Case Report

Vacuolar Change in the Thyroid Follicular Cells in BrlHan: WIST@Jcl (GALAS) Rats

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Abstract: Huge vacuoles in the follicular epithelial cells distributing diffusely in the thyroid gland were seen in 2 out of 36 males and 8 out of 82 females of BrlHan: WIST@Jcl (GALAS) rats examined histopathologically. The vacuoles showed negative reactivity by periodic acid-Schiff reaction and thyroglobulin-immunohistochemistry. Corresponding to the vacuoles, remarkable dilatation of rough endoplasmic reticulum was seen in electron microscopic observation. The animals in which the thyroid lesion was found had shown no abnormalities in behavior, body weight, food consumption, or hematological and biochemical examinations. Plasma concentrations of thyroid-stimulating hormone (TSH) and thyroid hormones (T3 and T4) were not different from those of normal rats. From these results, thyroid functions seemed to be kept normal in spite of the severe morphological change. Based on the character and incidence of the thyroid lesion, it was presumed to be related with a certain genetic factor of one or several parents of breeding rats rather than food contamination or infection. (*J Toxicol Pathol* 2001; **14**: 253–257)

Key words: rough endoplasmic reticulum, vacuole, thyroid gland, BrlHan: WIST@Jcl (GALAS) rats

BrlHan: WIST@Jcl (GALAS) rats, so called Wistar Hannover GALAS rats, have been used in toxicity studies in Europe. This strain has several beneficial characters such as a higher survival rate and a smaller body weight than those of other strains, for instance, Sprague-Dawley, Wistar, and Fischer 344 rats. A higher survival rate (about 80% for 2 years) is advantageous for long term-toxicity studies, and a smaller body weight is worthy to use in preliminary toxicity studies of new drugs when the quantity of compounds is limited. In our laboratory, this strain has been recently used in preliminary toxicity studies. In the studies, we found a lesion of the thyroid gland in 2 out of 36 males and 8 out of 82 females. One female bearing the lesion was from non-treated groups. We considered the lesion as a spontaneous change since the quality and the degree were similar to that in the non-treated animal.

All animals were supplied from CLEA, Japan, Inc. at 5 weeks of age. Animals examined were from 6 lots (4 lots for

4 preliminary toxicity studies and 2 lots for the accumulation of background data at 8 weeks of age). They were housed in stainless steel cages in a room maintained at $23 \pm 2^\circ\text{C}$, $55 \pm 15\%$ relative humidity and a 12-hr-light and -dark cycle, and had free access to a standard laboratory diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and tap water. Blood samples were withdrawn from the abdominal aorta under sodium pentobarbital anesthesia and the animals were killed by exsanguination at 8 weeks of age.

Many organs including the thyroid gland were removed and weighed (the organs of one rat was not weighed). The organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H.E.) for light microscopic observation. In addition, sections of the thyroid and pituitary glands were processed for periodic acid-Schiff (PAS) reaction. In 6 normal rats and 8 rats with thyroid lesion, immunohistochemical staining was performed on sections of the thyroid gland for thyroglobulin (rabbit anti human thyroglobulin polyclonal antibody, DAKO Japan Co., Kyoto, Japan) and on sections of the pituitary gland for thyroid-stimulating hormone (TSH; mouse anti human TSH monoclonal antibody, NICHIREI Co., Tokyo, Japan) by a streptavidin-biotin complex method. In 2 rats with thyroid lesion, small pieces from a lobe of the thyroid gland were

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fixed in 2% glutaraldehyde, post-fixed in 1% osmium acid, and embedded in Epok 412 for electron microscopic observation. Plasma concentrations of TSH and thyroid hormones (T3 and T4) were measured in the 14 rats by using radioimmunoassay-kit (Spac-S TSH Kit, TFB Inc., Tokyo, Japan) and ELISA-kits (Total (T3) EIA Kit and Total (T4) EIA Kit, ICN, Orangeburg, New York), respectively.

The thyroid lesion was found in 4 out of 6 lots. In any rats bearing the thyroid lesions (thyroid-lesion rats, hereafter), no abnormalities were seen in behavior, body weight, food consumption, or hematological and

biochemical examinations except for the increase in the thyroid organ weights (Table 1). Plasma concentrations of TSH and the thyroid hormones showed no differences between the thyroid-lesion rats and normal rats (Table 2). In macroscopic observation, both lobes of the thyroid gland were enlarged. In microscopic observation, huge vacuoles in the follicular epithelial cells were distributed diffusely in the thyroid gland. The vacuoles contained slightly eosinophilic substance and were located at the basal site in the follicular cells. They showed negative reactivity by PAS reaction (Fig 1-d), and immunohistochemistry for thyroglobulin (Fig 1-f).

Table 1. Absolute Organ Weight of the Thyroid Gland in the Rats with the Thyroid Lesion

Sex	Group	Animal No.	Thyroid weight (mg)
Male	Normal (n=7)	—	18.9 ± 7.2
	With the thyroid lesion (n=2)	4	39.3
		5	35.4
			37.4 ± 2.8
Female	Normal (n=17)	—	14.5 ± 2.7
	With the thyroid lesion (n=7)	9	31.2
		10	34.3
		11	31.8
		12	24.4
		13	30.6
		14	29.1
		15	26.5
			29.7 ± 3.3

Mean ± SD.

Table 2. Plasma TSH and Thyroid Hormone Levels (T4 and T3) in the Rats with the Thyroid Lesion

Sex	Group	Animal No.	TSH (μU/ml)	T4 (ng/ml)	T3 (ng/ml)
Male	Normal (n=3)	1	<0.1	39	1.7
		2	<0.1	39	1.6
		3	<0.1	52	1.8
	With the thyroid lesion (n=2)		<0.1	43 ± 8	1.7 ± 0.1
		4	<0.1	43	2.0
		5	<0.1	41	2.3
Female	Normal (n=3)		<0.1	42 ± 2	2.2 ± 0.2
		6	<0.1	50	1.7
		7	<0.1	37	1.5
	With the thyroid lesion (n=6)	8	<0.1	60	2.2
			<0.1	49 ± 11	1.8 ± 0.4
		9	<0.1	41	1.7
		10	<0.1	46	1.9
		11	<0.1	49	1.8
		12	<0.1	56	2.0
		13	<0.1	37	2.4
		14	<0.1	41	2.0
			<0.1	45 ± 7	2.0 ± 0.3

Mean ± SD.

Fig. 1. Thyroid gland from a normal rat (a, c, e) and a rat with the lesion (b, d, f). (a); Normal thyroid. (H.E., $\times 200$). (b); In the thyroid with the lesion, huge vacuoles are seen in the follicular epithelial cells, and the nuclei are located in the apical portion. The cell height is increased compared with that of normal thyroid (Fig. 1-a). (H.E., $\times 200$). (c); In the normal thyroid, the colloid is positive for PAS reaction. (PAS, $\times 200$). (d); The vacuoles are negative for PAS reaction. (PAS, $\times 200$). (e); The follicular epithelial cells and colloid are positive for thyroglobulin. (thyroglobulin stain, $\times 200$). (f); The vacuoles are negative for thyroglobulin. (thyroglobulin stain, $\times 200$).

The nuclei of the cells were shifted toward the luminal side and the cell height was increased compared with that of the normal follicular epithelial cells (Fig 1-b). The amount of the luminal colloid seemed not to be changed compared with

that of normal animals. The degree of the lesion was mostly similar among the thyroid-lesion rats.

In electron microscopic observation, rough endoplasmic reticulum (r-ER) was dilated extensively at the

Fig. 2. Electron micrographs of the thyroid glands in the rats with the lesion. a: In the basal portion, dilatation of rough endoplasmic reticulum (r-ER) is remarkable. (Bar=2 μ m). b: In the apical portion, no abnormalities except slight dilatation of r-ER are seen. (Bar=1 μ m).

basal site in the follicular epithelial cells (Fig 2-a). At the apical portion, there were no apparent changes except for slight dilatation of r-ER (Fig 2-b).

The number of TSH-positive cells in the pituitary gland was not changed compared with normal animals. In addition, no changes were found in any other organs than the thyroid gland in the histopathological examination performed in routine toxicity studies. Therefore, we consider that the thyroid functions in the thyroid-lesion rats were kept normal at 8 weeks of age when the animals were sacrificed.

Takaoka *et al.*¹ have reported a spontaneous case with dilated r-ER in thyroid follicular cells of a female Fischer 344 rat. In their observation, the rat showed no abnormalities in clinical signs, macroscopic pathology, hematology, or biochemistry, but the slight increases in body weight and the weights of the thyroid and pituitary glands. They have reported the swelling of the follicular cells with eosinophilic cytoplasm, the shifting of the nucleus toward the luminal side, and the dilatation of r-ER. They concluded that the thyroid functions in the rat was normal since no changes were observed in the amount of the colloid, TSH staining of the pituitary gland, or the electron microscopy except the dilatation of r-ER. This thyroid lesion was mostly similar to our findings. However, in our observation, the thyroid weight was increased remarkably and the body weight was unchanged compared with the normal animals.

Takaoka *et al.*¹ defined the thyroid lesion as intracytoplasmic edema since the dilatation of r-ER is caused by mild injuries with changes in ion and water movement, although they suggested that the substance contained in the dilated r-ER might be the precursory proteins of thyroglobulin. By their explanation, it is not clear whether the cause of r-ER dilatation is due to the change of water movement or the retention of the precursory proteins. We think it is hard to conclude that the changes in ion and water movement had occurred only from the observation of the dilatation of r-ER. Therefore, we consider that it is appropriate to describe the dilatation of r-ER objectively as vacuolar change in the light microscopic finding.

The dilatation of r-ER in the follicular epithelial cells has been reported in the animals with genetical hypothyroidism (the *rdw* rat², the *hyt/hyt* mouse³, the *cog/cog* mouse⁴). In the *rdw* rats, huge vacuoles were observed at the basal site in the follicular epithelial cells, and the nucleus was shifted toward the luminal side². These changes are similar to our findings, however, in the *rdw* rats, the decrease in the thyroid weight, the small follicular lumen

without thyroglobulin, and the lowered level of thyroid hormones with elevation of TSH in serum have been reported. None of these changes were seen in our observation.

In the dilated r-ER of the *rdw* rats, the presence of thyroglobulin has been observed². However, thyroglobulin was not detected in the dilated r-ER by immunohistochemical staining in our observation. The dilatation of r-ER is generally considered to occur due to 1) a rate of synthesis of secretory products in excess of that which can be handled by the transport mechanism, 2) a defect in the transport system, such as a mechanical or enzymic abnormality in the r-ER that hinders the egress of normal amounts of secretory material, and 3) the production of an abnormal secretory product with which the normal transport mechanism is unable to cope⁵. From our results, it is hard to estimate the substance contained in the dilated r-ER or, furthermore, the cause of r-ER dilatation.

As the cause of the thyroid lesion, food contamination or infection was considered not to be feasible since the lesion was seen in a similar degree and any morphological changes indicative to progress to the vacuolation were not seen in the thyroid gland of remaining rats. Furthermore, the incidence of the lesion was relatively low as 8.5% (10/118) for considering it was induced by the above causes. From these facts, a certain genetic factor of one or several parents of breeding rats is thought to be related.

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