaminase in the liver (Segal et al., 1962).

Previously the authors reported that the activities of the hepatic glucose-6-phosphatase and hepatic transaminase were found to be decreased in rats with acute CCl4 liver damage and to be increased in rats with alloxan diabetes and steroid diabetes, compared with normal control rats. The decreased C14-incorporation from alanine-1-C14 into blood glucose and liver glycogen in rats with CCl4 liver damage, especially in rats with acute CCl4 liver damage is in accord with the enzymatic changes observed in previous studies (Oji and Wada, 1963). The administration of cortisone in such liver damaged rats caused the increase of C14-incorporation into blood glucose, especially in acute CCl4 damage group. These findings suggest that cortisone enhances gluconeogenesis also in rats with liver damage just as in those without liver damage.

On the other hand, C14-incorporation into liver glycogen did not increase in acute CCl4 poisoning rats treated with cortisone, although cortisone caused the increase in C14-incorporation into liver glycogen in chronic CCl4 liver damaged rats than in the normal group.
This difference on the deposition of liver glycogen may be associated with the increase of blood glucose or glycosuria.

These findings would indicate that steroid diabetes occurs more frequently in patients with liver damage, especially in those with acute stages of liver disease, because newly-formed glucose-6-phosphate is abnormally diverted from the formation of glycogen to the elevation of blood glucose.

SUMMARY

C\textsuperscript{14}-incorporation into blood glucose and liver glycogen from C\textsuperscript{14}-labelled alanine was studied in rats with acute or chronic C\textsubscript{Cl\textsubscript{4}} liver damage.

In rats with C\textsubscript{Cl\textsubscript{4}} liver damage, C\textsuperscript{14}-incorporation from alanine into blood glucose and liver glycogen was found to be decreased, especially in rats with acute C\textsubscript{Cl\textsubscript{4}} liver damage. Such a disturbed C\textsuperscript{14}-incorporation was improved by the administration of 2.5 mg of cortisone to rats with chronic C\textsubscript{Cl\textsubscript{4}} poisoning. However, in rats with acute C\textsubscript{Cl\textsubscript{4}} liver damage, cortisone treatment caused a marked increase in C\textsuperscript{14}-incorporation into blood glucose and failed to increase C\textsuperscript{14}-incorporation into liver glycogen.

The latter finding may be related to the increased incidence of steroid diabetes in patients with liver damage.

REFERENCES

Oji, N. (1963b). 5th Japan Diabetic Society
Oji, N. and M. Wada (1962). *Clinic All Around* 12, 769. (In Japanese)