Enamel Structure in the Molar of Giant Panda (*Ailuropoda melanoleuca*)

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Abstract

The giant panda (*Ailuropoda melanoleuca*) is a herbivorous mammal that feeds on bamboo, although this species is now considered to belong to the carnivorous family Ursidae. In this study, enamel from the molars of the giant pandas was examined and compared with enamel from dogs (*Canis lupus familiaris*), and the effects of phylogeny and feeding were investigated based on the enamel structure. The molar morphology of the giant panda is blunt, has a broad occlusal surface, and is adapted to vegetative diets, whereas the morphology of the Hunter-Schreger bands (HSB) near the enamel surface correspond to a zigzag pattern, which is characteristic of carnivores that feed on bones. This tendency was stronger in comparison with HSB in dogs. The morphology of enamel prisms was also similar, and distinct rows of enamel prisms were found at the boundary between the transverse and longitudinal bands of HSB. For dietary adaptation of molar morphology in the giant panda, preservation of internal structure was strongly recognised.

Introduction

Hunter-Schreger bands (HSB) are alternately repeated transverse and longitudinal bands of enamel prisms in the enamel of many mammalian teeth; they have been shown to be related to tooth morphology and to correspond to the mechanical strength required for mastication (1, 2, 3, 4). Kawai (1) categorized the morphology of HSB according to an animal's classification, while Fujita (5) categorized HSB by focusing on an animal's eating habits. Subsequently, in addition to the horizontal type, which is almost perpendicular to the tooth axis, the vertical type (6, 7, 8) which is parallel to the tooth axis, the island type (9, 10), in which the transverse and longitudinal bands are distributed in a patchy manner were found, and conclusively they were classified into five types (9, 11), including the irregular type seen in elephants and the rodent type, which shows a characteristic direction of enamel prisms. HSB have a three-dimensional extent in the enamel, and their morphology changes according to

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Fig. 1. Dentition of Giant panda (*Ailuropoda melanoleuca*). (From Thenius E, 1969)

the enamel layer in which they occur. Koenigswald et al.(12) have classified enamel by “Schmelzmuster” described the spatial distribution of HSB types and prism direction. Stefen(13, 14) showed morphological changes in HSB by layer after conducting comparative research among carnivores, and classified the wavy structure of HSB observed in the surface layer into three types, which was contrasted with the eating habit. In particular, a steep wavy shape, referred to as the zigzag-type, was suggested to be related to the structural strength necessary for the masticatory pressure needed to crash bones.

The giant panda (*Ailuropoda melanoleuca*) is a large herbivorous mammal that feeds mainly on bamboo. Although the special dietary habits and tooth morphology (Fig. 1) (15) are of interest, there have not been many histological studies of teeth (16–18). There have been various theories about the taxonomic position of giant pandas, but they are now considered to belong to the Ursidae family (19–21). In this study, we confirmed that the molar enamel of the giant panda has characteristics in common with the enamel of the dog (*Canis lupus familiaris*) (22, 23), which is more carnivorous. Clues were sought to investigate the relationship between changes in external form and the effects of eating habits and phylogeny.

Materials and Methods

Molars of giant pandas (*Ailuropoda melanoleuca*) from Pleistocene cave deposits in Guangxi, China were used as

samples. Although the enamel surface of the molars was slightly worn, a sufficiently thick enamel was present for each cusps. Enamel thickness was measured in the mid-ventral portion of the crown. Specimens were prepared as longitudinal, transverse, and tangential sections of the enamel. The tangential surfaces were parallel to the dentinoenamel junction (DEJ) and inclined from the DEJ to the enamel surface. Each sample was cut out using a diamond cutter, embedded in polyester resin, and polished using a rough to a medium abrasive, further to a grain size of 3 μm , 1 μm and then 0.25 μm of a diamond paste, to obtain a mirror surface. The polished surfaces were etched using 1/20 N hydrochloric acid for 20 to 40 s, then Au or Au-Pd was sputtered and observed under a reflection optical microscope (Zeiss, Stemi 2000-CS) and a scanning electron microscope (SEM) (Hitachi, S-2700, S-3400N) at an acceleration voltage of 15–20 kV.

Results

Morphology of molars

Five distinct cusps were confirmed in the mandibular first molar, and the development of the cingulum was remarkable. The mandibular third molar had a triangular shape of about 20 mm on one side and a nearly flat occlusal surface with no cusps and a slight depression in the center (Fig. 2). When light was applied from the side of the molars, wavy HSB were observed in some areas (Fig. 3).

Fig. 2. Lower left M3 Scale: 5mm

Fig. 3. Reflected light microscopy of tubercle. Appearance of undulating or acute HSB. Scale: 500 μm

Optical microscopy

In the reflected light observation of the sample which polished the inclined surface along the tangent plane and sputtered Au-Pd, horizontal HSB which were almost perpendicular to the tooth axis were observed, and the fold of the HSB were confirmed (Fig. 4a). The HSB changed width as they approached the enamel middle layer, and the angle of the waviness became acute as they approached the surface, repeating the bifurcation. Finally, the waviness of the HSB increased, the transverse and longitudinal bands ran from a U shape to a V shape, and continuity in the horizontal direction became indistinct. In the enamel surface layer the zigzag form could be seen, and the continuity in the horizontal direction was indistinct.

SEM imaging: Tangential section

In the tangential section, HSB showed wavy shape, but the boundary between the transverse and longitudinal bands was indistinct (Fig. 5a). The cross-sectional shape of the enamel prisms was hexagonal to oval, and the interprismatic substance was also clear (Fig. 5b). The boundary between the transverse and longitudinal bands of HSB showed that some areas of the enamel prisms could be distinguished from each other by their cross-sectional morphology, while other areas could be identified by slight differences in the crystal arrangement

(Fig. 5b, c). The cross-section of enamel prisms was hexagonal to round with a diameter of 6 to 7 μm , and the interprismatic substance was also developed, with a width of 1 μm (Fig. 5d).

SEM imaging: Longitudinal section

The thickness of the enamel was about 1.3 mm at its thickest point, in the middle of the enamel. In the longitudinal section of enamel, HSB was continuous, with slight curvature from the DEJ to nearly one-third of the way to the enamel surface, and then lined-up radially with enamel prisms to the surface (Fig. 6a). HSB began in a direction almost perpendicular to the DEJ, and the transverse and longitudinal bands were clear with little change in width (Fig. 6b). At another site, an irregular sequence was observed near the DEJ (Fig. 6c). A transition of enamel prisms in the transverse and longitudinal bands into swirling patterns was observed (Fig. 6d).

In another section, two to three rows of continuous enamel prisms from the DEJ to the surface layer were observed in a ladder pattern (Fig. 7a). The enamel prisms forming the boundary between the transverse and longitudinal bands were longitudinally arranged in rows, which became clear from about 20 to 30 μm on the DEJ side and continued to the surface layer to about one-third of the enamel prisms. The width of each band was about ten-times that of the enamel prisms (Fig. 7a-c).

Fig. 4. Reflected light microscopy of oblique tangential section.

a: Giant panda, b: Dog

Upward is the enamel surface

Appearance of undulating or acute HSB.

Bands of HSB change from a U shape to a V shape, and continuity in the horizontal direction became indistinct.

Fig. 5. SEM micrograph of tangential section of giant panda molar.

- a: Both zones of HSB run undulating
- b: Boundary between zones of HSB different direction from adjacent bands.
- c: Boundary between zones of HSB different direction from adjacent bands.
- d: Appearance of prisms. 6-7 μm diameter.

The morphology of the enamel prisms ranged from hexagonal to elliptic with diameters of 6 to 7 μm , and the interprismatic substance was also clear (Fig. 7b, d).

Discussion

The dentition of the giant panda (*Ailuropoda melanoleuca*) is shown in Fig. 1. The molars of Ursidae reflect their tendency to be omnivorous, and the molars of the giant panda have broad occlusal planes with rounded and some secondary cusps suitable for herbivory (Fig. 2), which is different from those of canines which have carnassial teeth. In terms of function, they are thought to enhance the mechanical strength of enamel for mastication (2, 3, 4). Therefore, differences in feeding and mastication

patterns may lead to differences in HSB morphology as well as in tooth morphology. Differences in HSB morphology were classified by Kawai(1) into carnivorous, hoofed, primate, and rodent types based on comparative anatomy studies. Fujita(5) noticed a relationship with feeding on the basis of the classification of Kawai(1) and categorized HSB morphology into a carnivorous type, a herbivorous type, and an omnivorous type, and added the rodent typology which was difficult to unify.

The morphology of HSB found in the enamel of most mammals is horizontal HSB, which are almost perpendicular to the tooth axis. However, in rhinoceroses, vertical HSB types parallel to the tooth axis have been reported (10). HSB were classified into five types: including is-

Fig. 6. SEM micrograph of longitudinal section of giant panda molar. (D: dentin)

a: HSB run parallelly from DEJ to 1/3 surface of enamel and prisms run radial course to the surface.

b: HSB run parallelly from DEJ.

c: Almost hexagonal shape of cross section of prisms with thick interprismatic substance can be observed.

d: Prisms run irregularly near the DEJ

d: Island-like prism decussation

land-like(9–11), characterized by mottled transverse and longitudinal bands; rodent type, with characteristic enamel prisms, and irregular type, such as elephant molars (4, 6–8). Giant pandas and domestic dogs (*Canis lupus familiaris*) belong to the horizontal HSB type, and depending on how light is applied, undulating HSB can be observed on the enamel surface by reflected light microscope (Fig. 3) (24).

Stefen(13, 14) showed that among carnivores, the HSB of hyena enamel are of the moderate horizontal type in the deep to middle enamel layers, whereas they become zigzag-type (V-shaped) in the superficial enamel layers.

This indicates that the types of HSB change in the enamel layer, and the enamel prisms change the course and organization developmentally.

When the morphology of HSB at different depths of enamel is examined in the tangential section of the molar tooth surface of the giant panda, the wave angle tends to become an acute angle from the enamel middle layer to the surface layer, and it becomes V-shaped near the enamel surface (Fig. 4a). The morphology of HSB near the enamel surface of the giant panda corresponds to the zigzag pattern suggested by Stefen(13, 14). The angle of HSB in the giant panda is more acute than that of HSB in

Fig. 7. SEM micrograph of longitudinal section of giant panda molar. (D: dentin)

- a: HSB run paralelly from DEJ to 1/3 surface of enamel and prisms run radial course to the surface.
 b: Each zone of HSB is about ten prisms wide. Boundary between bands is clear by means of intermediate prisms (Arrow).
 c: Each zone of HSB run different direction.
 d: Appearance of prisms. Almost hexagonal shape of cross section of prisms with thick interprismatic substance can be observed. 6-7 μm diameter.

dogs (Fig. 4a, b). According to Stefan (13, 14), the tendency of this wave motion to become steep is understood to be a structural adaptation of HSB in response to pressure on the enamel, and it is also assumed that the structure guarantees the mechanical strength necessary for mastication in relation to the ossiphagous feeding habits of hyena. In both species, the difference in conditions such as bifurcation and convergence is observed near the DEJ and from the middle layer to the surface layer, and the change in structure becomes clearer by comparing the longitudinal and tangential sections of enamel.

In the SEM image of the longitudinal section, the ar-

range of enamel prisms near the DEJ was irregular due to the disorder of the banded structure (Fig. 6c, d). Such aggregation of enamel prisms during the early stages of enamel formation is indicative of island-like HSB. These findings indicate that ameloblasts first undergo irregular aggregation at an early stage of enamel histogenesis, followed by an island-like HSB in the deep layer that transitions to typical cingulate movement (9-11). According to comparative anatomical classification of HSB, typical island-shaped forms are found in the fossil proboscids *Deinotherium* and *Barytherium* (9-11) (Cenozoic Tertiary). The similar morphology observed in the

Fig. 8. SEM micrograph of longitudinal section of dog molar.

a: Each zone of HSB is about seven prisms wide. HSBs reach the enamel surface. Arrow: DEJ (From: Fukuda R, Suzuki K, 1999)

b: Boundary between bands is clear by means of intermediate prism row (Arrow). (From: Kikuchi R, Suzuki K, 2002)

early enamel development of the giant panda can also be interpreted as the histogenetic expression of phylogenetic changes in HSB.

The SEM image of the longitudinal section showed the existence of row of enamel prisms as a boundary between both bands of horizontal HSB. This morphology was also confirmed in the dogs used for comparison (Fig. 8). This structure raises a question whether the enamel prisms stay in the band to which they belong or shift to the adjacent band in the transverse and longitudinal bands of HSB. Hanaizumi et al. (25–27) investigated three-dimensional enamel prisms of canine tooth-germs and reported the presence of enamel prisms with greater movement at the center of each zone and less movement at the borders of the HSB, while studies of rhinoceroses with well-defined enamel prisms have shown that the enamel prisms can shift (28, 29). From the viewpoint of the strength of enamel, it is considered that the zigzag HSB developed even in the herbivory, because the hard material of bamboo requires to reinforce the enamel of the giant panda.

In comparison with dogs, the giant panda wave showed an acute angle in the HSB of the tangential section. In the longitudinal HSB, it was confirmed as a common point that there were two to three rows of enamel prisms in the boundary of transverse band and longitudinal band of HSB. The cross-sectional shape of the enamel prisms was also similar.

On the other hand, *Ursavus* of the Ursidae family, is regarded as systematically valid, but zigzag HSB are not known in this group (14). In the HSB of the giant panda, there is a difference in which enamel prisms radiate to the surface in the one-third of the surface layer, while HSB continue to the surface in dog molar. The remarkable difference is that the enamel thickness of the dog is about 0.5 mm, while that of the giant panda is 3 times as thick.

In this study, we confirmed that giant pandas are adapted to a bamboo-based vegetable diet, but also that their enamel has carnivorous characteristics despite having a low cusp molar morphology. In particular, it was confirmed that giant pandas have the zigzag-type of HSB in the tangential section, as observed in ossiphagous species like hyena, and it was also proven that there was a common point with the enamel of the dog in the internal structure of their molar. How the movement of a group of ameloblasts is organized in adaptation to phylogeny and feeding habits to form these three-dimensional structures, including the HSB, will be examined from the viewpoint of enamel histogenesis in the future.

Conflicts of interest

The authors have no potential conflicts of interest.

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