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Endocrinological Studies and Potential Biomedical Uses of Antlers

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Abstract Antlers from deer species are alternative animal by-products. Due to the oriental trade, the velvet antler industry is rapidly emerging in North America. The unique biological property of antler with a deciduous natural phenomenon offers the valuable model of biomedical research. Growing antlers showed different structures according to cell populations consisting of mesenchymes, chondroblasts, chondrocytes and osseous tissues from distal to proximal portions of main beam. Their structures were different from two tissues, cartilage and bone, in growing antlers. Zone of maturing and calcifying chondrocytes referred as upper section was invaded by osteoblasts indicating the occurrence of endochondral ossification. The cartilaginous tissues were gradually replaced by osseous tissues downward. The bony tissues referred as the middle and base sections in this thesis contained spongy bone and cortical bone structure in the difference of the degree of mineralization and the thickness of cortical bony in adjacent to outer velvet layer. In addition, the endocrinological regulators such as testosterone, prolactin, growth hormones and other growth factors are actively involved in the unique deciduous nature shown in the growth and development of antler.

Key words: Antler, Deer, Chondrocytes, Endochondral Ossification, Growth factors

Introduction

Velvet antlers have been known as the most precious source of traditional oriental medicine for many centuries. Health conscious people believe that velvet antlers have various biological activities to stimulate our physiological body conditions and protect us against diseases. This belief is important to keep the antler industry growing and to develop a new value-added antler product other than the conventional use of antlers. The modern use of antlers as a functional food is expanded into North America and Europe as well as in Asia. International markets demand velvet antler of which quality is determined by specific size, color, shape and mineralization. Thus, following the casting of the previous set of hard antlers, antlers from deer species are harvested at 65~85 days when the main beam just begins to bulb at the 4th tine, depending on the photoperiod in various regions. The harvested antler in a growing stage is composed of soft cartilaginous tissues in distal portion and hard bony structures downward [63]. Antlers grow in length by endochondral ossification, and in diameter by intramembranous ossification [33,40,48,61]. The growth of antler depends on the proliferation and maturation of chondrocytes that occur in distal portion of antlers. Antlers are probably the fastest growing non-pathological tissues among mammals. For example, the maximum growth rate of antlers of elk (*Cervus elaphus*) is approximately 2.75 cm/day [26]. They are suggested to be a useful model for the study of bone growth and metabolism [11,27]. Antlers can be divided into four sections, tip, upper, middle and base which are used differently to treat diseases such as anemia, arthritis, hypercholesteremia, and cancer or to promote health as traditional oriental tonics [62].

However, little is known about the biological properties of antler circulated in markets. This review attempts to deliver the understanding of antler growth with the deciduous nature based on the endocrinological regulation and to potentiate the feasibility of antlers as biomedical uses.

General Understandings of Antlers

In the webster International dictionary, antler is defined as the branched deciduous horn of any animal of the deer family. The term, antler, comes from the Latin anteoculae, meaning "in front of the eyes". In the orient, antlers refers to soft growing tissues, terms of "nog yong" in Korea, "lu rong" in China, "rokujo" in Japan or "pantui" in Russia, and hard antlers before cast off, terms of "lu jiao" in China or "nog gag" in Korea. Both soft and hard antlers have been

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used for the treatment of various diseases in oriental medicine [58].

Structure

Antlers are appendages of the skull, composed of a solid bony core and supported on skin covered pedicles (protuberance of the frontal bone) which are permanent tissues [41]. The formation of primary antlers from males except reindeer can be initiated after pedicle development from the periosteum of the frontal bones of almost all members of Cervidae [27]. While the antler is growing or regenerating, it is covered by a skin with a dense of fine hair that leads to the term "velvet antler" which can be applied to the growing tissue. After growth is finished, the velvet skin peels off to be known as hard antler.

Horn and Antler

Antlers differ from the keratinous horns of the Bovidae in that horns are permanent [21], while antlers are deciduous with a cycle of regeneration, proliferation, mineralization and casting off [5,41]. Growing antler has fine hair known as velvet. Cervids such as sika, red deer, caribou, wapiti and moose have antlers, while bovids including mountain goat, bighorn sheep, bison and pronghorn antelope possess horns [35]. With the exception of caribou (*Rangifer tarandus*), antlers are found only on males [42,49]. A few (accounting for about 1%) of female deer grow antlers because of excessive testosterone [37]. On the other hands, horns are permanent structures composed of a bony core and an outer sheath of keratin, the same material found in fingernails and hooves [35]. The horns of bovids never branch, whereas antilocaprid (pronghorn) horns have a forward-projecting prong.

Function of Antler

A number of theories of antler function have been proposed in behavioral aspects [14]. During the breeding season, antlers threat or intimidate rivals to occupy female deer. On the other hand, antlers function as display organs, determining social hierarchy among males without actual battle. As status symbols, they dictate the rank order in bachelor groups which determines priority for food and shelter [6]. It has been suggested that antlers evolved as thermoregulatory organs [57]. Although antlers may have some role in heat dissipation (the growing tips of antlers are often the warmest parts of the body), a primary evolutionary role is refuted by the fact that Roe deer (*Capreolus capreolus*) and some tropical deer grow their antlers in winter [22]. The olfactory projection theory [6] postulates that antlers dissipate pheromones from rich sebaceous glands in velvet to mark boundaries in territorial species.

Taxonomy of Deer

The Cervidae, a family in taxonomy, is currently classified into 4 subfamilies: Cervinae with four genera (*Axis, Cervus, Dama,* and *Elaphurus*), Hydropotinae with one genus (*Hydropotes*), Muntiacinae with two genera (*Elaphodus* and *Muntiacus*) and Odocoileinae with nine genera (*Alces, Blastocerus, Capreolus, Hippocamelus, Mazama, Odocoileus, Ozotoceros, Pudu* and *Rangifer*). In Oriental classification, deer for the traditional use of antlers are limited only to Cervus species (*Cervus nippon* and *Cervus elaphus*) according to the Oriental Countries' Specification for natural medicines.

The family Cervidae is represented in North America by the elk (derived from the Germanic "elch", which refers to the European moose) or wapiti (a Shawnee Indian name meaning "white rump") (*Cervus elaphus*), moose (an Algonkan Indian word for "eater of twigs") (*Alces alces*), caribou (a Micmac Indian name, which refers to reindeer) (*Rangifer tarandus*), black-tailed deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*). This family dates from the Miocene in the Old World except the genus Odocoileus in the New World [23]. Wapiti, moose and caribou in North America have counterparts in Europe and Asia. There are no European or Asian counterparts to the genus Odocoileus. The wapiti is a common name of the American elk larger than the European red deer, which exceeds several-fold in size [24].

Endocrinological Regulation of Antlers

The seasonality of the antler cycle depends on the changes in the photoperiodicity [25,46]. In the retina, the photic stimulus becomes an electrical message which is further transformed in the pineal gland into a chemical signal, the hormone melatonin [8,55]. Melatonin is primary messenger of photoperiodically dependent periodicity. Thus, the formation of primary antlers is initiated after pedicle development from the periosteum of the frontal bone [1,7,34,39,41]. The initial of antler growth occurs at the tip (distal portion of main beam) containing reserve mesenchyme which can be proliferated and differentiated into chondroblasts and chondrocytes which become cartilaginous tissues, followed by maturation, hypertrophy and calcification as shown in long bones [59]. The growth of antler for approximately 3～4 months is suspended with the increase of massive mineralization of the cartilaginous tissue. Due to the mineralization, velvet skins become dead. The hard antlers are retained over the winter period until the time of casting.

Hormones

Studies of the antler cycle have been extensively reported as below. The close relationship of the antler growth to the reproductive cycle of the male deer is important for the seasonal levels of hormones. Antler growth is seasonal in boreal and temperate species of deer. For example, in the wapiti (*Cervus elaphus*) the velvet antler grows in spring and is hard for breeding season in autumn. Previous antlers are cast in spring. The seasonal cycle is closely related to the change of day length and reproductive cycle of the male deer regarding nutrient status [29,43,56,65] and hormones such as melatonin [15,67], testosterone, estrogen [3,31,38], thyroxine, prolactin, growth hormone, cortisol [4,30,50] and insulin growth factor.

Testosterone

Antler tissue is a target organ for steroidal hormones [10]. The antler growth period is associated with minimal seasonal levels of testosterone. Testosterone known as prohormone to be metabolized to other androgens and estrogens stimulates antlerogenic periosteum to produce pedicle in the onset of antlerogenesis [53]. During the rapid mineralization phase in 2 ~ 3 weeks, a slow rise of testosterone levels was observed [7]. Shedding of velvets was observed during the period of high testosterone levels [32]. Finally, a rapid reduction of testosterone levels after the rut is manifested by the casting of antlers [7].

Thyroid Hormones

The thyroid is a large endocrine organ that functions mostly to control the growth metabolism. The thyroid hormones are very simple modifications of the amino acid tyrosine. Iodide is added to one or two spots on the amino acid and then two of the modified tyrosines are combined to form one of the two thyroid hormones, thyroxin (T4) or triiodothyronine (T3). Both T4 and T3 can stimulate osteoblastic activity during the mineralization of antler [53]. A polypeptide hormone calcitonin functions in calcium maintenance to decrease the levels of calcium in the blood. The high level of calcitonin was found in the phase of rapid antler growth, while it decreased in mineralization period [13,28].

Prolactin

Pituitary hormones contain mainly prolactin (PRL) and growth hormone (GH). Prolactin synchronizes timing of reproductive activity with seasonal changes. PRL, one of the pituitary hormones, acts synergistically in long-day breeders such as the roe deer, antagonistically in short-day breeders such as white-tailed deer or red deer and inhibits the rise of testosterone levels in blood. By keeping testosterone level low, PRL is preventing the premature onset of antler mineralization and initiation of the rutting behavior [8].

Growth Hormone

Growth hormone directly has a stimulatory effect on antlerogenesis. It promotes the secretion of Insulin-like growth factor (IGF) which has target organ in growing antler tissues and stimulates directly antler growth. Gonadotropin releasing hormone stimulates secretion of luteinizing hormone and follicle stimulating hormone. The luteinizing hormone stimulates secretion of testosterone by the Leydig cells of the testes and testosterone derivatives such as 5 a-dihydrotestosterone and estradiol [16].

IGF

Insulin-like growth factors (IGFs) have amino acid sequences similar to insulin and function as mitogens. Extensive studies on IGFs have been related to antler growth [16,17,50,52]. IGF-I was found to have a mitogenic effect on the proliferation of antler cells cultured obtained from the tip section [47]. It stimulates the proteoglycan synthesis forming the extracellular matrix during the antler development. The tip section of antler contains the IGF-I receptors supporting the function of IGF-I [17]. In addition, levels of IGF-I in serum increased at the antlerogenesis, but decreased after the breeding season. This finding is positively related with the change of testosterone level [16]. IGF-I concentrations in plasma are high, and positive correlations have been found among plasma IGF-I, live weight gain, and antler growth rate in red deer [66].

Epidermal Growth Factor

Epidermal growth factor (EGF) has the effect on mitogenic to many cell types. EGF stimulates the fast tissue growth. It has been found in relatively high concentrations in antler velvet [36].

Bone Morphogenetic Proteins

In embryonic and post-fetal life bone development is initiated by one of several glycoproteins named Bone Morphogenetic Proteins (BMPs) resulting in the local differentiation of endochondral bone. During the rapid growth of antler, BMPs' gene is responsible for expression of proteins involved in the differentiation of antler tissues [18,19,33].

Other growth factors

Antler growth is very rapid, and the growing tip is composed of fibroblasts which divide and differentiate into chondroblasts and eventually osteoblasts. Fast growing tissues contain a great amount of growth factors such as fibroblast growth factor (FGF), nerve growth factor (NGF), and others [12]. FGF plays a prominent role in skeletal and nervous systems development. NGF exerts chemotropic and chemotropic activities on sensory and sympathetic neurons. NGF was the first neurotropic factor identified. It is derived from and regulates the density of innervations of the target tissues. TGF alpha stimulates growth of microvascular endothelial cells. Thus, several other growth factors little studied in the velvet antler have effects on antler growth and development. The study of their possible role has to be conducted to understand the antler growth.

Histological Observations of Antler Growth

The growth of antler depends on the endochondral ossification in length and the intramembranous ossification in diameter [2]. In the former, cartilage first calcifies to form bones in perpendicular growth, while mesenchymes in periosteum become osteoblast and then bones in horizontal growth. To understand the development of the rapidly growing antler which is approximately 2.75 cm/day in the case of elk (*Cervus elaphus*) [24], there are histological observations related to the growth of antlers of various breeds [2,20, 39,45,59,63] on the ossification compared to the long bones.

In the present study, the main beam of a growing antler of wapiti (*Cervus elaphus*) can be divided into four major zones by cell populations through microscopic observation (Fig. 1.). The distal tip section (approximately 1 ~ 1.5 cm from top of the antler) contained the distinct region (Fig. 2a ~ 2d) including those of reserve mesenchymes adjacent to the dermis, prechondroblasts, chondroblasts and immature chondrocytes. The cellularity was apparently highest in the zone of prechondroblasts, and gradually decreased as cells matured. In the zone of prechondroblasts, both cell proliferation and development of eosinophilic structures appeared to be active (Fig. 2b). The direction of blood flow is from distal to proximal section, through cartilage down to bone. The zone of tip section in antlers is a growth center by development with the proliferation and differentiation of mesenchymal cells into chondroblasts and chondrocytes, followed by cartilage maturation, hypertrophy and calcification in upper section, downward.

The upper section containing chondrocytes as major tissues and primary spongiosa as minor tissues was comprised of columns of clustered maturing chondrocytes separated by perivascular spaces containing blood vessels. The size of chondrocytes, which was larger than that of chondrocytes in the tip sections, increased as cells mature. Matured hypertrophied chondrocytes adjacent to perivascular spaces were found to lose nuclei staining with Hematoxylin (Fig. 3). These cells appeared to be calcifying chondrocytes. Osteoblasts were seen adjacent to the calcifying cartilage (Fig. 3). The number of calcifying chondrocytes increased in proximal direction with concomitant decrease of the area of cartilaginous tissue. These observations suggested the occurrence of endochondral ossification undergoing development

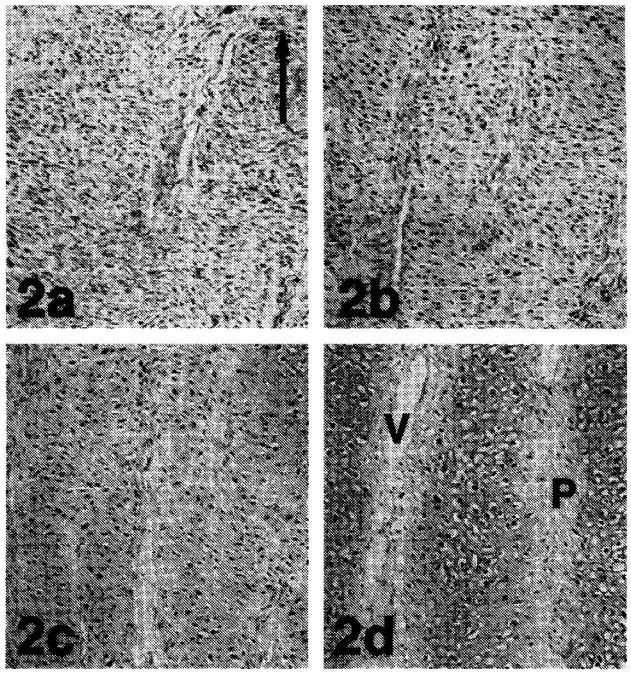

Fig. 2. Tip section in a growing antler showing four apparent cell types. Longitudinally sectioned tissues were stained with Hematoxylin and Eosin. a: Reserve mesenchyme. b: Prechondroblasts containing perivascular tissues with eosinophilic structure (E). c: Chondroblasts invaded by blood vessels (V). d: Immature chondrocytes invaded by blood vessels (V). Long arrow points distal. magnification × 330.

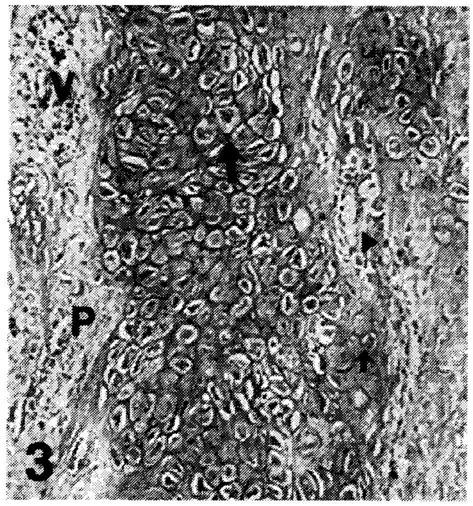

Fig. 3. Upper section in a growing antler showing matured hypertrophic chondrocytes (large arrow). Calcifying chondrocytes (small arrow) and lining osteoblasts (arrow head) adjacent to the perivascular space (P) and blood vessels (V), indicating endochondral ossification. Longitudinally sectioned tissues were stained with Hematoxylin and Eosin. magnification × 106.

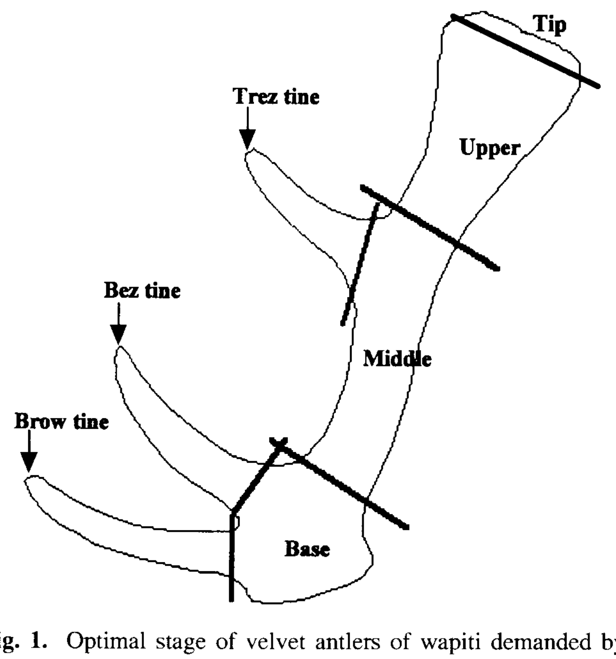

Fig. 1. Optimal stage of velvet antlers of wapiti demanded by international markets showing four sections used in the current research.

of cartilage, calcification, and osseous replacement, sequentially. From this study, cells in the tip section still proliferate into cartilaginous tissues and mature chondrocytes in upper sections were observed to calcify. The process of endochondral ossification includes several events including growth, maturation and calcification of chondrocytes followed by invasion of bone forming cells, osteoblasts (Fig. 3).

The middle and base sections contained bony structures including spongy bone with osteoid on its margin (Fig. 4a), and cortical bone structure (Fig. 4b). The cortical bony tissues were covered with tissues of cellular periosteum containing mesenchymal cells, which differentiate into bone forming cells, osteoblasts. The difference of both sections is a degree of mineralization and is a thickness of cortical bony tissue adjacent to outer velvet layer. The middle section includes the stage of mostly primary spongiosa in calcifying zones, while the base section dissected at approximately 3 cm from pedicle was observed to contain thicker cortical bony tissues than the middle section did, indicating hardening of tissues by calcium accumulation. The intramembranous ossification is dependent on the differentiation of mesenchymal cells into osteoblasts in the cellular periosteum and is responsible for latitudinal growth of periphery of main beam in growing antlers (Fig. 4.). Osteoblasts form osteoid tissues which are subsequently mineralized to form bone spicules.

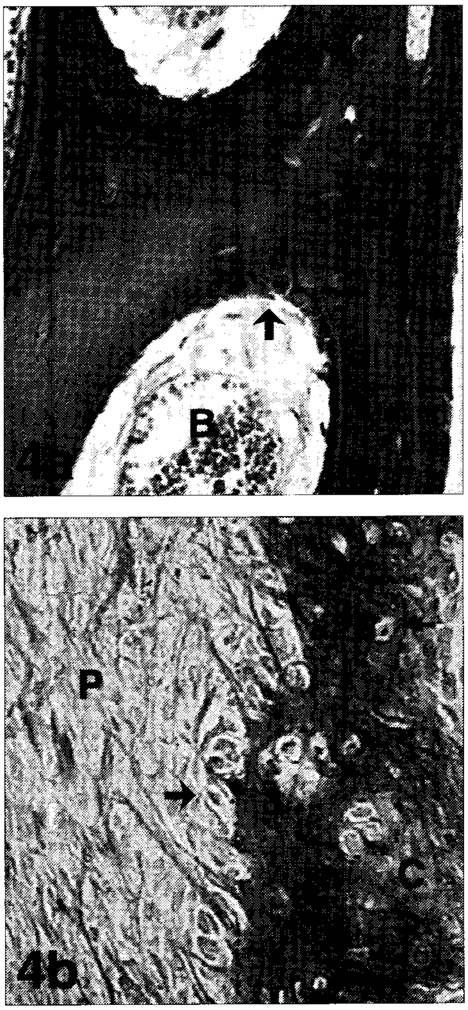

Fig. 4. Bony structure in base section. a: Bony trabeculae of spongiosa lined by osteoid (arrow). B: bone marrow. b: Cortical bony structure (C) covered with periosteum (P). Small arrow points osteocytes. In the periosteum (P), mesenchymal cells differentiate into bone forming cells, osteoblasts (large arrow). Because of many similarities in staining patterns between the base and middle sections, the micrograph of the latter is not shown. Longitudinally sectioned tissues were stained with Hematoxylin and Eosin. magnification × 330.

The process of endochondral ossification involves several events including growth, maturation and calcification of chondrocytes followed by invasion of bone forming cells, osteoblasts. The intramembranous ossification is dependent on the differentiation of mesenchymal cells into osteoblasts in the cellular periosteum.

Antler Use as Biomedical Model

Studies of Aging

A report showed the results of treating a small group of elderly men with recombinant human growth hormone (GH) for six months [51]. In that study, GH increased lean body and bone mass, decreased adiposity, and apparently restored skin thickness to that of a 50-year-old man. The treatment of GH may improve mood, libido, and muscle strength as anti-aging therapy. The potential use of antler growth factors as anti-aging is associated with somatic changes due to the declination of the secretory capacity of GH.

Osteoporosis

Osteoporosis is a metabolic bone disease associated with increases in bone resorption and with decreases in bone formation due to the aging. Finding appropriate therapies are not straightforward because of this complex pathophysiology. Recently, osteoporosis represents a vast therapeutic market that has been recognized by the medical profession and pharmaceutical industry. Antler development associated with hormonal changes may provide a unique model to study osteoporosis in that it takes only a few weeks to transform an embryonic-like blastemitic and cartilaginous tissue to fully developed Haversian lamellae. During that short period a huge amount of calcium and phosphate is transferred into the antler bone, a large proportion of which is taken from skeletal deposits in ribs, sternum, and vertebrae [9]. The demineralization of skeletal bones in deer can replenish the calcium deposits in the skeleton after antler mineralization is achieved. The endoclinological approach is very useful to understand the osteoporosis using deer as an animal model. In addition, bone morphogenetic proteins (BMPs) have effects on osteoblasts differentiation, whereas TGF stimulates osteoblast proliferation but decreases differentiation. In antler, BMP-2 acts as an autocrine factor in osteoblast proliferation as well as enhancing the expression of the structural proteins of the bone matrix, such as type-1 collagen, osteopontin, osteocalcin, and bone sialoprotein [44].

Osteoarthritis

Osteoarthritis (OA) is a true disease of cartilaginous disorder that affects over 50 million North Americans. Natural osteoarthritis occurs in human and a variety of animal species including horses, pigs, dogs and rabbits. It is characterized by loss of matrix proteoglycans, fibrillation of cartilage surface, and eventually loss of collagenous matrix to expose underlying bone. The etiopathogenesis of OA is probably related to mechanical wear, failure of the chondrocytes to maintain a balance of matrix synthesis and degradation, and extracartilaginous factors such as bony remodeling and synovium-mediated event [54].

Current therapy for patients with OA is primarily palliative and aimed at reducing pain, retarding inflammation, and maintaining joint function. Treatments currently in use have not been shown to modify the pathologic processes that occur in the tissue. Recently, a new concept has been advocated, in which the aim of treatment is to stimulate cartilage repair and, at the same time, inhibit cartilage breakdown. During the rapid growth and development of antler, there are numerous interactions between tissues and growth factors. Cells produce the matrix molecules which are similar with components consisting of articular cartilage found in human and animal [64]. Thus, understanding the mechanism of growth factors and matrix components is crucial to obtain a useful information to prevent or cure the osteoarthritis.

Conclusion

This review provided basic information on the growth and development of antlers. Antlers at 65～85 days of growth were histologically examined by light microscope. The distal tip section contained reserve mesenchymes adjacent to the dermis, prechondroblasts, chondroblasts and immature chondrocytes as shown in other reports. Zone of maturing and calcifying chondrocytes referred as upper section was invaded by osteoblasts indicating that antler growth undergoes both endochondral ossification and intramembranous ossification.

Growth factors as a possible pharmaceutical agent were found in growing antlers during the fast growth period. The tip section known as the growth center of antlers contains various growth promoters such as EGF, FGF, IGF-I, TGF, and others. During the endochondral ossification, BMPs are actively involved in antler growth and development. We conducted *in vitro* experiment whether water-soluble extract from antler tip sections may contain growth promoter (s) to stimulate the growth of bovine skin fibroblasts [60].

The opportunity for development of biomedicine using antlers appears vast with strong demand in North America and Europe as well as Asia. Thus, the future antler industry largely depends upon concerted R&D efforts among researchers (Government, Industry and University) by exploring the thousands years old medicinal claims of velvet antlers using modern scientific research tools, and by further developing biomedical products.

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