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Case Report

Spontaneous Vacuolar Degeneration of the Thyroid Follicular Epithelium in Cynomolgus Monkeys

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Abstract: Vacuolar degeneration of the thyroid follicular epithelium was observed in two untreated female cynomolgus monkeys assigned to control groups. In light microscopy, large vacuoles containing a homogenous substance occupied the basal region of the epithelium, and the nuclei had shifted toward the apical region. The vacuoles showed negative reactions to PAS and thyroglobulin. Electron microscopic observation revealed dilatation of the rough endoplasmic reticulum corresponding to the vacuoles. The plasma TSH, T3 and T4 levels determined for the samples kept frozen were within the normal ranges, suggesting that the thyroid function was kept intact. (DOI: 10.1293/tox.24.229; J Toxicol Pathol 2011; 24: 229–232)

Key words: vacuole, rough endoplasmic reticulum, thyroid epithelium, cynomolgus monkey

Cynomolgus monkeys (*Macaca fascicularis*) are widely used in preclinical toxicology studies due to their phylogenic relationship to humans. Since a limited number of animals are used in nonrodent toxicity studies, it is difficult to distinguish test-compound-related lesions from spontaneously occurring changes. A vacuolar change in thyroid follicular cells is known to be one of the spontaneous changes in Wistar Hannover GALAS rats[1–3], and it has been observed by electron microscopically, as dilatation of the rough endoplasmic reticulum (r-ER). Furthermore, similar findings have been reported in mice[4], rats[5,6] and humans[7,8] with congenital goitar and hypothyroidism. To the best of our knowledge, little information is available on naturally occurring lesions of the thyroid gland in nonhuman primates. In histopathological examinations of cynomolgus monkeys in toxicity studies, severe vacuolar degeneration of the thyroid follicular epithelium was observed in 2 out of 182 females (1.01%) and 0 out of 186 males assigned to control groups. This case report describes histopathological, immunohistochemical and electronmicroscopic characteristics of the vacuolar changes in the thyroid follicular epithelial cells. Further hormonal determination was also conducted on one of these animals.

All animals were obtained from the Primate Quality Control Center, Ina Research Philippines, Inc., and were 2–3 yrs old at necropsy. The animals were necropsied routinely after exsanguination under anesthesia by intravenous injection of pentobarbital. The first (Case 1, age: 2 yrs) of the two female monkeys was treated subcutaneously with a vehicle (saline) for 2 weeks, and the second monkey (Case 2, age: 2 yrs) was treated orally with a vehicle, a mixture of 0.2 w/v% polyoxyethylene hydrogenated castor oil 60 (HCO-60) and 0.5 w/v% sodium carboxymethyl cellulose (CMC), for 13 weeks. The thyroid glands and other organs were fixed in 10% neutral-buffered formalin and processed routinely into histological sections. Thyroid sections were stained with hematoxylin and eosin (H.E.), periodic acid-Schiff (PAS) and an immunohistochemical marker for determination of thyroglobulin (rabbit anti-human thyroglobulin polyclonal antibody, Dako Japan Inc.) by the peroxidase-labeled polymer method using an EnVision kit (Dako Japan Inc.). For electronmicroscopic examination of these animals, several tissue masses were dissected from the formalin-fixed thyroid glands. Then they were refixed with 2.5% glutaraldehyde and 1% osmium tetroxide, embedded in epoxy resin, sectioned and stained with 1% uranyl acetate and lead citrate. In one of these two animals (Case 2) and in 4 intact animals (age: 2 yrs) in the control group of the same toxicity study, the thyroid stimulating hormone (TSH), triiodothyronine (T3) and tetraiodothyronine (T4) levels in plasma were determined in samples obtained at week 7 and 13 of the treatment period using a chemiluminescent immunoassay

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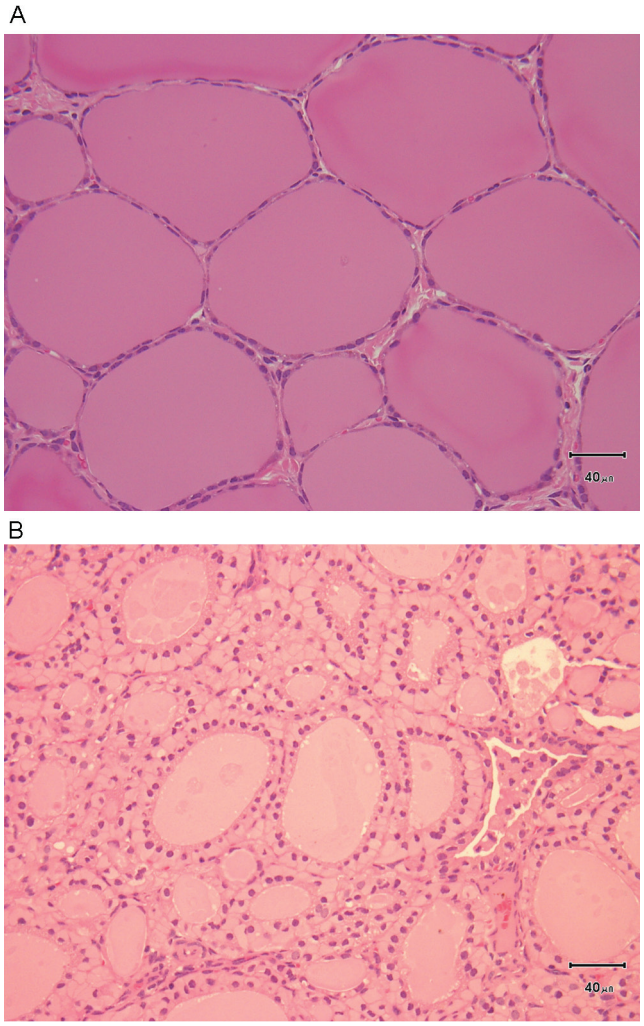

Fig. 1. Thyroid gland from an intact cynomolgus monkey (A) and a monkey in the control group exhibiting changes (B: Case 1). A large vacuole is observed in the basal region of each follicular epithelial cell, and the nuclei are characteristically located in the apical region adjacent to the follicular lumina (B). H.E. staining. The scale bars indicate 40 μm.

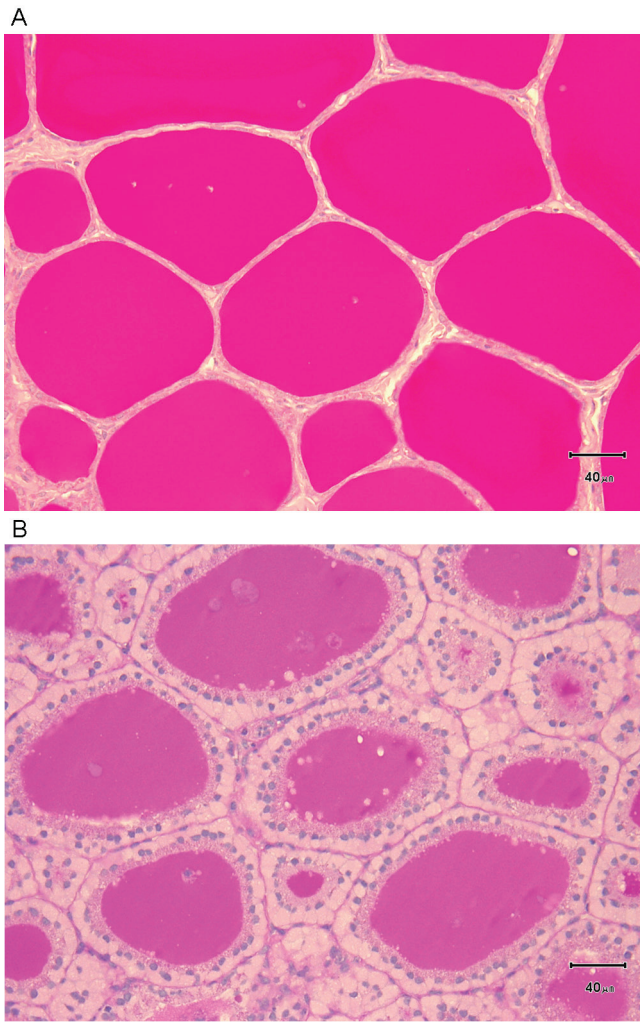

Fig. 2. Thyroid gland from an intact cynomolgus monkey (A) and a monkey in the control group exhibiting changes (B: Case 2). The colloid reacted positively (A, B), and the vacuoles reacted negatively (B). PAS reaction. The scale bars indicate 40 μm.

kit (Chemilumi ACS-TSH III, T3 and T4, Siemens Medical Solutions Diagnostics, Inc.). The studies were conducted in compliance with the "Partial Amendments to the Law for the Humane Treatment and Management of Animals (Law No. 68, Jun. 22, 2005, Japan)" and the "Guidance for Animal Care and Use" of Ina Research Inc. and in accordance with protocols reviewed by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc., which is fully accredited by AAALAC International (Accredited Unit No. 001107).

In the two monkeys, no macroscopic abnormalities in the thyroid glands were observed at necropsy, and the organ weights were within the normal range (Table 1). In light microscopy, a large vacuole was observed at the basal region of each follicular epithelial cell and was compressing the nuclei toward the apical region. As a result, the nuclei were characteristically located in the apical region adjacent to the follicular lumina (Fig. 1). An homogeneous substance was observed in the vacuoles, and the substance showed negative reactions to PAS (Fig. 2) and anti-thyroglobulin immunostaining (Fig. 3). Electron microscopy revealed that the dilatation of the r-ER corresponded to the vacuoles observed in light microscopy (Fig. 4). The plasma levels of TSH, T3 and T4 in the monkey with thyroid changes were not apparently different from the other control values (Table 2). In addition, no abnormalities were seen macroscopically or microscopically in any other organs including the pituitary glands of these two females.

Large vacuoles at the basal region have been reported in female Fisher 344 rats[5] and Wistar Hannover GALAS rats of both sexes[1–3]. The vacuolar degeneration reported here was very similar to the findings that vacuoles reacted negatively to PAS and thyroglobulin in the reported rat cases[1,5]. On the other hand, dilatation of r-ER in the follicular

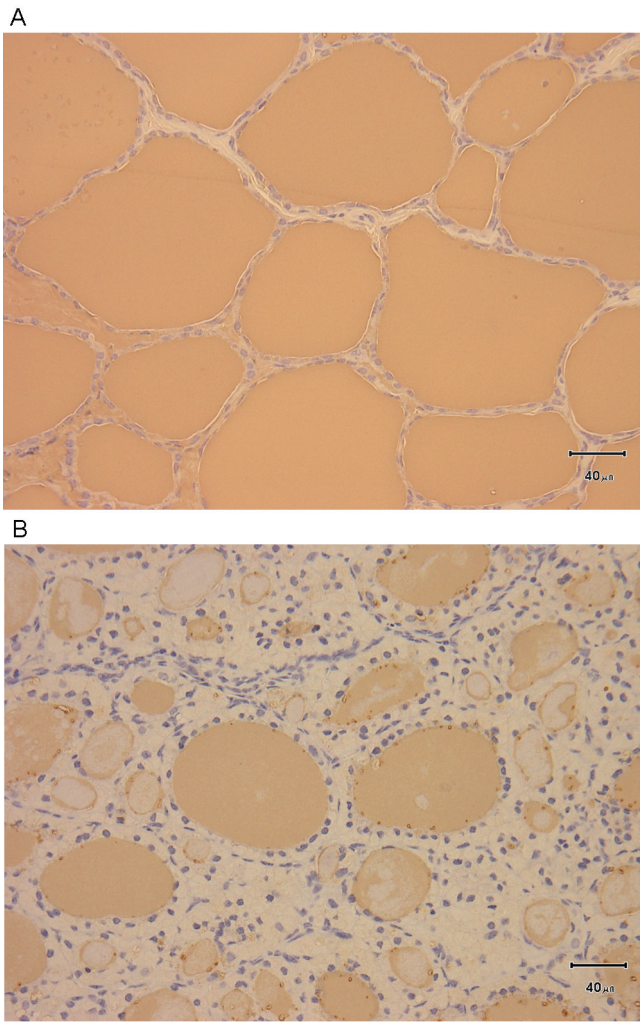

Fig. 3. Thyroglobulin immunohistochemistry of the thyroid gland from an intact cynomolgus monkey (A) and a monkey in the control group exhibiting changes (B: Case 1). The colloid reacted positively (A, B), and the vacuoles reacted negatively for thyroglobulin (B). The scale bars indicate 40 μm.

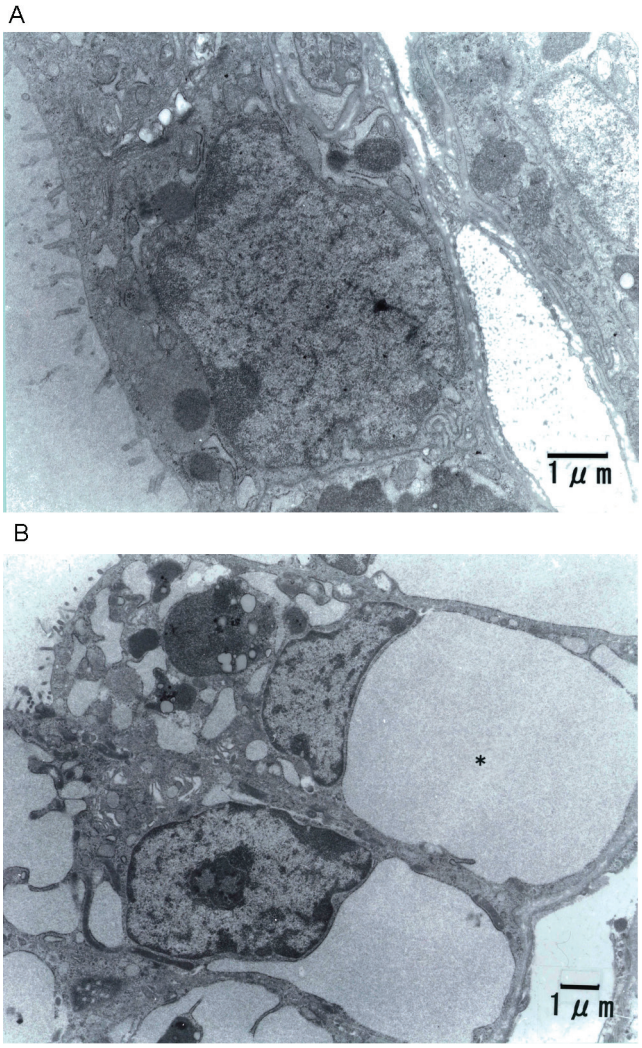

Fig. 4. Electronmicrographs of the thyroid follicular cells from an intact cynomolgus monkey (A) and a monkey in the control group exhibiting changes (B: Case 1). In the basal portion, the dilatation of the rough endoplasmic reticulum (r-ER), corresponding to the vacuoles (*), observed by light microscopy is remarkable. The scale bars indicate 1 μm.

Table 1. Body and Thyroid Weights

Group	Body weights (kg)	Thyroid weights (mg)	
		Left	Right
Control (n = 23 [a])	2.42 ± 0.22 [b]	189 ± 66 [b]	183 ± 66 [b]
Case 1	2.70	151	167
Case 2	2.60	141	168

[a] Twenty-three out of the 182 female control animals were weighed.
[b] Mean ± SD

epithelium has been reported in animals with genetic hypothyroidism[2–4,6,9]. However, this differed from the reports that vacuoles reacted positively to thyroglobulin in rats with hypothyroidism[2,3]. A large amount of r-ER is present at the basal region of the follicular epithelium and synthesizes thyroglobulin. Dilatation of the r-ER might be the process through which thyroid hormone synthesis or secretion was affected. However, the plasma TSH, T3 and T4 levels in the monkeys were within the normal ranges in the present study. Furthermore, no abnormalities were seen in blood biochemistry or organ and body weights. The animals were found to be healthy in clinical observations. From these results, it is suggested that the function of the thyroid glands with vacuolar degeneration was maintained to a degree that still allowed the animals to remain in a healthy condition. It is uncertain whether vacuolar degeneration is part of a process leading to further cell lesion or merely a morphological change remaining in a stable state. It is also unknown whether the change originated genetically or was brought about by other causes.

We believe this case report provides useful information for the practice of histopathology in toxicity studies. It is important to gain a wide knowledge of spontaneous lesions

Table 2. Plasma TSH and Thyroid Hormone (T3, T4) Levels

	Weeks [a]	Case 2	Intact animals in the control group (n=4)				
			Animal Nos.				Mean ± SD
			1	2	3	4	
TSH (μIU/mL)	7	2.1	0.9	0.7	1.2	2.6	1.4 ± 0.9
	13	1.6	0.5	1.5	0.6	2.2	1.2 ± 0.8
T3 (ng/dL)	7	187	197	190	187	204	195 ± 8
	13	203	186	217	168	179	188 ± 21
T4 (μg/dL)	7	4.0	6.1	4.1	5.0	3.5	4.7 ± 1.1
	13	3.3	5.0	3.3	5.0	3.3	4.2 ± 1.0

[a] Blood samples were collected and analyzed at weeks 7 and 13 in the treatment period of the corresponding study.

in the monkey in order to make a correct toxic assessment when animals randomly assigned to drug treatment groups exhibit marked changes.

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