SHORT COMMUNICATION

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THE EFFECT OF SOME ANTIINFLAMMATORY DRUGS ON COLLAGEN OF RAT FIBROUS CARTILAGE

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Received 12 December, 1989, Revised 20 January, 1990

The use of SDS-polyacrylamide gel electrophoresis of the cyanogen bromide derived peptides from fibrous cartilage collagens enabled to calculate type I to type II collagen ratio in this tissue. Some of the investigated drugs (acetylsalicylic acid, colchicine) changed this ratio without having a significant effect on total collagen content in fibrous cartilage.

Molecular polymorphism of collagen is one of the most challenging problems in biochemistry of this protein. At present at least 12 genetically distinct collagens isolated from different tissues are known [1, 2]. Normal fibrous cartilage contains type II collagen and a small amount of type I collagen [3] which is present in significant quantities in the healing tissues in experimentally injured joints [4].

Since antiinflammatory drugs are known to affect the metabolism of some connective tissue compounds [5] and glucocorticoids inhibit collagen biosynthesis [6], it was decided to study the effect of some antiinflammatory drugs on collagen content in rat fibrous cartilage and on type I to type II collagen ratio in this tissue.

The use of sodium dodecyl sulfate polyacrylamide gels to separate the peptides obtained by cleavage with cyanogen bromide from various collagens has proved to be one of the most reliable ways of distinguishing between type I and II collagens [7]. In our studies reported in this paper we used this method to determine relative proportions of type I and II collagens in fibrous cartilage of rats treated with some antiinflammatory drugs.
MATERIALS AND METHODS

Female rats of Wistar strain weighing 80 g were used in the experiments. The animals were divided into 6 groups of 6 rats each, fed with standard diet and kept at 22°C. The following substances were administered orally by stomach intubation to the rats: Group I, control, 0.9% NaCl, 4 ml; Group II, prednisone (Encorton, Polfa), 1.0 mg/kg; Group III, colchicine (Colchicum-Dispert, Kali-Chemie), 0.025 mg/kg; Group IV, acetylsalicylic acid (Aspirin, Bayer), 50 mg/kg; Group V, indomethacin (Metindol, Polfa), 2.5 mg/kg; Group VI, phenylbutazone (Butapirazol, Polfa), 20 mg/kg.

The above drugs were administered in single daily doses for 30 days. The animals were then killed and samples of ribbon fibrous cartilage were excised. The material was homogenized in water using Polytron homogenizer, lyophilized and submitted to cyanogen bromide cleavage as described by Epstein et al. [8]. The samples of lyophilized material (20 mg) were heated in 70% (v/v) deoxygenated formic acid at 60°C for 1 h. Cyanogen bromide was added to the solution to the final concentration of about 50 mg/ml under nitrogen, the mixture was left at room temperature for 24 h and then lyophilized.

The lyophilized material was dissolved in 0.063 M Tris/HCl buffer, pH 6.8, containing 3% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.01% bromophenol blue, to the concentration of 4 mg/ml. The mixture was incubated in a water bath at 60°C for 1 h.

SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli [9]. The above mixture (50 μl) was used for electrophoretic separation on 12.5% gels. The gels were then stained for 3 h with 0.25% (w/v) solution of Coomassie brilliant blue R-250 in methanol:acetic acid:water (5:1:10 by vol.) and destained in 7% acetic acid for five days. The areas of relevant bands were cut out, hydrolysed in 6 M HCl, and hydroxyproline was determined by the method of Bergman & Loxley [10]. The following bands were obtained: band A (α1(II)CB10) and band B (α1(II)CB11); α1(I)CB7; α1(I)CB8 [7], (Fig. 1). Taking into consideration the aminoacid composition of type I and II collagens, its molecular mass, subunit composition and the amount of hydroxyproline residues in CNBr peptides of band A and B [11, 12], to evaluate the percentage of type II collagen in the sample, we used the equation:

\[ X = \frac{4a}{4b + a} \times 100 \]

where X is type II collagen content (in %), and a and b, hydroxyproline content in band A and B respectively.

Protein was determined by the microbiuret method.
RESULTS AND DISCUSSION

SDS-polyacrylamide gel electrophoresis of CNBr peptides of type I and type II collagens from fibrous cartilage of rats treated with some antiinflammatory drugs is shown in Fig. 1. In bands A and B hydroxyproline content was determined and the ratio of type I to type II collagens calculated using the procedure described in Materials and Methods.

As it is shown in Table 1, acetylsalicylic acid caused a decrease and colchicine an increase of type I to type II collagen ratio in fibrous cartilage of rats. It is worth noting that none of the drugs investigated (except phenylbutazone) had any effect on the total collagen content in this tissue.

Our observations suggest that treatment with acetylsalicylic acid or colchicine has a bearing on the quantitative relationship between type I and type II collagen in fibrous cartilage, but not on the total collagen content in this tissue.

Antiinflammatory drugs are known to affect the metabolism of some connective tissue compounds. Thus, one of the important considerations in the evaluation of the effect of antiinflammatory drugs on cartilagous tissue should be the type of collagen present and quantitative relationship between collagen of different types.

It seems that the advantage of the present technique over the method of O'Driscoll et al. [7] is that it allows analyses to be more accurate. The precision of the previously described technique [7] where densitometric scanning of CNBr peptides stained with Coomassie brilliant blue was performed, depends on the time of destaining and the presence of non collagenous proteins (proteoglycans) with similar electrophoretic mobility as

![Fig. 1. SDS polyacrylamide gel electrophoresis of CNBr peptides of type I and type II collagens from fibrous cartilage of rats treated with some antiinflammatory drugs: 1, control; 2, acetylsalicylic acid; 3, phenylbutazone; 4, indomethacin; 5, prednisone; 6, colchicine. For comparison, CNBr peptides of type I collagen are presented (S). The bands represent: A [α1(II)CB10] and B [α1(II)CB11; α1(I)CB7; α1(I)CB8]]
Table 1

Collagen content and type I to type II collagen ratio in the fibrous cartilage of rats treated with some antiinflammatory drugs. The results obtained were evaluated statistically by Student's t-test. Mean values ± SD are presented (n=6)

<table>
<thead>
<tr>
<th></th>
<th>Collagen (% of protein total)</th>
<th>Type I (% of total collagen)</th>
<th>Type II (% of total collagen)</th>
<th>Type I to type II collagen ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.5 ± 1.6</td>
<td>15 ± 3</td>
<td>85 ± 3</td>
<td>0.18</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>32.0 ± 1.1</td>
<td>5 ± 4*</td>
<td>95 ± 4*</td>
<td>0.05</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>44.0 ± 2.3*</td>
<td>17 ± 4</td>
<td>83 ± 4</td>
<td>0.20</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>33.1 ± 1.7</td>
<td>17 ± 3</td>
<td>83 ± 3</td>
<td>0.20</td>
</tr>
<tr>
<td>Prednisone</td>
<td>28.3 ± 2.7</td>
<td>22 ± 6</td>
<td>78 ± 6</td>
<td>0.28</td>
</tr>
<tr>
<td>Colchicine</td>
<td>28.9 ± 1.6</td>
<td>45 ± 5*</td>
<td>55 ± 5*</td>
<td>0.82</td>
</tr>
</tbody>
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* P<0.05 compared with control values.

that of the determined CNBr peptides, which may disturb the real ratio of type I to type II collagen. Determination of hydroxyproline in band A and B eliminates such factors, since in the collagenous tissue hydroxyproline is present only in collagen.

REFERENCES


