Distribution of Thyroid Gland C Cells at Fractures in Thoroughbred Racehorses

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The distribution of thyroid gland C cells in 171 Thoroughbred racehorses (117 males, including 7 geldings and 47 mares) with a fracture was compared with that in 87 Thoroughbred racehorses without a fracture. No significant difference was found between the number of C cells in the fractured and non-fractured groups. But comparison of male and female fractured groups revealed a tendency to an increase in the number of C cells in 2-year, 4-year and 6-year male racehorses. The number of C cells at the time of fractures of the third metacarpal bone, third metatarsal bone, proximal sesamoid and first phalanx of the fore and hind limbs exceeded the number for fractures of the scapula, pelvis, humerus, femur, radius, tibia, corpus and tarsus, thereby pointing to differences between the bones in calcitonin sensitivity. A positive correlation was found between the frequency of racing starts and the number of C cells in 2-year, 3-year and 4-year-old horses with a bone fracture. No such correlation was found in Thoroughbred racehorses aged 5 years or more, suggesting that horses which do not show an increase in the number of C cells at a young age might have high tolerance of frequent participation in races.

Key words: distribution of C cells, fracture, immunohistopathology, Thoroughbred racehorses, thyroid gland

It has been reported that the majority of bone fractures in racehorses derive from pathological changes in bone characteristics rather than from sudden accidents [3, 5, 9, 10]. Furthermore, it has been reported that the serum calcitonin level in racehorses with a bone fracture is significantly higher than that of healthy horses [1] but there have been few reports of investigations of bone disease based on endocrinological considerations. In the present study, we compared the thyroid gland tissue of Thoroughbred racehorses with fractured bones with that of non-fractured horses by means of an immunohistochemical investigation with anti-human calcitonin serum to determine the number of C cells at the time of the fracture. We also examined the effect of the fracture site and the frequency of race starts on the change in the number of C cells.

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Materials and Methods

Thoroughbred racehorses

A total of 258 animals, consisting of 171 horses (117 males including 7 geldings and 47 mares) with fractured bones and 87 horses without fractured bones were investigated in this study. The most common age groups of horses with fracture were 2-year and 3-year-olds numbering 53 (36 males, 17 mares) and 63 (42 males, 21 mares), respectively (Fig. 1). The 171 fractured horses had suffered a fracture of one of the bones in the 4 limbs during training or racing in the period from 1980 to 1992, and based on the judgment of an extremely low chance of recovery had been the subject of euthanasia. When fractures of more than one bones were found in a single horse, these were regarded as separate fractures resulting in a total of 258 fractures in 171 horses. Furthermore, multiple breaks in a single bone were regarded as a single fracture. Stratified by bone type, there were 50 cases of third
metacarpal bone fracture, 49 cases of the lateral proximal sesamoid of forelimb fracture, and 22 cases of third metatarsal bone fracture (Fig. 2). To assist in evaluating the significance of the C cell value in a group with fractures, control thoroughbred racehorses (a group without fracture), which were killed by intravenous injection of an overdose of barbiturates for reasons related to accidental disorder (fracture, colic or sudden death) were employed.

**Light microscopy**

Sagittal thyroid gland sections, which present the maximum surface area, were prepared. These tissue sections were fixed in a 10% neutral buffered formalin solution, embedded in paraffin by the routine method, cut into thin sections and then double-stained with...
hématoxylin-eosin (HE) stain.

**Immunohistochemistry**

Thyroid gland tissue samples obtained from all of the study subjects were subjected to immunohistochemical staining with a rabbit polyclonal antibody (Zymed Laboratories, Inc., San Francisco, CA, diluted 50-fold with PBS) specific to human calcitonin. Formalin-fixed, paraffin-embedded tissue was thinly sectioned, deparaffinized and then treated with methanol containing 3% hydrogen peroxide to eliminate endogenous peroxidase activity. After allowing the sections to react with the primary antibody overnight at 4°C, they were bound to peroxidase-labelled amino acid polymer (Histofine Max PO Kit, Nichirei Co., Tokyo, Japan), and then subjected to a coloration reaction with diaminobenzidine substrate. Contrast staining was carried out by means of hematoxylin.

**C cell assay**

The number of C cells was assayed by means of an immunohistochemical staining with an antibody specific to calcitonin. The assay was carried out in accordance with the method of Yoshikawa et al. [11]. More specifically, the number of positively stained cells per unit area in 7 fields of view was counted and the mean number of stained cells was derived for the entire area as shown in Fig. 3. Statistically significant intergroup differences were investigated by t-test.

**Results**

**The fractured group**

The follicles were mainly small in size and oval in shape and the follicle epithelium was formed from 1 to several layers of cells (Fig. 4a). The follicular epithelial cells were cuboidal in shape and clusters of small follicles containing numerous C cells were observed in the periphery of the follicles (Fig. 5a). With the exception of young horses, the follicles of the horses without bone fractures were large, and were covered with a layer of flattened epithelial cells (Fig. 4b). C cells were scattered around the follicle epithelium, but there tended to be a smaller number of C cells than in the fractured bone group (Fig. 5b).

**Number of C cells at the fracture**

The number of C cells among the fractured bone population, consisting predominantly of 2-6-year-old horses, was generally high (Table 1). Comparison of the number of C cells in male and female horses in the fractured bone group revealed a higher number among males and statistically significant differences ($P<0.05$) between males and females in the 2-year, 4-year and 6-year-old age groups (Fig. 6).

**Number of C cells by site of bone fracture**

No significant correlations were found between the number of C cells and the site of fracture among the
Fig. 4.  a: Fractured bone group. Thyroid gland consists of small follicles of cuboidal follicular epithelium cells. HE. × 360.  
   b: Non-fractured bone group. Thyroid gland consists of large follicles and follicler cells which are flattened. HE. × 360.

Fig. 5.  a: Fractured bone group. Numerous C cells are observed in the periphery of the follicles. Immunostaining for calcitonin. × 360.  
   b: Non-fractured bone group. Small C cells are scattered around the follicles. Immunostaining for calcitonin. × 360.
fore- and hind-limbs and the right and left sides. Nevertheless, there was a tendency for the number of C cells to be higher among the fractured third metacarpal bone, third metatarsal bone, right and left lateral proximal sesamoid and first phalanx bones than in the fractured scapula, pelvis, humerus, radius, tibia, corporeal and tarsal bones (Figs. 7 and 8). Based on this finding, the number of C cells was compared in 2 to 6-year-old horses, which constituted the major portion of the study population, in the proximal bone group with fractures superior to the corporeal and tarsal and the distal bone group with fractures inferior to those in these bones. The results were $47.8 \pm 4.2$ (cells/mm²) in the proximal bone group and $72.2 \pm 5.4$ (cells/mm²) in the distal bone group, thereby showing a noticeably increased number of C cells in the distal bone group with fractures ($p<0.01$) (Fig. 9).

**Frequency of race starts and the number of C cells**

Of the total of 171 horses with fractures, 131 young horses aged 2 to 6 years (with a total of 187 fractures) were investigated to compare the frequency of race starts in the past with the number of C cells. A tendency for an increase in the number of C cells in parallel with an increase in the number of race starts was seen in the fractured horses aged 2, 3 and up to 4 years (Fig. 10) but no correlation between the two parameters was observed in horses aged 5 years and up to 6 years.

### Table 1. Calcitonin-positive cells at the Fracture

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Years)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractured</td>
<td>Mean of values</td>
<td>76.2</td>
<td>71.3</td>
<td>55.3</td>
<td>63.2</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>5.2</td>
<td>3.2</td>
<td>6.1</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Number of horses</td>
<td>70</td>
<td>96</td>
<td>32</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Non-fractured</td>
<td>Mean of values</td>
<td>47.7</td>
<td>49.7</td>
<td>25.0</td>
<td>64.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>17.0</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of horses</td>
<td>140</td>
<td>21</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

![Graph](image.png)

**Fig. 6.** Comparison of number of calcitonin-positive cells in male and female horses in the fractured bone group.
Discussion

It has been reported that the blood calcitonin level is significantly increased in racehorses with a bone fracture relative to the level in healthy horses [1]. But there has been no pathological research conducted on the morbidity of thyroid gland C cells, which play a role in bone metabolism and in the formation of the lesion causing the fracture. In the present study, we
conducted a comparative investigation of C cell kinetics in 171 racehorses who had a bone fracture during training or when running in a race, and 78 racehorses without any bone disease or a history of endocrinological disease.

A tendency for a decrease in the number of C cells with age was seen in the bone fracture group, but between the ages of 2 and 5 years the number of C cells increased to above the normal level seen in the non-fractured group as shown in Table 1 and Fig. 6. This
suggests that the number of C cells does not increase in 5-year-old horses with fractures compared with horses without fractures. It was thought previously that the occurrence of a bone fracture in racehorses is caused by the excessive biodynamic load during racing [6], but it has also been reported that based on the results of analysis of bone strength and density, most bone fractures are the result of an existing morbid lesion rather than deriving from a spontaneous lesion [3, 5, 9, 10]. Calcitonin, which is secreted by C cells, is a hormone that suppresses the movement of calcium from bone [2]. Therefore the high C cell value in the bone fracture group suggests the possibility that change in bone properties is influenced by calcitonin. Furthermore, the number of C cells in males with a fracture was much higher than in females with a fracture, with statistically significant differences (P<0.01) in the 2-year, 4-year and 6-year-old groups. The finding that females in the non-fractured group showed a tendency to have a lower number of C cells than males, indicates that the influence of calcitonin on change in bone properties is more sensitively expressed in males [11]. The higher incidence of fracture in males in Thoroughbred racehorses supports the speculation (Kaneko et al. 1990. *Equine Science* 27: 107–141, in Japanese). There was no statistically significant difference between the right and left limbs or the fore- and hind limbs in the distribution of C cells. But the group with a distal bone fracture had a remarkably and statistically higher number of C cells (P<0.01) at the time of bone fracture than the groups with fractures at other sites. In the case of fracture of the lateral proximal sesamoid, changes in the blood concentrations of parathyroid gland hormone and calcitonin were found to differ, suggesting that calcitonin adjustment was different at the time of fracture of a large bone and the lateral proximal sesamoid [1, 8]. In the present study, comparison of the number of previous races with the number of C cells in 131 racehorses revealed that 2-year, 3-year and 4-year-old racehorses had a tendency for an increase in the number of C cells with an increase in the frequency of races. As a result of the movement load, bone tissue hardness of the carpus and proximal sesamoid increased, thereby indicating that the bones had become more fragile [12, 13]. It appears that factors contributing to distal bone fracture are focal osteopetrosia or osteosclerosis due to calcium movement [4, 5], and generating factors in proximal bone groups with fractures are fatigue and stress fractures. These fractures might be associated with increased focal bone porosity which decreases the stiffness of the bone [7]. C cells and bone calcium are thought to respond to resist excessive movement load. Nevertheless, since calcitonin and bone calcium have a mutual feedback relationship [2], future studies should investigate which of these factors causes the response to movement load. In racehorses aged 5 years or more, no specific relationship between the frequency of races and the number of C cells was found. This might be explained by suggesting that individuals which do not show an increase in C cells at a young age have bones that resist fracture.

**Acknowledgments**

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**References**

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