EFFECTS OF SULFONAMIDE ON THE PITUITARY-THYROID GLAND

1. MORPHOLOGICAL CHANGES OF THYROID GLAND AND VARIATION IN PLASMA THYROXINE AND TRIOIODOTHYRONINE

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Accepted March 7, 1983

Abstract—Morphological changes of the thyroid gland on light and electron microscopy as well as concomitant variation in plasma T₃ and T₄ levels after oral treatment with sulfonamide at a dose of 2 g/kg for 30 consecutive days were examined in male Wistar rats. Several rats were treated with the same dose by the same route for 15 days and then put to the restoration experiment for 15 days.

Plasma T₃ and T₄ levels significantly decreased as compared with the control in the period from the 1st day to the 30th day of the administration. A significant increase in thyroid weight was seen from the 5th day. On the 30th day, the weight was at last about eight times as heavy as that of the control. Histologically, hyperplasia of the follicles which were lined with high columnar cells was observed. The epithelial cells showed considerable pleomorphism and colloid was virtually absent at this time.

As early as on the 3rd or 5th day, the follicular cells were high-columnar in shape on electron microscopy and there was an increase in rough-surface endoplasmic reticulum, the cisternae of which were dilated. Swelling of mitochondria and the absence of colloid were also noted. There were no secretory granules in the cytoplasm and numerous long microvilli projected into the lumens. On the 30th day, the follicular cells became remarkably high-columnar and had small and ovoid nuclei at their base. In the restoration study, numerous dense secretory granules and colloid droplet profiles appeared in the cytoplasm.

Key words: Sulfonamide, thyroid gland, T₃, T₄, rat
INTRODUCTION

It was reported by Mackenzie, J. B. et al. (1941) and Mackenzie, C. G. et al. (1943) that sulfonamides produced thyroid gland hyperemia and enlargement in rats, mice, and dogs. In these studies, they observed a reduction in colloid and an increase in the height of the follicular epithelium. Similar changes were also shown after oral treatment of rats with various sulfonamides (Astwood et al., 1943). Swarm et al. (1973) informed that the rats receiving sulfamethoxazole had nodularity of or adenoma formation in the thyroid gland after 52 or 60 weeks. However, ultrastructural study of the effect of sulfonamide on the thyroid gland of rat has not yet been made.

On the other hand, it is well known that the administration of sulfonamide to rats inhibits the activity of thyroid iodide peroxidase and then the synthesis of thyroid hormone in the thyroid follicular cells (Yamamoto, 1966).

The author investigated the correlation between the light and electron microscopic observations of thyroid gland and the variation in plasma thyroxine and triiodothyronine in male Wistar rats following high-dose consecutive administration of sulfonamide.

MATERIALS AND METHODS

Male Wistar rats were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals. Following the one-week preliminary holding period, healthy and wellgrown rats of 5 weeks (weighing 110±10 g) were selected for this experiment. The animals were housed in metal cages, five in each cage, and maintained in an air-conditioned room (temperature : 23±2°C; humidity : 55±5%). Food (Funahashi Farm) and tap water were accessible ad libitum. Sulfadimethoxine (ST) was used after being suspended in 0.5% aqueous carboxymethyl-cellulose (CMC). The first group was given an oral treatment of ST at a dose of 2 g/kg every day (ST-treated group). In order to investigate reversibility, the second group was given an oral treatment of ST at the same dose for 15 days and then put to the restoration experiment for the equal number of days (restoration group). The third group was given daily 1 ml of 0.5% CMC/100 g B. W. by the same injection route (control group). All rats were weighed daily.

On the 1st, 3rd, 5th, 7th, 10th, 15th, 20th, 25th, and 30th day, 6 rats of the first group were anesthetized with ethyl ether and blood was collected from the femoral vein. For light microscopy, materials were fixed with 10% buffered formalin and embedded in paraffin. Thin sections were stained with hematoxylin eosin (HE), periodic acid-Schiff (PAS) and Azan Mallory (AZAN).

For electron microscopy, small pieces of the thyroid from representative samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 and postfixied in 1.0% osmium tetroxide. Blocks were dehydrated in a graded series of alcohol and embedded in Epon 812 according to the method of Luft (1961). Semi-thin sections were stained with toluidine blue for light microscopy. Ultra-thin sections cut with an
Morphological changes of thyroid gland

ultramicrotome (Sorval Porter-Blum MT 2-B) were doubly stained with uranyl acetate and lead citrate to be examined with an HS-9 electron microscope (Hitachi, Tokyo).

The blood was centrifuged at 2700 rpm for 10 min and the plasma separated was used for chemical determination of activities of triiodothyronine (T₃), thyroxine (T₄) (radio-immunoassay method), and total cholesterol (enzyme method).

After 5, 10, and 15 days of restoration, 6 rats of the second group and on the 1st, 10th, 20th, and 30th day of treatment, 5 rats of the third group were dissected in a similar fashion (Table 1). All rats were starved for 16 hr before the dissection.

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<tr>
<th>Group</th>
<th>Days</th>
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**RESULTS**

1) **Body weight**

All rats survived throughout the experimental period. The growth rate appeared significantly lower in the ST-treated group than in the control after the 6th day of administration. Rats of the former group showed a decrease in weight by about 30 to 60 g compared with the control on the 30th day. In the restoration experiment for 15 days, the growth rate in the restoration group was greater than that in the control (Fig. 1).

2) **Plasma T₃ and T₄**

From the 1st to the 30th day of treatment, the ST-treated group was lower in plasma T₃ and T₄ values than the control. Especially T₄ could not be detected after the 7th day. These changes almost reverted to normal after 10 or 15 days of restoration (Table 2).

3) **Plasma total cholesterol**

The ST-treated group showed an increase in plasma total cholesterol on the 3rd day of treatment and maintained the increased value to the 30th day. After restoration for 10 days, however, this value reverted to normal (Table 2).

4) **Macroscopical findings of thyroid**

From the 3rd day of treatment, enlargement and hyperemia of the thyroid were observed in the ST-treated group. On the 30th day, these changes were more remarkable.

5) **Thyroid weight**

A significant increase in thyroid weight was recognized in the rats of the ST
Table 2  Plasma levels of T₃, T₄ and total cholesterol in male rats after oral treatment with ST (2 g/kg)

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<td>ST2g/kg × 30days</td>
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</table>

Mean ± S. D.
* Significant difference from control (p<0.05)
** Significant difference from control (p<0.01)
R: Restoration group
Morphological changes of thyroid gland

treated group from the 5th day of treatment. On the 30th day, the weight was about
eight times as heavy as that in the control. In the restoration experiment for 15 days
in the second group, the weight had a tendency to reversion. At the end of the 30-day
experimental period, the thyroid weight of the restoration group was about half of that
of the ST-treated group (Table 3).

6) Histopathological findings of thyroid gland

ST-treated group:

On the 1st day of treatment, thyroid follicles were distended with colloid and
epithelial cells were almost cubic (Fig. 2). On the 3rd or 5th day, hyperplasia of the
follicles lined with columnar cells was observed, while colloid was virtually scanty. In
addition, interstitial blood vessels considerably proliferated. Around the 7th or 10th
day, these changes became more intense and the follicles varied considerably in size but

<table>
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<th>Table 3</th>
<th>Thyroid weight treated orally with ST 2 g/kg (mg)</th>
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<tbody>
<tr>
<td>Group</td>
<td>Days</td>
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<tr>
<td>ST 2g/kg×30 days</td>
<td>1</td>
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<td>2(R)</td>
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<tr>
<td>ST 2g/kg×15 days</td>
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<tr>
<td>Control</td>
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Mean±S.D.
* Significant difference from control (p<0.05)
** Significant difference from control (p<0.01)
R : Restoration group

<table>
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<tr>
<th>Table 4</th>
<th>Histopathological findings of thyroid gland treated orally with ST 2g/kg</th>
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<tbody>
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<td>Observations</td>
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<tr>
<td>Hyperplasia of follicles</td>
<td>1</td>
</tr>
<tr>
<td>Columnar epithelium</td>
<td>2</td>
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<tr>
<td>Pleomorphism of follicular cells</td>
<td>3</td>
</tr>
<tr>
<td>Absence of colloid</td>
<td>4</td>
</tr>
<tr>
<td>Intestinal blood vessel proliferation</td>
<td>5</td>
</tr>
<tr>
<td>Full of eosinophilic colloid</td>
<td>6</td>
</tr>
<tr>
<td>Large follicles</td>
<td>7</td>
</tr>
<tr>
<td>Vacuolation of colloid</td>
<td>8</td>
</tr>
</tbody>
</table>

− Negative
+ Slight changes
++ Moderate changes
+++ Marked changes
generally tended to be small. The hyperplastic columnar epithelial cells were found to form papilliferous projections into the lumens (Fig. 3). After the 15th or 20th day, the epithelial cells became remarkably hyperplastic and colloid was absent in them. On the 25th or 30th day, all these findings were very marked and some follicular cells showed pleomorphism.

The above results are shown in Table 4.

Restoration group:

After 5 days of restoration, follicles filled with eosinophilic colloid were rather large and lined with flattened epithelial cells. Some vacuoles were observed in the periphery of colloid (Fig. 4). However, hyperplasia of follicles and proliferation of blood vessels became slight, though these had been remarkable during the administration period. After 10 or 15 days of restoration, the follicles well-filled with colloid were larger in size than those in the control and lined with flattened epithelial cells (Fig. 5).

The above results are also shown in Table 4.

7) Electron microscopy of thyroid gland

Control group:

Follicular cells of the thyroid were cuboidal in shape. In the cytoplasm of these cells, the cisternae of rough-surface endoplasmic reticulum (rER) were dilated and well-developed Golgi apparatus, numerous mitochondria, and electron dense granules were seen.

In addition, many microvilli projected into the colloid filling the lumen.

ST-treated group:

On the 1st day of treatment, no significant changes were observed in comparison with the control group. On the 3rd or 5th day of treatment with ST, follicular cells were high-columnar in shape, and in the cytoplasm of these cells, there was an increase of rER, the cisternae of which were dilated and contained colloid-like materials (Fig. 6). In addition, swelling of mitochondria was found. Around the 7th, 10th or 15th day, increase of rER, in which cisternae were dilated, were found in more plenty and numerous long microvilli projected into the lumens, where colloid was not seen (Fig. 7). There were no secretory granules in the cytoplasm, but in the Golgi apparatus, no remarkable changes were found in comparison with the control. On the 20th, 25th or 30th day, the follicular cells were more columnar as compared with the early stage of treatment, and small and ovoid nuclei were seen at their base. And numerous round or oval mitochondria were surrounded by the dilated cisternae of rER (Fig. 8).

Restoration group:

After 5 days of restoration, epithelial cells became flat. Some cisternae of rER were dilated and almost all follicles were filled with colloid. After 10 days of restoration, the follicles were lined with flattened epithelial cells, those lumina of which were large and well filled with colloid. A number of small and dense secretory granules appeared in the cytoplasm. After 10 or 15 days of restoration, a few round colloid droplet profiles were found to be enclosed in the cytoplasm of these epithelial cells (Fig. 9).
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Fig. 2  The 1st day of treatment. Follicles are distended with colloid, and epithelial cells are almost cubic in this thyroid gland. H. E. stain × 400

Fig. 3  The lesion of thyroid gland on the 10th day of treatment. Hyperplastic columnar epithelial cells are found, and colloid is virtually scanty. H. E. stain × 200
Fig. 4  The lesion of thyroid gland after 5 days of restoration. Vacuoles of the colloid (arrows) are recognized.  
H. E. stain ×400

Fig. 5  The lesion of thyroid gland after 15 days of restoration. Large follicles filled with eosinophilic colloid and flattened epithelial cells are found.  
H. E. stain ×400
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Fig. 6  The lesion of follicular cell on the 5th day of treatment. Dilated cisternae of rER, containing colloid-like materials therein are seen. \( \times 17800 \)

Fig. 7  The lesion of follicular cell on the 10th day of treatment. Numerous long microvilli are seen, and dilated cisternae of rER are found more abundantly. \( \times 16900 \)
Fig. 8  The lesion of follicular cell on the 30th day of treatment. Numerous round or oval mitochondria are surrounded by the dilated cisternae of rER.  $\times 16900$

Fig. 9  The lesion of follicular cell after 15 days of restoration. Round colloid droplet profiles (arrows) are found.  $\times 3300$
DISCUSSION

Several investigators have described the morphological changes of the thyroid gland following sulfonamide administration to rats (Mackenzie, C.G. et al., 1943; Astwood et al., 1943; Swarm et al., 1973; Honda et al., 1973; Lagler et al., 1976). They reported a reduction in colloid and an increase in the height of the thyroid epithelium.

In the present study, the author found almost similar changes in the thyroid.

Oral administration of ST induced thyroid hypertrophy and the thyroid weight was about eight times as heavy as that in the control on the 30th day of treatment. Histologically, hyperplasia of the follicles lined with high columnar cells was observed and colloid was absent in the follicles. These findings were first recognized on the 3rd day and became intense with the repeat of administration.

In addition, decreases in plasma $T_3$ and $T_4$ levels were observed from the 1st day of treatment. These results of decreases in plasma $T_3$ and $T_4$ levels were almost coincident with the histological findings that colloid was absent.

It can be considered that lower plasma $T_3$ and $T_4$ caused an increase in plasma thyro–trophin as a result of the removal of the negative feedback mechanism. It can be also considered that the increase in plasma thyro–trophin induced thyroid hypertrophy.

All the above results suggest that ST has a direct effect on the thyroid gland and inhibits the synthesis of thyroid hormone in follicular cells. Yamamoto (1966) reported that sulfonamide is an anti–thyroid compound.

In addition to the above-mentioned, some of the hyperplastic columnar epithelial cells formed papilliferous projections into the follicles. And some follicular cells showed pleomorphism. Swarm et al. (1972) reported, studying the administration of sulfamethoxazole to rats and monkeys, that lung metastases were observed in some rats sacrificed after 60 weeks’ treatment, but not in monkeys after 52 weeks’ treatment. Bridges et al. (1968) and Akimoto et al. (1975) reported that the metabolism of sulfonamides was different from species to species. It can be surmised that the long term treatment of rats with this compound induced the increase of pleomorphism of thyroid follicular cells.

On electron microscopy, small and ovoid nuclei were seen at the base of the follicular cells and in the cytoplasm, numerous swollen mitochondria were surrounded by dilated cisternae of rER. On the other hand, there were no secretory granules in the cytoplasm, but no remarkable changes were found in the Golgi apparatus in comparison with the control. These findings were recognized on the 3rd or 5th day of oral treatment with ST 2 g/kg and increased more and more in substance with the repeat of administration.

It is well known that sulfonamide inhibits the peroxidase activity of thyroid follicular cells (Yamamoto, 1966). The peroxidase activity of thyroid follicles reported until now is localized in the following sites: perinuclear cisternae, cisternae of rER, inner lamellae of the Golgi apparatus, some apical vesicles, and external surface of microvilli.
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(Strum et al., 1970; Fujita et al., 1972; Tice et al., 1974; Takahashi et al., 1977).

In the present study, it would be suggested that the peroxidase activity was inhibited at some organelles of thyroid follicular cells. On the other hand, the oral administration of aminotriazole to rats (Tsuda, 1975) and dogs (Hikosaka, 1975) produced thyroid gland hyperemia and diluted cisternae of rER were found. It is also well known that aminotriazole (Alexander, 1959 a, b) and aminogluthimide (Studer et al., 1970) are anti-thyroid compounds which are similar to sulfonamide in functions to the thyroid gland.

After 5 or 10 days of restoration, the follicles filled with eosinophilic colloid were rather large and lined with flattened epithelial cells. Some of the follicles were vacuolated at their edges. The vacuolation is occasionally found in the case of Basedow’s disease (Anderson, 1971). On the restoration experiment of 10 or 15 days, the thyroid gland was hyperfunctional and there was a change of plasma T₃ and T₄ values. Moreover, many dense secretory granules and colloid droplet profiles found on electron microscopical observations during the restoration experiment were similar in substance to the changes in the fine structure of the rat thyroid following thyrotropic hormone administration (Wetzel et al., 1965; Seljelid et al., 1968). It would also be suggested that the thyroid gland was hyperfunctional.

From the above facts, it is concluded that plasma T₃ and T₄ levels significantly decreased already on the 1st day of ST administration. The thyroid weight was increased and hyperplasia of follicles lined with columnar cells was observed on the histological examination. In addition to this, an increase in rER, the cisternae of which were dilated, was found on electron microscopy. All these changes tended to revert to normal during the restoration period.

ACKNOWLEDGEMENT

The author is greatly indebted to Prof. F. Uchino for his kind guidance and encouragement throughout this study.

Thanks are also due to Dr. Ishihara, Dr. Kohayashi and Dr. Kamei for their valuable advices.

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