Calcification and Fracture of Costal Cartilage in Beagles

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ABSTRACT. Calcification and fractures of costal cartilages were investigated in beagle dogs of both sexes aged from 3 months to 15 3/4 years. By soft x-ray radiography, calcification and minute fractures of costal cartilages were already observed at 6 months of age regardless of sexes, with a subsequent development with age. Complete fractures were frequently observed in dogs aged more than 11 years. By the analysis of four costal cartilages (1st, 5th, 9th, 12th or 13th), it was demonstrated that after 6 months of age the water content decreased rapidly together with marked increase in the organic matrix, ash, calcium and phosphorus contents. Histological examination of undecalified specimens revealed that the matrix in the central zones was already calcified and minute fractures therein occurred at 6 months of age. These changes gradually expanded from the central to the peripheral zone with age. At 9 months of age, the calcified cartilage altered to an osseous tissue similar to that of trabecular bones, showing a normal histomorphological dynamics thereafter. It is concluded that the observed sequence of calcification and fracture of costal cartilage is a normal and age-related change which occurs after 6 months of age. — KEY WORDS: aging, beagle, calcification, costal cartilage, fracture.

Calcification and fractures of costal cartilages in old beagle dogs in our breeding colony are frequently seen with a lack of such reports in available literature, though similar phenomena have been already reported in humans [1, 2, 5-7, 9-11] and rats [1, 8]. The present report describes the process of calcification and the occurrence of fracture of costal cartilages in beagle dogs with the advance of age.

MATERIALS AND METHODS

Radiographical examination was carried out on the costal cartilages of a total of 103 beagle dogs of both sexes aged from 3 months to 15 3/4 years. Then, 49 dogs out of them were sacrificed for gravimetric, chemical and histological examinations of costal cartilages (Table 1).

All animals were kept in an air-conditioned room at 22±1°C, with a relative humidity of 55±5% in a 12 L and 12 D photoperiod. All dogs were given a commerical dog diet (150 g for young—250 g for adult) and water ad libitum.

Before sacrifice, the dogs were given tetracycline (25 mg/kg body weight) and calcein (8 mg/kg body weight) for later histological examination of the pattern of calcification. After autopsy, the soft x-ray radiographs were taken of the 1st to 13th costal cartilages.

The 1st, 5th, 9th and 12th or 13th costal cartilages were selected as subjects for chemical analyses. Some parts of these cartilages were weighed to know the wet weights. Then, they were dried in an oven at 90°C for 24 hr to measure the dry weights, followed by incineration in an electric furnace at 600°C for 24 hr to measure the ash weights. The calcium and phosphorus contents were measured by o-cresolphthaleincomplexone method (calcium C-test kit, Wako Co. Ltd.) and molyb-
Table 1. Animals used

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>6 &quot;</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>9 &quot;</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>4 years</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>11-15½ years</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

denun blue method (phosphor B-test kit, Wako Co. Ltd.), respectively. The water, organic matrix, ash, calcium and phosphorus contents were calculated as follows:

water content (%) = \[ \text{[wet weight (w.) - dry w./wet w.] \times 100} \]

organic matrix content(%) = \[ \text{[dry w. - ash. w./wet w.] \times 100} \]

ash content(%) = \[ \text{ash w./wet w. \times 100} \]

calcium content (%) = \[ \text{calcium w./wet w. \times 100} \]

phosphorus content (%) = \[ \text{phosphorus w./wet w. \times 100} \]

The remaining parts of costal cartilages were stained with villanueva bone stain (VBS) [12], dehydrated with ethanol and acetone, and embedded in methylmethacrylate. These undecalcified specimens were sectioned at 10 μm with the inner bred cutter (Maruto Co. Ltd.). The sections were ground by the glass plates for grinding (Asashi glass Co. Ltd.) and observed under a refexed fluorescence microscope.

RESULTS

Radiographic findings: Radiographically, the 5th to 9th costal cartilages were calcified in all dogs aged more than 6 months. Fractures were often observed in some of the 8th to 10th costal cartilages in the dogs more than 11 years of age as shown in Fig. 1.

Soft x-ray radiographic findings: Percentages of calcified and fractured costal cartilages in dogs of various ages are shown in Table 2. As there was noted little significant sex difference, the total values of both sexes are presented. Calcification of costal cartilages was not seen at 3 months of age, but seen at 6 months when most of costal cartilages already showed calcification and minute or incomplete fractures (Fig. 2). The fractured costal cartilages looked like “bamboo” with segments of 5 mm or more intervals (Fig. 3). The calcification and fractures developed with age, as shown in Table 2. Complete fractures were often seen in some of the 4th to 11th costal cartilages in all dogs more than 11 years of age (Figs. 4 and 5).

Contents of water, organic matrix, ash, calcium and phosphorus: Percentages of water, organic matrix, ash, calcium and phosphorus contents in the 1st, 5th, 9th and 12th or 13th costal cartilages are shown in Fig. 6.

The water content of all costal cartilages decreased rapidly from 3 to 9 months, remaining flat thereafter until 4 years. In dogs more than 11 years of age the water content of the 5th, 9th and 12th or 13th costal cartilage was lower than that of the 1st costal cartilage. The organic matrix content increased to reach the peak at 9 months and decreased slightly thereafter. The ash content increased continuously with age in all cartilages, though it was relatively low in the 1st cartilage.

The calcium and phosphorus contents in the 5th, 9th and 12th or 13th costal cartilages increased markedly from 3 to 6 months. Subsequent increases were observed in the 12th or 13th cartilage. In the 1st cartilage the calcium content increased gradually with age, but the phosphorus content remained constant.

Histological findings: At 3 months, chondrocytes were small and flat and located in the lacunae surrounded by matrix which was labelled with tetracycline and calcine (Fig. 7). They became larger and more numerous at 6 and 9 months (Figs. 8 and 9). Calcification began to occur in the interterritory
Fig. 1. X-ray radiograph showing calcification and fractures (arrow) of costal cartilages in a female dog at 11 years of age.

Fig. 2. Soft x-ray radiograph showing calcification of the 2nd to 11th costal cartilages in a dog 6 months of age.

Fig. 3. Soft x-ray radiograph showing calcification and minute fractures like "bamboo segments" of the 7th to 9th costal cartilages at 9 months of age.

Fig. 4. Soft x-ray radiograph showing the complete fractures in the 9th and 10th costal cartilages in the same dog presented in Fig. 1.

Fig. 5. Soft x-ray radiograph showing the complete fractures in the 10th and 11th costal cartilages in a male dog aged 11\text{\textfrac{1}{2}} years.

along the median axis of costal cartilages by 6 months (Figs. 10–12). Minute fractures were found in the calcified parts at 6 months (Fig. 13). At 9 months, the calcified parts altered to osseous tissues similar to those of normal trabecular bones in the central regions of the cartilage, showing a normal histomorphological dynamics [3]. In the periphery of the cartilage, large chondrocytes were still retained (Figs. 14 and 15).
Table 2. Percentages of calcified and fractured costal cartilages at various ages observed by soft x-ray radiography

<table>
<thead>
<tr>
<th>Age</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13th</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>Cal.</td>
<td>0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>I.F.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>Cal.</td>
<td>25</td>
<td>92</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>I.F.</td>
<td>0</td>
<td>11</td>
<td>25</td>
<td>42</td>
<td>67</td>
<td>75</td>
<td>92</td>
<td>83</td>
<td>83</td>
<td>75</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>9 months</td>
<td>Cal.</td>
<td>45</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>78</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>I.F.</td>
<td>0</td>
<td>18</td>
<td>36</td>
<td>64</td>
<td>82</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>4 years</td>
<td>Cal.</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>I.F.</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>83</td>
<td>40</td>
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<tr>
<td>11–15³/₄ years</td>
<td>Cal.</td>
<td>29</td>
<td>86</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>I.F.</td>
<td>0</td>
<td>14</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>C.F.</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>29</td>
<td>71</td>
<td>71</td>
<td>43</td>
<td>71</td>
<td>71</td>
<td>14</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

a) Cal.; Calcified.
b) I.F.; Incomplete Fracture.
c) C.F.; Complete Fracture.

The bone marrow was formed in the center of the ossified tissue at 4 years (Fig. 16). Calcification and ossification progressed from the central to the peripheral region of the costal cartilage. The degree of fracture increased with age (Fig. 17). Chondrocytes were still observed in the periphery of the cartilage at 15 ³/₄ years.

**DISCUSSION**

Sanders [9] suggested that calcification of costal cartilage was caused by abnormal calcium metabolism, based on the fact that the formation of gallstones, calcification of the middle valves, fat calcium infarction of the kidney were seen at the same time. In the present study, however, calcification of costal cartilages was detected by x-ray radiographs in all dogs more than 6 months of age together with neither calcification of soft tissues nor abnormal calcium metabolism. Since calcification of costal cartilages has been reported to occur without metabolic disorders and bone diseases in humans [2] and rats [7], it was thought that similar calcification of costal cartilages might occur in normal beagle dogs.

Dearden et al. [2] reported that in humans calcification of costal cartilages began to appear at 2–4 years of age and proceeded to 81 years of age. Werner [9] supplemented that calcifying changes were observed at a high incidence (more than 95%) in humans 35–45 years of age. Smith et al. [7] demonstrated that the calcium and phosphorus contents in the costal cartilages increased in rats from 20 days on. These reports suggest that calcification of costal cartilages began to occur in humans and rats before weaning. However, calcification was not observed in weaning beagle dogs 3 months of age in the present study. This suggests that the calcification begins later in beagle dogs than in humans and rats.

Dearden et al. [2] described that the content of chondroitin sulfate decreased with age, and that the content of hyaluronidase resistant materials (presumably keratan sulfate) increased initially in the central zones and subsequently in the peripheral zones, becoming minute or absent in the central zone of aged costal cartilages. They stated, therefore, that such age changes in
Fig. 6. The percentages of water, organic matrix, ash, calcium and phosphorus contents in the 1st, 5th, 9th and 12th or 13th costal cartilages at various ages.

the content of these organic materials were related to the process of calcification in the matrix of cartilages. Futami et al. [4] reported that the remodeling of proteoglycan and consumption of glycogen occurred during calcification in bovine costal cartilages. On the other hand, Smith et al. [8] mentioned no constant relationships between quantitative changes in the organic and inorganic cartilage components in rats. In the present study, however, the organic matrix, ash, calcium and phosphorus contents in the costal cartilage were significantly larger in dogs more than 6 months of age than in those at 3 months of age. This suggests that calcification of costal cartilages may be related to the increased amount of inorganic substances except for the 1st costal cartilage.

Though the organic matrix contents in the 1st costal cartilage tend to increase with age (Fig. 6), calcification occurs later and more slowly than in the other cartilages. Adversely in humans, the 1st costal cartilage is calcified earlier than the other ones [10]. The reason why the 1st costal cartilage differs in calcification from the other ones still remains unknown [11].

To be noted in particular is alteration of the calcified parts of cartilages to osseous tissues similar to those of normal trabecular bones [3], the alteration showing a normal histomorphological dynamics. This osseous alteration may be of some use in the compensation of the decreased activity of calcium metabolism with age, and may also contribute in part to the prevention of calcified parts from enlarging and assist in keeping normal flexibility of costal cartilages in respiration.

Many investigators [5-7, 9-11] reported sex difference in the calcifying pattern of the
costal cartilages. They observed that calcification of human costal cartilages began at the center of the cartilage in men and at the periphery in women with a higher degree of calcification. In the present study on the beagle dogs, however, the calcification began at the center of costal cartilages in both sexes.

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Fig. 13. A minute fracture (A) observed in the calcified part of the 9th costal cartilage at 4 years of age. VBS. \times 200.

Fig. 14. Ossification of a calcified part observed in the central area of a costal cartilage at 9 months of age. Chondrocytes remain in the periphery of the cartilage. VBS. \times 200.

Fig. 15. Osseous tissues labelled with tetracycline (A) and calcin (B) showing a remodeling pattern at 4 years of age. VBS. \times 200.

Fig. 16. Bone marrow (A) observed in the center of the ossified part at 4 years of age. VBS. \times 200.

Fig. 17. A fracture (A) and calcification (B) progressing to the peripheral zone. Chondrocytes are still observed in the periphery of the cartilage at 153/4 years of age. VBS. \times 100.

REFERENCES

要約

ビーグル犬における肋軟骨の石灰化と骨折：福田 俊（放射線医学総合研究所内部被ばく研究部）——ビーグル犬における肋軟骨の石灰化とこれに伴う骨折の発生について、3ヶ月から15ヶ月までの齢発を用いて検索した。軟X線写真では、6ヶ月齢には石灰化および微細な骨折が始まっており、それらは加齢とともに進行し、11ヶ月齢以上の個体では完全な骨折が観察された。第1、5、9、12あるいは13肋軟骨についての成分の含有量を分析した結果、6ヶ月齢以降水分は減少を、有機成分は増加を示した。灰分、カルシウムおよびリンは第1肋軟骨以外の部位では6ヶ月齢以降に顕著な増加を示し、非脱灰組織標本の観察では6ヶ月齢には、肋軟骨中心部の軟骨細胞層の基質で石灰化および微細な骨折が生じており、その後それらは中心部から辺縁部へと増大した。9ヶ月齢以降、石灰化部分が骨梁体と同様な組織変化を示す骨化組織に変化した。測定したすべての項目とも有意な雌雄差はみられなかった。


