HORMONAL DISREGULATION MECHANISM IN THE RAT THYROID TUMOR INDUCED BY DINICONAZOLE

Shunji HOSOKAWA, Jun NAKAMURA, Seiichi ITO,
Masakazu MURAKAMI, Mariko INEYAMA, Kaoru YOSHIOKA,
Tomoya YAMADA, Takaki SEKI, Masatoshi MATSUO and Hirohiko YAMADA

*Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,
1-98, 3-Chome, Kasugade-Naka, Konohana-Ku, Osaka 554, Japan*

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ABSTRACT—To assess the toxicological significance of thyroidal tumor observed slightly in a long-term rat study with diniconazole, (*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, a 3-month subacute feeding study was conducted in male Crj: CD (SD) rats by administering diniconazole in diet at concentrations of 0, 100, 1,000, or 2,000 ppm. Examinations mainly for thyroid functions were performed at Weeks 2, 4 and 13. Measurement of serum hormone levels revealed continuous decreases in serum thyroxine (T_4) and free T_4 levels at and above 1,000 ppm and increase in serum thyroid stimulating hormone (TSH) level at 2,000 ppm concurrently with liver weight and hepatic UDP-glucuronyltransferase (UDP-GT) increases at and above 1,000 ppm. No changes were observed in serum triiodothyronine (T_3) and free T_3 levels. Increase in thyroid uptake of ^{125}I and organification of ^{125}I in the thyroid at 2,000 ppm and thyroid follicular cell hyperplasia at and above 1,000 ppm were also observed. However, no compound-related changes were observed in autopsy and organ weight in the thyroid. Based on the above results, diniconazole induces increases in the hepatic UDP-GT activity and the thyroid hormone excretion from the liver. The increased excretion of thyroid hormones causes decrease in serum T_4 and free T_4 levels, triggering the feedback mechanism of the pituitary gland, promotion of TSH release from the pituitary gland and increase in serum TSH level. The increased serum TSH level probably leads to increased ^{125}I uptake of thyroid and thyroid follicular cell hyperplasia. Thus, the thyroid tumorigenesis in rats treated with diniconazole is due to the secondary overstimulant effect on the thyroid by increased serum TSH level. The toxicological significance in humans is extremely low and it is unlikely that diniconazole would increase thyroid tumor in humans even if diniconazole were to alter normal thyroid hormone level in humans.

Key words : rat thyroid tumor, mechanism, thyroid hormone, TSH,
UDP-glucuronyltransferase, diniconazole.

INTRODUCTION

Diniconazole, (*E*)-1-(2, 4-dichlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol, is a new agricultural fungicide developed by Sumitomo Chemical Co., Ltd. A two-year dietary toxicity and oncogenicity study in CD rats with diniconazole (Spicer and Wazeter, 1989) revealed a slight increase (8/50, 16%) in the incidence of benign thyroid tumor only in the male highest dietary group of 2,000 ppm associated with an increase in liver weight and centrilobular hypertrophy of hepatocytes. Subacute (3-month) feeding study in rats demonstrated an increase in diffuse follicular cell hyperplasia with an increase in thyroid and liver weight and centrilobular hypertrophy of hepatocytes at and above 1,000 ppm (Murakami et al., 1984). Moreover, diniconazole has effects on various parameters relating to liver functions including liver weight, UDP-glucuronyltransferase (UDP-GT), cytochrome P-450 and b_5 contents, and bile flow rate (Isobe et al., 1991). Similar hepatic effects and thyroid changes have been reported for the microsomal enzyme inducers in rats (Bastomsky, 1977; Comer et al., 1985; Sanders et al., 1988; McClain et al., 1989; Capen and Martin, 1989). The mechanism of this thyroid change by these microsomal enzyme inducers has been shown to be a secondary or compensatory one in response to an increased rate of hepatic metabolism and clearance of thyroid hormones. The decreases in blood thyroid hormone levels trigger the feedback mechanism in the pituitary gland, promoting thyroid stimulating hormone (TSH) release from the pituitary gland and leading to an increase in blood TSH level (Emerson et al., 1989). The finding that administration of TSH caused rapid proliferative response of rat thyroid gland (Bybee and Tuffery, 1989) suggests prolonged elevations of TSH induce an increase of follicular cell tumors. Moreover, a recent document of thyroid follicular cell carcinogenesis (Hill et al., 1989) reviewed in accordance with the U. S. Environmental Protection Agency procedures and has been approved for publication describes that some of thyroid tumors in rats are due to increased serum TSH level caused by increased breakdown of thyroid hormones resulting from

the increased level of liver enzyme activity. To evaluate rat thyroid tumorigenesis by diniconazole based on this hypothesis, this paper presented the results of thyroid uptake of ^{125}I , serum thyroid hormone and TSH level, hepatic UDP-GT activity and histopathological examination in thyroid when male CD rats were fed diniconazole in diet at 0, 100, 1,000 or 2,000 ppm for 3 months.

MATERIALS AND METHODS

Chemicals: Diniconazole, (*E*)-1-(2, 4-dichlorophenyl)-4,4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol (Lot No. PCG-86073) was synthesized by Sumitomo Chemical Co., Ltd. (Osaka, Japan) and was more than 95.6% pure by HPLC. $\text{Na } ^{125}\text{I}$ and [^{125}I]-thyroxine ($46.6 \mu\text{Ci/nmol}$) were purchased from Amersham (Buckinghamshire, U.K.). Thyroxine (T_4), uridine 5'-diphosphoglucuronic acid (UDPGA) and β -glucuronidase were purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

Animals and housing: Male Sprague-Dawley-derived CD rats purchased from Charles River Japan Inc. (Kanagawa, Japan), approximately 6 weeks old upon arrival, were acclimatized for 1 week to laboratory conditions with temperature $24 \pm 2^\circ\text{C}$, relative humidity $55 \pm 10\%$, lighting cycles of 12 hr (8:00–20:00) and frequency of ventilation 10 times or more/hr prior to the start of the study. The animals were approximately 7 weeks old at the start of treatment. Feed and water were available *ad libitum* using stainless feeder and automated water supply system, respectively. Rats were housed 2 animals per cage in suspended aluminum cages with stainless steel wire-mesh floors during the treatment period.

Diet preparation: Diniconazole was administered in mixture with powdered basal diet sterilized by irradiation with ^{60}Co (Oriental Yeast Co., Tokyo, Japan). Since the test compound was stable within the basal diet for 6 weeks (Murakami et al., 1984), the mixture was prepared at intervals of 2 or 4 weeks. The premix was made by blending with appropriate amounts of the test compound and basal diet in a mortar and pestle. This premix and additional quantity of diet were then blended in a mixer (SAN-EI Seisakusho Co., Tokyo, Japan). The mixtures were tested for the homogeneity of the test compound at the

beginning of administration, and the concentrations were analysed every month (Sumika Chemical Analysis Service Ltd., Osaka, Japan). The results of analysis were satisfactory.

Experimental design : One hundred and forty-four male rats were divided into eighteen groups of eight rats each and fed diniconazole in diet at concentrations of 0, 100, 1,000 or 2,000 ppm and examinations for thyroid functions mentioned later were performed at Weeks 2, 4 and 13. The dosage of 2,000 ppm at which a slight increase in the incidence of thyroid tumor was observed in a combined chronic toxicity and oncogenicity study in rats with diniconazole (Spicer and Wazeter, 1989) was employed as the highest dosage level. The dosage of 1,000 ppm at which thyroid weight increase and thyroid hyperplasia were demonstrated in 13-week subacute toxicity study in rats (Murakami et al., 1984) was selected as the intermediate dosage. The dosage of 100 ppm at which no compound-related changes were observed in the above studies was chosen as the lowest dosage.

Clinical symptoms, body weights and food consumptions : Clinical symptoms and body weights of all rats were monitored once a week. On the day of autopsy, body weights of all sacrificed rats were recorded. Food consumptions for 7 continuous days were examined once a week per cage.

Thyroid iodine uptake and organification : Eight rats of the control and 2,000 ppm group were injected intraperitoneally with Na ^{125}I in physiological saline solution (about 80 nCi/0.2 ml) at Week 2, 4 and 13. At 24 hr after the injection, rats were killed by exsanguination and the thyroids were removed. After weighing, radioactivity of ^{125}I in the thyroids were measured by the gamma-counter (Packard Instrument Co., U.S.A.). The amount of protein-bound ^{125}I in the thyroids was also measured at Week 13. The thyroids were homogenized individually in 0.5 ml ice-cold solution of 0.15 M NaCl-1 mM KI. The radioactivity in a 0.1 ml aliquot of each homogenate was determined with the gamma-counter. The protein in the remaining 0.4 ml of homogenate was precipitated by addition of 0.4 ml of 10% trichloroacetic acid and was centrifuged for 10 min at 1300 $\times$ g. The resulting pellet was washed once by resuspending in 5% trichloro

acetic acid and centrifuged. The amount of protein-bound ^{125}I in the pellet was determined with the gamma-counter.

Serum TSH, T_3 and T_4 concentrations : At 2, 4 and 13 weeks, 8 animals per group were killed by decapitation and blood was collected to remove the serum by centrifugation for measurement of blood levels of thyroid stimulating hormone (TSH), total triiodothyronine (T_3), total thyroxine (T_4), free T_3 and free T_4 by RIA (radioimmunoassay). All samples for measurement were preserved below -30°C until measurement. The serum level of TSH was measured by NIADDK rat TSH radioimmunoassay kit (antibody ; anti-rTSH-RP-2) supplied by Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, U.S.A. Serum total T_3 , free T_3 , total T_4 and free T_4 levels were measured in Special Reference Laboratory, Tokyo, Japan using Solid Phase RIA Kit (Dainabot Co., Tokyo, Japan, Ciba-Corning Diagnostic Co., Medfield, U.S.A. or Amasham Corp., U.K.).

Autopsy, organ weights and histology : After blood collection, the liver and thyroids were isolated and examined macroscopically. The wet liver weights were measured, while the thyroid weights were measured after fixation in 10% neutral buffered formalin solution. Hematoxylin and eosin-stained paraffine embedded sections of thyroids were examined under light microscope.

Hepatic UDP-GT activity : The livers from 5 animals of each group were removed, weighed and homogenized individually in ice-cold 50 mM Tris-HCl-0.154 M KCl buffer (pH 7.4). The homogenate was centrifuged at 10,000 $\times$ g for 20 min at 4°C and the resulting supernatant fraction was centrifuged at 105,000 $\times$ g for 60 min at 4°C . UDP-glucuronyltransferase (UDP-GT) activity toward T_4 as substrate was determined in the resulting pellet by measuring the appearance of thyroxylglucuronide as described by Comer et al. (1985). The reaction mixture containing 0.03 ml of microsomal suspension (1.5 mg of protein), 20 mM UDP GA, 33 μM T_4 and 4.2 μM [^{125}I] T_4 (46.6 $\mu\text{Ci/nmol}$) in 100 mM Tris-HCl buffer (pH 7.4) with 20 mM MgCl_2 was incubated for 30 min at 37°C . The reaction was terminated by addition of 0.2 ml ice-cold 75% ethanol/water. Protein was removed by centrifugation and 40 μl of the resulting supernatant fraction was chromatog-

raphed on thin-layer silica gel plates with butanol saturated with 2 N NH_4OH as described by West et al. (1965). The area of the plates corresponding to the thyroxylglucuronide were scraped and the radioactivity was determined with a gamma-counter. Boiled microsomal suspensions were prepared through the procedure to serve as background control.

Statistical analysis: Analysis of variance in one-way classification was performed on body weight, food consumption, thyroid uptake of ^{125}I , serum thyroid hormone levels, hepatic UDP-GT activities and organ weights. Any data showing significant differences at a level of 5% were further tested using the LSD (least significant difference) method for any significant differences from the control group.

RESULTS

Clinical symptoms and body weights: No significant differences were observed in the incidence of clinical symptoms between the treated and

control groups. Statistically significant decrease of body weight was observed in animals fed 2,000 ppm diniconazole at Week 1, however, no significant differences were seen thereafter. In the 100 and 1,000 ppm groups, the body weight changes were comparable to the control group during the study (Table 1).

Food consumptions and compound intake: Decreased food consumption was observed only at Week 1 for the 2,000 ppm group. The average amounts of compound intakes for the 100, 1,000 and 2,000 ppm group calculated from the food consumption and body weight were 5.3, 54 and 108 mg/kg/day, respectively.

Thyroid weights and thyroid iodine uptake: Thyroid weight changes and thyroid ^{125}I uptake at Week 2, 4 and 13 are shown in Table 1.

Neither the thyroid weight nor necropsy examination of the thyroid showed statistically significant changes in the treatment groups. Increases in the thyroid uptake of ^{125}I were observed in the 2,000 ppm group at Week 13. The percentage of protein bound ^{125}I (PBI, an

Table 1. Effects of diniconazole treatment in male rats on thyroid weights, thyroid-to-body weight ratios and thyroid iodine uptake for 2, 4 or 13 weeks^a.

Feeding period (week)	Dose ^b (ppm)	final body weight (g)	Thyroid weight (mg)	Thyroid/body weight (mg%)	^{125}I uptake ^c /thyroid (%)
2	0	328 ± 19 ^d	25 ± 2.9	7.6 ± 0.81	8.3 ± 2.0
	100	339 ± 14	23 ± 3.3	6.8 ± 0.98	—
	1,000	332 ± 28	23 ± 4.3	6.9 ± 1.4	—
	2,000	327 ± 19 ^d	21 ± 3.2	6.5 ± 0.82	9.3 ± 2.7
4	0	382 ± 28 ^d	27 ± 2.4	7.0 ± 1.0	9.0 ± 1.3
	100	382 ± 28	26 ± 3.4	6.8 ± 1.1	—
	1,000	398 ± 25	30 ± 4.7	7.5 ± 1.2	—
	2,000	376 ± 35 ^d	29 ± 3.9	7.9 ± 1.1	7.8 ± 2.3
13	0	503 ± 36	30 ± 5.4	6.2 ± 1.1	12.0 ± 1.7
	100	501 ± 55	30 ± 4.0	6.1 ± 0.6	—
	1,000	477 ± 54	31 ± 5.8	6.6 ± 1.3	—
	2,000	494 ± 46	35 ± 7.9	6.9 ± 1.5	17.0 ± 4.4*

a : Rats were fed diniconazole in diet at 0, 100, 1,000 or 2,000 ppm.

Eight animals/group were sacrificed by exsanguination. Values represent mean ± S.D.

b : Concentration of diniconazole in diet.

c : ^{125}I uptake expressed as % of administered dose.

d : N=16 rats.

*: Significantly different ($P < 0.05$) from control (0 ppm).

Table 2. Effects of diniconazole treatment in male rats on serum T₄, free T₄, T₃, free T₃ and TSH level for 2, 4 or 13 weeks^a.

Feeding period (week)	Dose ^b (ppm)	T ₄ (μg/dl)	free T ₄ (ng/dl)	T ₃ (ng/dl)	free T ₃ (pg/ml)	TSH (ng/ml)
2	0	3.9 ± 0.8	2.6 ± 0.3	0.8 ± 0.1	1.4 ± 0.2	3.1 ± 0.8
	100	3.5 ± 0.5	2.6 ± 0.4	0.8 ± 0.1	1.3 ± 0.3	3.2 ± 1.9
	1,000	2.7 ± 0.3**	2.1 ± 0.3**	0.8 ± 0.1	1.3 ± 0.3	4.9 ± 3.1
	2,000 ^c	2.1 ± 0.4**	1.8 ± 0.3**	0.7 ± 0.1	1.4 ± 0.5	9.1 ± 4.1**
4	0	4.0 ± 0.8	2.8 ± 0.3	0.7 ± 0.1	1.3 ± 0.3	2.9 ± 1.5
	100	4.1 ± 0.7	2.8 ± 0.4	0.8 ± 0.2	1.3 ± 0.6	3.7 ± 2.5
	1,000	3.0 ± 0.8**	2.3 ± 0.4*	0.7 ± 0.1	1.1 ± 0.3	3.5 ± 1.5
	2,000	2.3 ± 0.4**	1.9 ± 0.2**	0.8 ± 0.1	1.3 ± 0.3	6.5 ± 3.9**
13	0	4.7 ± 0.9	2.5 ± 0.4	0.8 ± 0.1	1.5 ± 0.4	4.7 ± 3.8
	100	3.6 ± 0.7**	2.1 ± 0.3*	0.7 ± 0.1	1.3 ± 0.3	4.6 ± 1.6
	1,000	3.2 ± 0.8**	1.9 ± 0.2**	0.7 ± 0.2	1.4 ± 0.4	6.5 ± 4.6
	2,000	2.4 ± 0.5**	1.6 ± 0.2**	0.7 ± 0.1	1.4 ± 0.2	5.5 ± 1.5

a : Rats were fed diniconazole in diet at 0, 100, 1,000 or 2,000 ppm.

Eight animals/group were sacrificed by exsanguination. Values represent mean ± S.D.

b : Concentration of diniconazole in diet.

c : N=7 rats.

* : Significantly different (P<0.05) from control (0 ppm).

** : Significantly different (P<0.01) from control (0 ppm).

index for iodine organification) in the 2,000 ppm group at Week 13 was significantly increased (11%) compared to the control value (14%).

Thyroid hormone levels in serum : Table 2 shows the effect of diniconazole on thyroid hormone levels in serum.

Serum T₄ and free T₄ levels were significantly decreased in the 1,000 and 2,000 ppm groups at Weeks 2 and 4, and in the 100 and above groups at Week 13. Serum TSH level was significantly increased in the 2,000 ppm group at Weeks 2 and 4. No statistically significant differences in serum TSH level were observed at Week 13, since each 2 animals of the control and 1,000 ppm groups showed extremely high serum TSH values. However, the serum TSH level showed 5.52 ± 1.462 ng/ml (mean±S.D.) in the 2,000 ppm group and the value was approximately comparable to the value in the 2,000 ppm at Week 4. No statistically significant changes were observed in serum T₃ and free T₃ levels at Weeks 2, 4 and 13.

Liver weights and hepatic UDP-GT activities : Liver weight and hepatic UDP-GT activity

changes were shown in Fig. 1. Increased absolute liver weights were observed in the 1,000 and 2,000 ppm groups at Weeks 2 and 4 and in the 2,000 ppm group at Week 13. Increased relative liver weights were observed in the 1,000 and 2,000 ppm groups throughout the treatment period.

Measurement of UDP-GT activity using T₄ as a substrate demonstrated the significant increases in the enzyme activity per wet liver weight for the 2,000 ppm group at Week 2 and 4. At 13 weeks of treatment, the enzyme activity per liver weight was elevated at and above 1,000 ppm.

Gross observations of liver and thyroid histology : Liver enlargement was observed in the 2,000 ppm group at Week 2 and in the 1,000 and 2,000 ppm group at Weeks 4 and 13. Yellowish spots and points of liver were observed in the 1,000 and/or 2,000 ppm groups at Weeks 2, 4 and 13. In the histopathological examination for thyroid, diffused follicular cell hyperplasia was dose dependently observed in the 1,000 and 2,000 ppm groups at Weeks 4 and 13 (Table 3). The

Fig. 1. Effects of diniconazole treatment in male rats on liver weights and hepatic UDP-glucuronyltransferase (UDP-GT) activity (per absolute liver weight basis) for 13 weeks. Rats were fed diniconazole in diet at 0, 100, 1,000 or 2,000 ppm. Five animals/group were sacrificed by decapitation. Data represent mean $\pm$ S.D.

* : Significantly different from 0 ppm ($P < 0.05$).

** : Significantly different from 0 ppm ($P < 0.01$).

Table 3. Histopathological findings in thyroid treated with diniconazole for 2, 4 or 13 weeks^a.

Feeding period (week)	Observations	0	100	1,000	2,000 ^b
2	No. of animals examined	8	8	8	8
	No findings	5	4	6	4
	Remnant of ultimobranchial body	3	4	1	3
	Ectopic thymus			1	1
4	No. of animals examined	8	8	8	8
	No findings	5	5	5	2
	Remnant of ultimobranchial body	3	3	1	1
	Diffuse follicular cell hyperplasia	1		3	5
13	No. of animals examined	8	8	8	8
	No findings	5	7	3	1
	Remnant of ultimobranchial body	2		2	2
	Ectopic thymus				1
	Diffuse follicular cell hyperplasia	1	1	5	6

^a : Rats were fed diniconazole in diet at 0, 100, 1,000 or 2,000 ppm.

Eight animals/group were sacrificed by exsanguination. Values represent mean $\pm$ S.D.

^b : Concentration of diniconazole in diet. (ppm)

Photo. 1. Light micrograph of thyroid from rat treated with diniconazole 2,000 ppm for 4 weeks. Diffused follicular cell hyperplasia was observed. Hematoxylin and eosin ($\times 200$).

follicles were generally small and had little colloid (Photo. 1). Remnant of ultimobranchial body was observed in some rats of all groups. Ectopic thymus was sporadically observed. No test compound related effects were observed histopathologically in thyroid at Week 2.

DISCUSSION

Administration of diniconazole to male rats in diet for 13 weeks induced continuous decreases in serum T_4 and free T_4 level and increase in serum TSH level. Decreased thyroid hormone probably triggered the feedback mechanism in the pituitary gland, promoting TSH release from the pituitary gland and leading to an increase in serum TSH level.

It is well known that TSH promotes biosynthesis and secretion of thyroid hormones in the thyroid. An increase in TSH leads to an iodine uptake into the thyroid, production of peroxidase and thyroglobulin, and iodination of thyroglobulin, endocytosis of colloid droplets and

promotion of hydrolysis of colloid. Morphologically, long-term TSH stimulation induces resorption of colloid from the follicular lumen, increases in epithelial cell volume, hypertrophy and hyperplasia of the follicular epithelial cells in rats fed chronically a low iodine diet (Denef et al., 1981) or treated with goitrogen (Graham and Hansen, 1972). Furthermore, it is reported that TSH increased proliferation of the thyroid cell (Bybee and Tuffery, 1989). Proto-oncogenes existing in rat thyroid follicular cells are activated by increased stimulation of thyroid follicular cells by TSH (Tramontano et al., 1986). These facts suggest that TSH is acting as a growth factor. Chronic treatment with goitrogen or a low iodine diet induces thyroid follicular cell hypertrophy, hyperplasia and tumors in rats (Axelrad and Leblond, 1955; Jemec, 1980; Graham et al., 1982). These treatments decrease serum thyroid hormone levels and increase serum TSH level (Rognoni et al., 1982; Cooper et al., 1983 and 1984). Thyroid hormone treatment or hypophysectomy exerts and antagonistic effect

against the above changes including thyroid tumors (Yamada and Lewis, 1968 ; Nadler et al., 1970). The antagonistic changes are correlated with serum TSH levels. These facts suggest that chronic overstimulant TSH induced by goitrogen and iodide deficiency etc. produce a slight increase in the incidence of follicular cell tumors through a promotion mechanism. Therefore, diniconazole induced thyroid follicular tumor is also considered to be produced through a promotion mechanism of TSH.

In the present study we measured thyroid uptake of ^{125}I and organification of ^{125}I as a functional test of the thyroid. At Weeks 2 and 4, no significant difference in thyroid uptake of ^{125}I was observed between the 2,000 ppm group and the control group. However, the uptake and organification were significantly increased in the 2,000 ppm group at Week 13, indicating the thyroid function was maintained in an almost normal condition or slightly hyperactive. Morphologically, thyroid follicular cell hyperplasia was demonstrated in the 1,000 and 2,000 ppm group at Weeks 4 and 13. These changes in the thyroid function and morphology were likely to be induced by increased serum TSH level. Similar changes in serum hormone levels and morphology were observed in rats administered with thionamides such as methimazole (Owen et al., 1973 ; Cooper et al., 1984) and propylthiouracil (Sellers et al., 1953 ; Cooper et al., 1983). However, the effect on the thyroid produced by diniconazole was considered to be different from the finding by thionamides (Jemec, 1980 ; Akoso et al., 1982) which primarily inhibits the organification of iodide in the thyroid, since no suppression in the thyroid uptake of iodine and organification was observed in rats administered diniconazole.

Thyroid hormones are well known to be converted to various metabolites at the peripheral tissues of the whole body. Glucuronidation represents the primary reaction in excretion of thyroid hormones in rats (Akoso et al., 1982) and UDP-GT is known as an enzyme involved in the reaction. The induction of hepatic UDP-GT play an important role in the thyroid hormone homeostasis. The inducers of hepatic UDP-GT increase the biliary excretion of thyroxine glucuronide and cause a decrease in serum thyroid

hormone levels, an increase in serum TSH level and thyroid follicular cell hypertrophy and hyperplasia (Bastomsky, 1977 ; Semiler et al., 1989). The polycyclic hydrocarbon type inducers increase the UDP-GT activity to a higher level, have a greater effect on biliary thyroxine excretion, and have a correspondingly greater goitrogenic effect than the phenobarbital-like microsomal enzyme inducers in rats (Hill et al., 1989). McClain et al. (1989) found that 4-week treatment with 100 mg/kg phenobarbital increased liver weight (1.4 fold) and T_4 -UDP-GT activity per wet liver weight (2.2 fold) in rats. Bastomsky and Murthy (1976) observed that 11-day treatment with polychlorinated biphenyls (250 ppm in diet) markedly increased the activity per wet liver weight (5 fold) in rats. Our investigations for the diniconazole induction of UDP-GT in liver using T_4 as a substrate revealed significant increase at 2,000 ppm throughout the treatment period of 13 weeks. The elevation of activity per wet liver weight was most remarkable (4.3 fold above control value) at Week 4. Diniconazole might cause an increase in the hepatic UDP-GT activity and serum TSH level to higher level than phenobarbital.

The inducers of hepatic UDP-GT also produce thyroid follicular cell tumors through a promotion mechanism using an initiation-promotion model established by Hiasa et al. (1982) or by the chronic treatment of the inducer in rats. McClain et al. (1988) demonstrated that supplemental administration of thyroxine blocked the promoting effect of phenobarbital and suggested that the tumor promoting effect of phenobarbital was directly proportional to the level of plasma TSH. These facts indicate that the tumor promoting effect is secondary to hormone imbalance as a result of increased hepatic disposition of T_4 as opposed to a direct tumor promoting or carcinogenic effect in the thyroid gland (McClain et al., 1989 ; Swenberg et al., 1992).

There are marked species differences in thyroid gland physiology that must be taken into account in an evaluation of toxicological significance about diniconazole induced thyroid tumor in rats. In humans, the major carrier protein is thyroxine-binding globulin. The thyroxine-binding globulin has a very high affinity for T_4 . This specific carrier protein is absent and thyrox-

ine-binding prealbumin and albumin transport thyroid hormone in rodents. Therefore, more thyroid hormone is free of protein binding and is subject to metabolism and removal from the body in rats (Hill et al., 1989). The hepatic enzyme inducers probably enhance further the metabolism of thyroxine in rats. In the human study conducted by Ohnhaus et al. (1981), the 14-day administration of rifampicin, a hepatic enzyme inducer, caused a decreased serum T_4 level by 59%, however serum TSH level and the TSH response to thyrotropin releasing hormone (TRH) usually remained within normal limits. These data indicate that a decreased serum thyroid hormone level does not always enhance the release of TSH from the anterior pituitary gland in humans. On the other hand, phenobarbital caused increase in antipyrine clearance by 56%, however serum thyroid hormone and TSH levels were not significantly changed. Ohnhaus and Studer (1983) concluded that a decreased serum T_4 level is probably affected by increased biliary excretion in human treated with hepatic enzyme inducers and induction sufficient to increase antipyrine clearance by at least 60% was required before a change in steady state hormone levels occurred. These data indicate that thyroid hormone imbalances via hepatic enzyme induction in rats are probably more sensitive than humans.

It is an established fact that thyroid tumors are produced experimentally in animals through chronic elevated TSH level by low iodine diets and goitrogens etc. It is also well known that low iodine diet causes endemic goiter and high serum TSH level in human (Patel et al., 1973; Kochupillai et al., 1973). There have been attempts to correlate iodine deficiency with endemic goiter and thyroid cancer in human. For some observers, endemic goiters by iodine deficiency constitute a predisposing factor for cancer (Beahrs et al., 1951; Islanbecov et al., 1966; Mustacchi and Cutler, 1956; Thalmann, 1954; Wegelin, 1928), on the other hand, some observations tend to show that no definite correlation can be established between thyroid cancer and endemic goiter (Clements, 1954; Egloff, 1961; Kind, 1966; Pendergrast et al., 1961; Saxen and Saxen, 1954; Ramalingaswami, 1969). Although the epidemiological observations often yield discrepant results, these data suggest that

iodine-deficiency is insufficient by itself to cause thyroid cancer. In other words, chronic TSH stimulation probably would not induce thyroid cancers in humans.

Based on these experimental evidences and considerations, the thyroid tumorigenesis in rats treated with diniconazole is considered to be derived from the secondary overstimulant effect on the thyroid by increased serum TSH level. The toxicological significance in humans is extremely low and it is unlikely that diniconazole would increase thyroid tumor in humans even if diniconazole were to alter normal thyroid hormone level in humans.

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