STRUCTURE-ACTIVITY RELATIONSHIPS IN 5-SUBSTITUTED 3-AMINO-1, 2, 4-TRIAZOLES-INDUCED GOITERS IN RATS

Masaya Takaoka, Munehiro Teranishi, and Sunao Manabe
Laboratory Animal Science and Toxicology Laboratories, Sankyo Co., Ltd.

Naoaki Goto
Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo

Abstract: The goitrogenic actions of 5-substituted 3-amino-1, 2, 4-triazoles, 3-amino-5-mercapto-1, 2, 4-triazole (AMTZ), 3-amino-1, 2, 4-triazole-5-carboxylic acid (ATZC), 3, 5-diamino-1, 2, 4-triazole (DTZ), 3-amino-5-methylthio-1, 2, 4-triazole (AMTTZ), and 3-amino-1, 2, 4-triazole (ATZ) were compared in rats. ATZ, AMTZ, ATZC, and DTZ inhibited the thyroid peroxidase (TPO)-catalyzed oxidation of guaiacol in vitro, exhibiting the order of antithyroid activity ATZ>AMTZ>ATZC>DTZ. In in vivo experiments, ATZ, ATZC, or AMTZ inhibited TPO activity, and induced goiter accompanied by decrease in serum thyroid hormone and increase in serum thyroid-stimulating hormone in rats. These antithyroid activities were shown in order of ATZ>ATZC>AMTZ. The TPO inhibitory action of ATZC in vivo was weaker than that of AMTZ, but it was more persistent than AMTZ. The anti-TPO action of DTZ disappeared in vivo, and AMTTZ showed no effects in vitro and in vivo. These results show that ATZC, AMTZ, and DTZ potentially have the anti-TPO activity like as ATZ and the 5-position site of ATZ would have a very important meaning to determine the antithyroid action of ATZ. In addition, the results strongly suggest that the anti-thyroid action depends on the substitute but not on the parental compound. (J Toxicol Pathol 7: 429–434, 1994)
Key words: Goiter, 5-Substituted 3-amino-1,2,4-triazoles (ATZs), Thyroid peroxidase

Introduction

Antithyroid compounds were classified into three groups by their chemical structure, i.e., thioureylenes, amino-heterocyclic compounds, and polihydric phenols. Many comparative studies of antithyroid effects of these compounds in vivo and in vitro have been reported. Previous studies, however, have not clarified the antithyroid action is caused by whether the potency of parental structure or that of substituent.

3-Amino-1,2,4-triazole (ATZ) is a well-known goitrogen inhibiting thyroid peroxidase (TPO) activity in rats and the 5-position of ATZ was a determinant to inhibit liver catalase activity.

In this study, the antithyroid actions of 5-substituted ATZs and the parental compound were compared in rats.

Materials and Methods

Agents

Chemical compounds, ATZ, 3, 5-diamino-1, 2, 4-triazole (DTZ), 3-amino-1, 2, 4-triazole-5-carboxylic acid (ATZC), 3-amino-5-mercapto-1, 2, 4-triazole (AMTZ), and 3-amino-5-methylthio-1,2,4-triazole (AMTTZ) were purchased from Aldrich Chemical Company (Tokyo, Japan) (Fig. 1).
Animals

Fischer 344 (F344) adult male rats, weighing from 200 to 250 g, were purchased from a commercial breeder (Charles River Japan), and were fed a standard laboratory diet and given water ad libitum.

Inhibitory effects of TPO in vitro

The thyroids of one hundred male F344 rats fed with regular diets, were homogenized in amounts of 0.25 M sucrose and 18 mM EDTA equal to 9 times their weights in water chilled in ice. TPO activity was determined by the guaiacol assay system of Nagataki et al.\textsuperscript{10}. In the system, test compound solution contained thyroid homogenates, 0.2 M phosphate buffer (pH: 7.4), 0.1 M guaiacol, and hydrogen peroxide. The absorbance at 436 nm was measured against a blank using a UV-300 spectrophotometer (Shimadzu, Japan). The final concentration of test compounds in medium was from $10^{-2}$ M to $10^{-8}$ M. The protein contents in the thyroid homogenate were determined by the method of Lowry \textit{et al.}\textsuperscript{11}. TPO activity was shown by the absorbance changes of guaiacol peroxidation per protein contents. The 50% inhibition of the compounds was calculated with a computer program by Probit's methods.

Experimental program and pathological examination

The test compounds were dissolved in DMSO and administered once daily at a dose of 1.5 mmole/kg by gavage as a 7-day successive treatment. Twenty-four hours after the last treatment, blood samples for 3,5,3' triiodo-thyronine (T$_3$), thyroxine (T$_4$), and thyroid stimulating hormone (TSH) assays were collected from the abdominal aorta under ether anesthesia. All blood samples were centrifuged at 2,000 g for 10 min and sera were stored at $-20^\circ$C until use for hormone assays. Thyroids were removed immediately after blood sampling and were weighed individually, followed by fixation with 10% buffer formalin. Paraffin sections of these samples were made and stained with hematoxylin and eosin (HE).

Determination of the serum concentrations of T$_3$, T$_4$, and TSH

Serum concentrations of total T$_3$ and T$_4$ were determined by the tube coated radioimmunoassay (RIA : Daiichi Radioisotope Lab., Tokyo, Japan). RIA of rat TSH was done by the double–antibody method (Pituitary Hormones and Antisera Center, Harbor UCLA Medical Center), using NIDDK rat
Takaoka, Teranishi, Manabe, et al. 431

TSH–RP–3 as the standard. The minimal detectable concentrations were 0.2 ng/ml, 0.25 ng/ml and 2 ng/ml for TSH, T_3, and T_4, respectively.

Assay of TPO activity in vivo

The test compounds were dissolved in dimethylsulfoxide (DMSO: Wako Pure Chemical Co., Japan) and administered at a dose of 1.5 mmole/kg by gavage as a single treatment. Three, 6, and 24 hours after administration, the animals were killed by exsanguination and the thyroid glands were sampled, weighed individually, and then stored at -80°C until use. The thyroid of each rat was homogenized with ice cold 0.25 M sucrose containing 18 mM EDTA. Then TPO activity was determined according to guaiacol assay method as mentioned above.

Statistical procedures

Statistical analysis was performed with a computer program using multiple-comparison test.

Results

Effects on TPO in vitro

ATZ, AMTZ, ATZC, or DTZ inhibited the TPO-catalyzed oxidation of guaiacol, but AMTTZ did not (Fig. 2). The 50% inhibitory concentration for TPO of ATZ, AMTZ, ATZC, and DTZ were $5.2 \times 10^{-7}$ M (95% confidence limits: $0.41-0.61 \times 10^{-6}$ M), $4.9 \times 10^{-6}$ M ($4.52-5.56 \times 10^{-6}$ M), $1.7 \times 10^{-4}$ M ($1.33-1.92 \times 10^{-4}$ M), and $3.5 \times 10^{-4}$ M ($4.10-6.10 \times 10^{-4}$ M), respectively.

Pathological examination of thyroid glands

The average weights of the thyroid of ATZ-, ATZC-, or AMTZ- treated rats increased significantly in order of ATZ>ATZC>AMTZ, compared with those of control rats. No detectable changes were obtained in the weight of thyroid of rats treated with DTZ or AMTTZ (Table 1).

The thyroid of rat receiving ATZ, ATZC, or AMTZ histopathologically showed hypertrophy, proliferation of epithelial cells, decrease in colloid contents, and increase in follicular number. These changes were the most remarkable in ATZ-treated and the least in AMTZ-treated rats. No abnormalities were observed in both DTZ- and AMTTZ-treated rat thyroid (Fig. 3).

Serum T_3, T_4, and TSH concentrations

The concentration of serum TSH increased and T_4 decreased in rats treated with ATZ, ATZC, or AMTZ compared with that of control. These changes were the most remarkable in ATZ-treated rats and were the least in AMTZ-treated rats. Serum T_3 concentration decreased in rats given ATZ. No changes were observed in rats treated with DTZ or AMTTZ (Table 2).

Inhibition of TPO activity in vivo

TPO activities of rats administered with ATZ and AMTZ went down significantly 3 hrs after treatment. Inhibitory activity in AMTZ-treated rat was lower than that of ATZ, while in either

<table>
<thead>
<tr>
<th>Compound</th>
<th>Final body Weight (g)</th>
<th>Thyroid Weight (mg)</th>
<th>Ratio (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>270±16.7b</td>
<td>13.7±1.2</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td>ATZ</td>
<td>288±8.0</td>
<td>38.1±4.9**</td>
<td>13.1±1.3**</td>
</tr>
<tr>
<td>DTZ</td>
<td>277±10.7</td>
<td>14.2±1.7</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>ATZC</td>
<td>280±18.1</td>
<td>22.0±3.2*</td>
<td>7.8±0.4*</td>
</tr>
<tr>
<td>AMTZ</td>
<td>285±6.8</td>
<td>18.4±1.8*</td>
<td>6.6±0.2</td>
</tr>
<tr>
<td>AMTTZ</td>
<td>246±5.5</td>
<td>14.3±2.9</td>
<td>5.1±0.4</td>
</tr>
</tbody>
</table>

Note: a), thyroid to body weight ratio ; b), mean± S.D. (N=6); *, control vs. treated groups (P<0.05); **, ATMT vs. other treated groups (P<0.05)
ATZ–or AMTZ–treated rats, the activity gradually recovered with progress of time. However, the activity of rat given ATZC had been kept invariable from 3 hrs to 24 hrs after a single treatment. The TPO activity of either DTZ– or AMTTZ–treated rats was similar to that of control animals (Table 3).

Discussion

It is well–known that ATZ, having been used as a herbicide, is a goitrogen which inhibits TPO
activity in rats. The compounds induced a decrease in circulating thyroid hormone and an increase in serum TSH concentrations, which resulted in the enlargement of rat thyroid gland. In the 5-substituted ATZ presented here, AMTZ and ATZC showed inhibiting activity of TPO in in vitro and in vivo experiment. These facts are similar to ATZ-induced goiter in the previous study and literatures, demonstrating that ATZC and AMTZ are complete goitrogens in rats, like as ATZ.

In the study of 3-position of 1, 2, 4-triazole (TZ) derivatives, anti-TPO activity of these compounds both in vitro and in vivo were comparable. In this experiment, anti-TPO activity of AMTZ is stronger than that of ATZC in in vitro experiment. However, the pathological changes in AMTZ-treated rat thyroid are milder than those in ATZC. In addition, AMTZ-induced TPO inhibitory action was transient, while a weak anti-TPO action of ATZC kept to 24 hours after a single treatment. Thus, the ATZC-induced goiter may be caused by its persistent TPO-inhibitory action in vivo.

DTZ inhibited TPO activity in vitro, but no abnormalities were observed in the thyroidal function in DTZ-treated rats. These results suggest that DTZ may be structurally a potential goitrogen. On the other hand, AMTTZ showed no-effects in rat thyroid in in vitro and in vivo experiment just like TZ, suggesting that AMTTZ is not a goitrogen.

In the study of goitrogenic activities of 3-substituted TZs in rat, ATZ, mercapto-TZ, and nitro-TZ inhibited TPO activity, each at a different level. These results showed that the antithyroid action depends not on the structure of the parental compound but on the substitute. At present, AMTZ, ATZC, DTZ, and AMTTZ showed the same results of the comparative thyroid toxicity study of 3-substituted 1, 2, 4-triazoles (TZ).

Inactivation of bovine liver catalase by ATZ was accompanied by the binding of ATZ to a single histidine residue on 5-position. In the present study, anti-TPO action of 5-substituted ATZs was demonstrated to be weaker than that of ATZ in the order of ATZ > AMTZ > ATZC > DTZ. These results suggest that the 5-position of ATZ is the site which has serious effects to inhibit TPO activity in rats.

Rady and Buxeraud reported that antithyroid action was under the influence of electron-donor substitute. The potency of antithyroid action and the substitute of chemical structure were thought to have close relationships and expressed the action in various degrees. Antithyroid action of a mercapto moiety was stronger than that of an amino moiety on the 3-position of TZ. In this study, it was demonstrated that a mercapto moiety has stronger anti-TPO activity than an amino moiety on the 5-position of ATZ. In these results, the fact that the potency of antithyroid action of a mercapto moiety is stronger than that of an amino moiety is a general consideration in antithyroid action of TZ derivatives.

Table 2. Serum TSH, T3, and T4 Concentrations in Rats Treated with the Compounds for 7 Days

<table>
<thead>
<tr>
<th>Compound</th>
<th>TSH (ng/ml)</th>
<th>T3 (ng/ml)</th>
<th>T4 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.15 ± 0.08</td>
<td>0.85 ± 0.08</td>
<td>79.03 ± 5.08</td>
</tr>
<tr>
<td>ATZ</td>
<td>14.92 ± 1.90</td>
<td>0.45 ± 0.09</td>
<td>35.84 ± 7.54**</td>
</tr>
<tr>
<td>DTZ</td>
<td>2.17 ± 0.80</td>
<td>0.88 ± 0.08</td>
<td>70.60 ± 6.20</td>
</tr>
<tr>
<td>ATZC</td>
<td>6.46 ± 8.99</td>
<td>0.87 ± 1.00</td>
<td>46.57 ± 13.35*</td>
</tr>
<tr>
<td>AMTZ</td>
<td>3.90 ± 0.77</td>
<td>0.85 ± 0.08</td>
<td>56.34 ± 8.22*</td>
</tr>
<tr>
<td>AMTTZ</td>
<td>1.84 ± 0.27</td>
<td>0.86 ± 0.10</td>
<td>71.75 ± 11.16</td>
</tr>
</tbody>
</table>

Table 3. Thyroid-peroxidase Activities in Rats after a Single Administration of the Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>After a single treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 hrs</td>
</tr>
<tr>
<td>Control</td>
<td>32.22 ± 5.67* (mU/mg protein)</td>
</tr>
<tr>
<td>ATZ</td>
<td>7.24 ± 1.68**</td>
</tr>
<tr>
<td>DTZ</td>
<td>32.90 ± 0.60*</td>
</tr>
<tr>
<td>ATZC</td>
<td>22.47 ± 4.42*</td>
</tr>
<tr>
<td>AMTZ</td>
<td>21.41 ± 5.25*</td>
</tr>
<tr>
<td>AMTTZ</td>
<td>31.22 ± 4.59</td>
</tr>
</tbody>
</table>

Note: a), mean ± S.D. (N=6); *, control vs. treated groups (P<0.05); *, AMTZ vs. other treated groups (P<0.05)
substituent has more effective antithyroid action than aminoheterocyclic compounds with an amino moiety⁴. These comparisons, however, could not clarify whether the antithyroid effects caused by the potency of parental structure or substitute. From the present study, the differences of antithyroid effects between thiourelenes and aminoheterocyclic compounds may be related to the potency of substituents.

In conclusion, the structure–activity relationships between antithyroid action and substituent of the compound determine to induce goiters in various degrees, and the potential goitrogenic capacity of the compound is different from the manifestation of lesions. These results might provide useful basic data for the investigation of drug-induced thyroid toxicity.

Acknowledgments: The authors are indebted to Mr. Isao Igarashi and Mr. Naotoshi Yamamura for their valuable technical assistance.

References