An Aged Bull with Concurrent Thyroid C Cell Carcinoma, Adrenal Pheochromocytoma and Pituitary Chromophobe Adenoma

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ABSTRACT. A Japanese Black bull aged 20 years died following progressive loss of the body weight. Pathological examination disclosed multiple endocrine tumors including thyroid C cell carcinoma with metastases to the cervical lymph nodes and lung, adrenal pheochromocytoma and pituitary chromophobe adenoma in the pars distalis. The serum calcium content was as mildly low as 8.0 mg/dl at the terminal stage. The bull had daily ingested the ration containing 1.9 times the recommended calcium amount for 8 years and 120,000 units of vitamin D₃ for 5 years prior to death. The long-term dietary intake of moderately excessive calcium with vitamin D₃ might be related to the pathogenesis of the thyroid C cell carcinoma.

KEY WORDS: bull, multiple endocrine tumors, thyroid C cell carcinoma.

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Multiple endocrine neoplasia syndrome in humans that has a genetic basis as the etiology, is characterized by concurrence of neoplastic and hyperplastic proliferation within two or more endocrine glands including the thyroid gland and adrenal medulla, and thought to represent a simultaneous neoplastic transformation of multiple endocrine cell populations of neural crest origin [9].

Similar syndrome occurs frequently in populations of aged bulls developing thyroid C cell tumors and pheochromocytoma [18], occasionally accompanied by pituitary adenoma [1]. The thyroid tumors in bulls are also designated as ultimobranthial tumors, since the tumors are suggested to be derived from remnants and cell cords of the ultimobranthial body that can differentiate into both C cells and follicular cells [5, 10]. Although the etiology of the tumors is unknown, a possible relationship has been suggested between the long-term dietary intake of excessive calcium and the high incidence of the tumors in bulls [11].

To our knowledge, there are no reports on multiple endocrine tumors in aged bulls in Japan. This report describes clinicopathological and epidemiological findings of an aged bull affected with concurrent tumors of the thyroid, adrenal medulla and pituitary glands.

A Japanese Black bull aged 20 years in a bull center for artificial insemination in Iwate Prefecture died following the disease course of one year with progressive loss of the body weight. During the period the bull developed swelling of the right tarsocrural joint in January 2003, swelling of the left antebrachiocondylar joint and difficulty in standing in May and extensive edema in the subcutaneous tissue of the right pelvic limb in September, followed by inability in standing

in November and death in December. The animal did not respond to repeated administrations of antibiotics during the disease course. The average daily calcium content with standard deviation was $64.5 \text{ g} \pm 12.6$ in the ration fed to the bull during the period from the age of 12 to 20 years, and corresponded to 1.9 times the recommended calcium level for maintenance of bulls [13]. Vitamin D₃ of 120,000 units had been daily added to the ration from the age of 15 to 20 years. No tumors of the thyroid gland or adrenal medulla had been found in descendants of the bull.

Serum samples were gained from the examined bull in December 2002 and November 2003 when it was one month prior to death, and the serum calcium content was determined using an auto dry chemistry analyzer (Spotchem SP-4410, Arkray, Kyoto). The content decreased mildly from 9.3 mg in the former to 8.0 mg/dl in the latter.

Macroscopically, multiple tumors were found in the thyroid, adrenal medulla and pituitary glands. The entire thyroid gland weighing 109 g was almost completely incorporated by multiple tan nodules 5 to 20 mm in diameter and firm in consistency (Fig. 1). The normal thyroid tissues were markedly compressed and remained in the peripheral areas of the lobes and the internodular areas. The ventral cervical lymph nodes, including the cranial and middle deep cervical lymph nodes, were enlarged from 10 to 35 mm in diameter, and yellowish in color, replacing the original structure. Several discrete tan nodules 4 to 8 mm in diameter were also scattered in the cranial and middle pulmonary lobes.

Both adrenal glands were mildly enlarged with expansion of the medullary area. The left medulla contained a single well-defined nodule that was yellowish red in color and 8 mm in diameter. The architecture and volume of the cortical areas remained within normal range. The pituitary gland was mildly enlarged with a single nodule within the pars dis-

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Fig. 1. Extensive multinodular enlargement is seen in both thyroid lobes and isthmus.

tal. The nodule was yellowish brown in color and 7 mm in diameter.

In addition to the neoplastic lesions, there were chronic purulent periarthritis in the right tarsocrural and left antebrachicarpal joints, myocellulitis in the shoulder and posterior trunk and calcinosis in the aortic arch and abdominal aorta. No osteopetrosis was found in the vertebral column.

Tissue samples collected throughout the body were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections of tissue were stained with hematoxylin and eosin (HE). Sections from the adrenal medulla or pituitary glands were stained with Grimelius' method or aldehyde-thionin-periodic acid Schiff-orange G. Selected sections including the liver, kidney, thyroid, adrenal and pituitary

glands were stained with Congo red as well as the streptavidin-biotin-peroxidase (SAB) method. The SAB method was applied using a SAB kit (Nichirei Co., Tokyo) and monoclonal anti-AA amyloid (KYOWA, Tokyo), polyclonal rabbit anti-calcitonin (DakoCytomation, Kyoto) or polyclonal rabbit anti-adrenocorticotrophic hormone (ACTH, Advance Biofactures Co., New York, NY, U.S.A.) antibodies, as described previously [8, 19].

Histologically, many tumor nodules in the thyroid gland were present as discrete and expansive masses that were well circumscribed or partially encapsulated by a connective tissue. The tumor cells in the nodules were subdivided into discrete groups by connective tissue septa with capillaries. The predominant tumor cells in most nodules with high cellularity were polyhedral to spindle shaped with scanty cytoplasm that was lightly eosinophilic and indistinctly outlined. The oval or elongate nuclei had one or more nucleoli and evenly distributed chromatin (Fig. 2A). The tumor cells in other nodules with moderate cellularity were cuboidal or columnar with spherical or oval nucleus and abundant, lightly eosinophilic and finely granular cytoplasm, and occasionally assumed acinar structures without colloid-like materials. Mitotic figures were rare in both types of the tumor cells. Multiple foci of C cell hyperplasia were seen in the remaining normal thyroid tissues. A histological pattern similar to that of the thyroid tumor was also found in the metastatic lesions in the cervical lymph nodes and lung. Calcitonin antigens were present in the cytoplasm of tumor cells in the thyroid gland (Fig. 2A, inset) and metastatic lesions, as well as of hyperplastic C cells.

The adrenal tumor was confined to the left medulla and

Fig. 2. A) The tumor cells in the thyroid gland are subdivided by fibrous connective tissue septa and are polyhedral to spindle. HE stain. $\times 165$. Inset shows calcitonin antigens (arrowheads) in the cytoplasm of tumor cells. SAB method. $\times 660$. B) The tumor (right side) in the adrenal medulla is incompletely encapsulated by a fine connective tissue from the adjacent cells (left side), and consists of oval to slender tumor cells. HE stain. $\times 165$. C) The tumor cells in the pituitary gland are subdivided by capillaries and fine connective tissues and are cuboidal or polyhedral. HE stain. $\times 165$.

encapsulated by a fine and incomplete connective tissue with compression of the adjacent cells. The tumor cells were subdivided into small lobules by fine connective tissue septa and capillaries often distended with erythrocytes. The tumor cells were positive for Grimelius' method and varied from oval to slender shaped, predominant in the latter (Fig. 2B). The cytoplasm was lightly basophilic or amphophilic, finely granulated and indistinct in the outer boundary. The nucleus was rather rich in chromatin with one or two nucleoli. Mitotic figures were rare.

A multifocal hyperplasia was found in both adrenal medulla. The hyperplastic lesions were well demarcated from the adjacent tissue with slight compression, but not encapsulated and not so highly cellular as the tumor. The morphology and architectural arrangement of the hyperplastic cells resembled those of the tumor cells described above. No morphological abnormalities were observed in the adrenal cortical cells.

The tumor within the pars distalis of the pituitary gland was demarcated by a fine and incomplete connective tissue from the adjacent normal parenchyma with mild compression. The tumor cells were subdivided into small packets by many capillaries and fine connective tissues. The tumor cells were cuboidal or polyhedral with indistinctly demarcated cytoplasm (Fig. 2C). The cytoplasm was lightly eosinophilic and finely granular, but devoid of distinctive secretory granules and ACTH antigens with the applied stains. The nucleus was round or oval and hypochromatic with one or two prominent nucleoli. A few mitotic figures were seen. Acidophils were scattered within the tumor near the margin. The hypothalamus and thalamus were neither compressed nor invaded by the pituitary tumor.

There were moderate or mild amyloid deposits, positive for Congo red stain and AA amyloid antigen, in the renal glomeruli and splenic follicles, as well as the spaces of Disse in the liver and small vascular walls in the organs and tissues throughout the body. Amyloid was also deposited in the stroma of the remaining normal thyroid tissues, but not within the tumor. No parathyroid glands were examined histologically.

The present bull aged 20 years was affected with multiple endocrine tumors in the thyroid, adrenal medulla and pituitary glands, accompanied by hyperplasia of the thyroid C cells and adrenal medullary cells. Based on the criteria reported elsewhere [1–4, 6, 10], observed tumors were diagnosed as thyroid C cell carcinoma with metastases to the cervical lymph nodes and lung, adrenal pheochromocytoma and pituitary chromophobe adenoma in the pars distalis, respectively. The concurrence of the neoplastic and hyperplastic lesions within the thyroid gland and adrenal medulla appears to indicate the developmental process from hyperplasia to tumors, as described previously [4, 18, 20].

Functional chromophobe adenomas in the pituitary gland with hyperadrenocorticism are frequently found in dogs [7], but infrequently in other animal species [3, 15]. The present pituitary adenoma might be nonfunctional since there were no pathological evidences, such as presence of ACTH anti-

gens in the tumor cells and hypertrophy and hyperplasia of the adrenal cortical cells, although quantitation of blood levels of the major pituitary hormones is necessary to exact endocrinological evaluation of the tumor [2].

The amyloid often localized within thyroid C cell tumors is suggested to be produced by the tumor cell [17, 18] and to be chemically different from AA amyloid associated with chronic inflammatory processes [5, 14]. In the present bull with purulent periartthritis of the legs, amyloid deposits, positive for AA amyloid antigen, were observed in several organs and small vascular walls throughout the body including the remaining normal thyroid tissues, but not within the thyroid tumor. These results may suggest that the present case was also involved in systemic AA amyloidosis.

Serum calcium levels in aged bulls with calcitonin secreting thyroid tumors are slightly lower than in control bulls [1, 6]. The nearly normal calcium value is guessed to be possibly a result of the low turnover rate of bone in aged bulls and the compensatory mechanisms of other endocrine organs [5]. Serum calcium content in the present bull was as mildly low as 8.0 mg/dl one month prior to death. The level might be a reflection of disturbance of the compensatory mechanisms at the terminal stage.

The previous bulls with thyroid C cell tumors had ingested more than 3.5 times the recommended calcium amount for a long time [11], while the present bull had continuously ingested 1.9 times the recommended calcium amount and 120,000 units of vitamin D₃ that facilitated the intestinal absorption of calcium [12]. No thyroid tumors had been seen in the offspring of the present bull, although the occurrence pattern of autosomal dominant inheritance of the thyroid tumors is suggested in a family of Guernsey bulls [16]. It seems likely that the long-term dietary intake of moderately excessive calcium with vitamin D₃ might be related to the pathogenesis of the present thyroid C cell carcinoma. Further studies will be needed to accumulate knowledge of diseases limiting the usefulness of bulls kept in Japan.

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