Quantitation of type I and III collagen of liver in alloxan-induced diabetic rabbits

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Collagen is the most abundant protein in the body and it is the major macromolecular constituent of extracellular matrix, representing a family of genetically distinct molecules [1]. Soft organs such as pancreas and liver contain only a small amount of collagen, whereas in tissues such as skin and tendon, collagen account for over 70 per cent of the dry weight [2]. In vertebrates most tissues are composed of type I and III collagen. Connective tissue disorders are apparent in diabetics, and diabetes mellitus involves metabolic processes and produces profound changes in the biochemical composition and biochemical properties of the affected tissue [3]. Our interest has centered on hepatic tissue architecture and how mild experimental diabetes influence total collagen content and the relative proportions of major collagen types I and III in rabbit liver.

Twenty two male albino rabbits aged 1-0.15 years, weighing 1.4-2.0 kg were divided into two groups: a) Control group (12 rabbits) and b) alloxan-induced diabetic group (10 rabbits). The animals were fasted for 42 h before alloxan treatment and the diabetic group was treated once a week for a week for four weeks with alloxan at a dose of 120 mg/kg dissolved in 0.5 ml sterile saline. Blood glucose levels were measured before and after treatment for four weeks. After decapitation of the rabbits of both groups, the tissue samples were removed and defatted with acetone and ether. The dry defatted liver samples were hydrolyzed in 6 N HCl at 105°C for 16 h. Following hydrolysis, the hydroxyproline contents of the neutralized samples were measured by the spectrophotometric method of Tougaard [4]. Values for collagen content were derived from the hydroxyproline values by multiplying 7.46. Collagen types I and III were prepared from liver by solubilization with pepsin, followed by three times repeated differential salt precipitation with crystalline NaCl and dissolution. The dry defatted tissue samples were suspended in 0.5 M acetic acid and pepsin added at a ratio of 1:100 (10 mg/g dry tissue weight). Digestion was allowed to proceed for 24 h at room temperature. The insoluble residue was extracted a second time under similar conditions. The collagen was precipitated from the combined supernatant by addition of solid NaCl to achieve a concentration of urea was 1 M and the running gel at 7 hours 30 minutes. The mean blood glucose levels of alloxan treatment were found as 106.2 ± 5.5 mg/dl and 366.0 ± 9.3 mg/dl, respectively and the increase was found to be significant (p<0.001). Dry defatted weight of the liver samples of alloxan-induced diabetic rabbits did not differ from those of the control group. Alloxan diabetes did not induce a significant change (p>0.05) in total hepatic collagen and the relative amounts of collagen types I and III, after four weeks of treatment (Table 1).

Table 1. Percentage of collagen types in liver samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Collagen (mg/g dry weight)</th>
<th>Type I</th>
<th>Type III</th>
<th>Type I/III Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1</td>
<td>5.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>8.7</td>
<td>6.1</td>
<td>2.1</td>
<td>2.9</td>
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Total collagen content of non-diabetic and diabetic rabbits liver were found to be 0.79 % ± 0.14 and 0.84 % ± 0.08, respectively. SDS-PAGE revealed hepatic type I and also type III collagen to separate into three protein bands (α1, α2, γ) in both groups, whereas type I collagen of the other tissues generally divided into five protein bands (α1, α2, β1, β2, γ). SDS polyacrylamide gel electrophoresis revealed hepatic type I and III collagen to have homogeneous protein bands corresponding to molecular weights of 95,000-100,000 Daltons for both α1 and α2 chains [Fig. 1]. The obtained data provide a quantitative evaluation of collagen composition in diabetic animals which may be of interest in studies of connective tissue diseases involving the hepaticcellular degeneration.

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Fig. 1. Electrophoretic pattern of liver type I and III collagen on SDS-polyacrylamide gel.

Lane 1 Type III marker 2 Type I marker 3: M. W. markers 4 Type I + III marker 5: Control type I 6: Diabetic type I 7: Control type III 8: Diabetic type III

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