NOTE  Pathology

Ultrastructure of Aortic Elastic Fibers in Copper-Deficient Sika Deer (Cervus nippon Temminck)

Hiroyasu YOSHIKAWA1), Shueqin WANG2), Hirokazu SEO1), Tetsuro KUROTAKI1), Hideaki UEKI1) and Takashi YOSHIKAWA1)

1)Department of Veterinary Pathology, Faculty of Veterinary Medicine and Animal Sciences, Kitasato University, Towada City, Aomori 034–8628 Japan and 2)Department of Veterinary Medicine, Changchung University of Agriculture and Animal Sciences, Changchung, China

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ABSTRACT. Light microscopic and transmission and scanning electron microscopic observations were performed on the aortas of two 4- and 6-year-old deer affected with cervine ataxia and two 6-month- and 4-year-old healthy deer. Examination of the aortas from affected deer by transmission electron microscopy revealed the absence of distinct elastic laminae in the internal elastic lamina and tunica media, but discontinuous and irregular clumps of elastin were present. Scanning electron microscopy disclosed immature architecture of elastic fibers in the aortas from the copper-deficient deer, and the architecture was similar to that of a 6-month-old healthy deer.

KEY WORDS: aorta, copper deficiency, deer.

Cervine enzootic ataxia (CEA) is characterized by demyelination of the brain stem and spinal white matter and has been reported in the UK [16], U.S.A. [8], Australia [18], New Zealand [28], Sweden [20] and China [29]. The cause of the disease is suggested to be copper deficiency [7,19]. For cattle, pigs and chickens, on the other hand, aneurysm and arterial rupture due to degeneration of elastic fibers have been reported with copper deficiency [2, 7, 13, 14, 17, 26]. In order to clarify the pathogenesis of these arterial diseases in copper-deficient condition, fine structures of the aortas from deer affected with CEA were examined.

The animals were two 4- and 6-year-old Sika deer affected with CEA [29] and two healthy, 6-month- and 4-year-old deer. Aortic arch, thoracic aorta and abdominal aorta were examined.

Each part of the aortas was fixed in 10% buffered formalin, dehydrated in a graded alcohol, cleared in xylol, and embedded in paraffin wax. Tissue sections of 4–5 µm thickness were stained by hematoxylin and eosin (HE), toluidine blue and elastica Van Gieson’s stain (EVG). Scanning electron microscopic specimens were rinsed in several changes of two-fold diluted MacIlvaine’s phosphate-citrate buffer solution (pH 3.0), immersed in 0.5 or 1% tannic acid in the same buffer for approximately 2 hr, washed in several changes of distilled water and immersed in 2% OsO4 for approximately 2 hr. These specimens were dehydrated with ethanol in increasing concentrations, transferred to isoamyl acetate and dried in a critical point dryer with liquid CO2. All dried tissues were coated with platinum-paladium in an ion coater and examined under a scanning electron microscope (Hitachi S-450, HITACHI, Tokyo, Japan). Small tissue blocks of aortas for transmission electron microscopy were prefixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for approximately 1 day, postfixed for 1.5 hr with 1% OsO4 solution, dehydrated in a graded ethanol, and embedded in Epon 812. Semithin sections were stained with toluidine blue and observed with a light microscope. Ultrathin sections were stained with tannic acid, uranyl acetate and lead citrate, and examined in a transmission electron microscope (Hitachi H-7000, HITACHI, Tokyo, Japan).

In copper-deficient deer, the aortic wall was unevenly thickened, and elastic fibers in the tunica media were either fragmented and swollen or completely disappeared with only islands of swollen elastic fibers persisted (Fig. 1). The smooth muscle cells normally present between elastic lamellas were only sparsely distributed and contained metachromatic materials in the cytoplasm. Another feature of the affected aortas an abnormally large amount of collagen dispersed as thick wavy bundles throughout the wall.

Alterations elastic fibers were seen in aortas of all copper-deficient deer by transmission electron microscopy. The changes were observed in all tunicae, but varied in severity from one area to another in the same section. Elastic fibers appeared to have failed to coalesce to form the normal homogenous thick internal elastic lamina. The tunica media was characterized by the appearance of poorly formed lamellae of elastin (Fig. 2). They were discontinuous and irregular in thickness and surrounded by a prominent sheath of 100–110Å microfibrils. Between the lamellae were abnormally abundant collagen fibers, most of which were arranged in wavy bundles. In inner aortic media, several smooth muscle cells were interspersed among disorganized clumps of elastin and large bundles of collagen fibers.

In scanning electron microscopic specimens treated with tannic acid and osmium followed by critical point drying, the medial elastic laminae of 6-month-old healthy deer were approximately 1 µm thick and consisted of numerous microfibrils approximately 0.1–0.2 µm in diameter. Most of these microfibrils ran perpendicular to elastic laminae (Fig. 3a). The medial elastic laminae of 4-year-old healthy deer...
were increased in thickness, approximately 2–3 µm, and the microfibrils were attached to its smooth surface (Fig. 3b). In 6-year-old copper-deficient deer, on the other hand, elastic fibers in the tunica media remained thin and fine, arranged in an irregular and undulating manner, and surrounded by a reticular arrangement of thin and fine fibrils similar to those observed in the 6-month-old healthy deer (Fig. 3c).

Several reports have attributed the sudden death of pigs and chickens to arterial disorder in copper deficiency [7, 14, 26]. The mechanism has been reported to involve rupture of the arterial wall due to dysplasia of elastic fibers [7, 17, 26, 27]. No ultrastructural report has been available on the arteries of copper-deficient deer or their elastic fibrous disorder. Morphological changes such as fragmentation and irregular clumps of elastic fibers, as pointed out in the present study, are similar to the findings in pigs [4, 25, 26, 27], chickens [2, 15, 17, 21, 24], rats [12] and rabbit [13] in experimental copper deficiency. The major protein constituting elastic fibers of the aortic wall is elastin, which corresponds to 50% of the dry weight of the aorta [1]. Accordingly, this suggests that abnormality of elastin evolves a loss of arterial elasticity and an increase in fragility, which may induce circulatory disorder. An abnormality of the elastin architecture may be caused by the impaired activity of a Cu-containing enzyme (lysyl oxidase) involved in the formation of peptide cross-linking in the process of elastin synthesis [3, 5, 6, 9, 22, 23]. As observed by scanning electron microscopy, thinning and irregular arrangement of the arterial elastic fibers of the deer affected with CEA were similar to those observed in the 6-month-old healthy deer (Fig. 3c).

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REFERENCES


Fig. 3. (a) Six-month-old healthy deer: elastic fibers remain thin and fine, but a large amount of delicate and premature fibers adheres to their surface. (b) Four-year-old healthy deer: elastic fibers increase in thickness and stratify in a relatively regular manner. (c) Six-year-old CEA deer: elastic fibers remain thin and fine, arrange in an irregular and undulating manner, and surrounded by a reticular arrangement of thin and fine fibrils similar