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The Promoting Effects of Food Dyes, Erythrosine (Red 3) and Rose Bengal B (Red 105), on Thyroid Tumors in Partially Thyroidectomized N-Bis(2-hydroxypropyl)-nitrosamine-treated Rats

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The effects of erythrosine (Red 3), rose bengal B (Red 105) and thyroidectomy on the development of thyroid tumor were examined in male Wistar rats treated with N-bis(2-hydroxypropyl)nitrosamine (DHPN). Red 3 and Red 105 were used at 4% in the basal diet and were administered for 19 weeks from week 2 to 20. Thyroidectomy was performed by resection of the left lobe at week 4. Single injection of DHPN was performed intraperitoneally at 280 mg per 100 g body weight at the beginning of the experiment. Red 3 and Red 105 significantly promoted the development of thyroid tumors in thyroidectomized rats given DHPN, but had no significant effect in non-thyroidectomized rats. The incidence of thyroid tumors was 91% in rats with partial thyroidectomy, Red 3 and DHPN, 100% in rats with partial thyroidectomy, Red 105 and DHPN, and 64% in rats with partial thyroidectomy and DHPN. Serum TSH was 5.5±3.1 ng/ml in rats with partial thyroidectomy, Red 3 and DHPN, 2.1±2.2 ng/ml in rats with partial thyroidectomy, Red 105 and DHPN, and 1.5±0.5 ng/ml in rats with partial thyroidectomy and DHPN.

Key words: N-Bis(2-hydroxypropyl)nitrosamine — Thyroid tumor — Promoter — Erythrosine (Red 3) — Rose bengal B (Red 105)

Erythrosine (Red 3)[*3] (2,4,5,7-tetraiodofluorescein disodium salt, FD & C Red No. 3) and rose bengal B (Red 105) (9-3′,4′,5′,6′-tetrachloro - O - carboxyphenyl - 6 - hydroxy-2,4,5,7-tetraiodo-3-isoxanthone, FD & C Red No. 105) are red dyes widely used as coloring agents in food, soft drinks, drugs, and cosmetics (Fig. 1).

Food Chemical News (October 31, 1983) reported that Red 3 induced thyroid tumors in a few male Sprague-Dawley rats after lifetime feeding.[1] Ito *et al.* reported that $(C57BL/6N \times C3H/N)F_1$ male mice given 0.5% Red 105 for their life time had follicular adenoma in the thyroid at an incidence of 22.9%, which was significantly higher than that in the controls ($P<0.05$).[2]

Propylthiouracil (PTU),[3] 3-amino-1,2,4-triazole (AT),[4] phenobarbital (Pb),[5,6] 4,4′-diaminodiphenylmethane (DDPM),[7] 2,4-diaminoanisole sulfate (DAAS)[8] and potassium iodide (KI)[9] all cause enlargement of the thyroid and promote the development of thyroid tumors in DHPN-treated rats. Long-term treatment with PTU,[10] AT[11] and DAAS[12] induced thyroid tumors in rats. However, Red 3 did not promote the development of thyroid tumors in DHPN-treated male Sprague-Dawley rats.[13]

The purpose of the present experiment was to study the promoting effects of Red 3 and Red 105 on the development of thyroid tumors in DHPN-treated and thyroidectomized male Wistar rats.

MATERIALS AND METHODS

Chemicals and Diet DHPN (Nakarai Chemical Co., Kyoto) was injected ip into male Wistar rats at a dose of 280 mg/100 g body weight at the beginning of the experiment. Control rats received an injection of 0.5 ml saline/100 g body weight.

[*3] Abbreviations: Red 3, food red No. 3 — erythrosine; Red 105, food red No. 105 — rose bengal B; DHPN, N-bis(2-hydroxypropyl) nitrosamine; PTU, propylthiouracil; AT, 2-amino-1,2,4-triazole; Pb, phenobarbital; DDPM, 4,4′-diaminodiphenylmethane; DAAS, 2,4′-diaminoanisole.

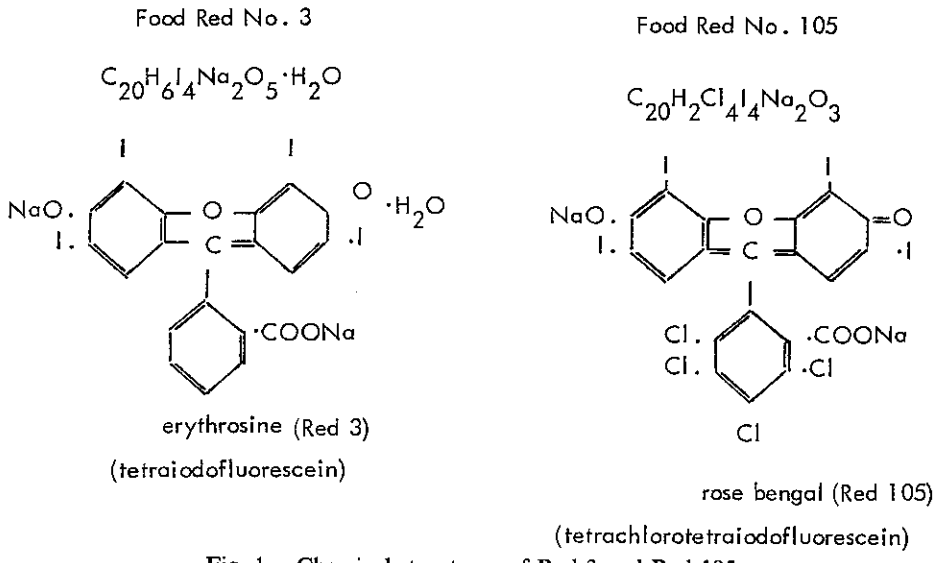

Fig. 1. Chemical structures of Red 3 and Red 105.

The dose of Red 3 (Sanei Chemical Ind., Osaka) or Red 105 (Benifuji Chemical Ind., Tokyo) was 4% in the basal diet (Oriental M, Oriental Kobo Co., Osaka) as in the previous report.[13] Red 3 and Red 105 in the basal diet were given for 19 weeks from week 2 to week 20.

Animals and Experimental Design A total of 170 male 6-week-old inbred Wistar rats were purchased from Kitayama Labes Animals Co., Kyoto, and were fed the basal diet. After 1 week, 168 healthy rats weighing 150–170 g were divided into 9 groups and were given the diets shown in Table I. All animals in groups 1, 3 and 5 had the left thyroid lobe resected at week 4.

The animals were housed in wire cages in an air-conditioned room at 24° and weighed weekly. The experimental duration was 20 weeks. Blood was collected under urethan anesthesia for assay of TSH and thyroid hormone, when the rats were killed. At autopsy, the liver, lungs, kidneys and thyroid gland were weighed and fixed in 10% buffered formalin. Serial sections of thyroid glands were prepared, stained with hematoxylin and eosin, periodic acid-Schiff, Van Gieson and Grimelius, and histologically examined. Liver, lung and kidneys were not examined histologically. The thyroid tumors were counted in the largest serial section. The histologic classification of thyroid tumors reported by Napalkov was used.[14]

The serum concentrations of T4, T3 and TSH in 5 rats of each group were measured by radioimmunoassay; T4 with the T4 Corning radioimmunoassay kit (Corning Medical, Corning Glass Works, Medfield, Mass.); T3 with T3 radioimmunoassay II (Dinabot RI Institute, Tokyo); and TSH by radioimmunoassay with specific rat TSH antiserum provided by Dr. A. F. Parlow (Department of Obstetrics and Gynecology, School of Medicine, Harbor General Hospital, Calif.) under the NIAMDD Rat Pituitary Hormone Program.

Results

Mean Body, Thyroid and Liver Weights Two rats in group 1, one in group 3 and three in group 5 died of hemorrhage after partial thyroidectomy. Two rats in groups 1 and 5, and one in group 3 lost both lobes of the thyroid. Therefore, the effective number of rats was 12 in group 1, 14 in group 3 and 11 in group 5. One in group 3 died of the injection of DHPN. One in group 7 died accidentally.

The mean final body, thyroid and liver weights in each group are shown in Table I. The mean final body weights in the experimental groups were lower than in the control group (group 9). The body weights in groups 1 and 3 were significantly lower than that in group 5. The body weights in groups 2 and 4 were lower than that in group 6, although that in group 2 was not significantly different. The body weights in groups 7 and 8 were lower than that in group 9, but the weight in group 8 not significantly different.

Table I. Effective Numbers of Rats and Means of Final Body, Thyroid and Liver Weights in Thyroidectomized and Non Thyroidectomized Rats Treated with DHPN, Red 3 and Red 105

Group	Treatment			No. of rats	Final BW (g)	TW (mg)	TW/BW×10⁴	LW/BW×10²
	DHPN	TD	Red 3 or 105					
1	+	+	Red 3	12	$331\pm20^{x,C,b,\gamma)}$	$34\pm22^{x,B,a)}$	$1.04\pm0.67^{x,B,a,\beta)}$	$2.9\pm0.2^{B,b)}$
2	+	−	Red 3	19	$343\pm25^{A,\gamma)}$	18 ± 7	$0.54\pm0.19^{A,a)}$	3.1 ± 0.3
3	+	+	Red 105	14	$316\pm21^{C,c,\gamma)}$	$29\pm18^{B,a)}$	$0.90\pm0.54^{l,A,a)}$	$2.4\pm0.2^{m,C,c,\gamma)}$
4	+	−	Red 105	20	$328\pm23^{C,c,\gamma)}$	19 ± 11	$0.58\pm0.35^{A)}$	$2.7\pm0.1^{C,c,\gamma)}$
5	+	+	—	11	364 ± 21	13 ± 7	0.36 ± 0.21	3.2 ± 0.2
6	+	−	—	20	352 ± 12	$20\pm3^{B)}$	0.57 ± 0.08	3.2 ± 0.5
7	−	−	Red 3	19	358 ± 25	$20\pm3^{B,a)}$	$0.58\pm0.10^{A,\beta)}$	$3.0\pm0.2^{A)}$
8	−	−	Red 105	20	$357\pm15^{a)}$	$15\pm2^{c,\gamma)}$	$0.42\pm0.06^{c,\gamma)}$	$2.5\pm0.1^{C,c,\gamma)}$
9	−	−	—	20	$369\pm20^{b)}$	$18\pm1^{A,b)}$	$0.50\pm0.05^{A,b)}$	$3.0\pm0.2^{A)}$

DHPN, N-bis(2-hydroxypropyl)nitrosamine; TD, thyroidectomy; BW, body weight; TW, thyroid weight; LW, liver weight.
Analysis of significance of differences by *t*-test: *x*) $P<0.05$ (compared with group 2); *l*) $P<0.05$, *m*) $P<0.01$ (compared with group 4); *A*) $P<0.05$, *B*) $P<0.01$, *C*) $P<0.001$ (compared with group 5); *a*) $P<0.05$, *b*) $P<0.01$, *c*) $P<0.001$ (compared with group 6); α) $P<0.05$, β) $P<0.01$, γ) $P<0.001$ (compared with group 9).

Table II. Incidence of Thyroid Tumors in Thyroidectomized and Non Thyroidectomized Rats Treated with DHPN, Red 3 and Red 105

Group	Treatment			No. of rats	No. of animals with tumors (%)					
					Adenoma			Carcinoma		
	DHPN	TD	Red 3 or 105		F	P	S	F	P	S
1	+	+	Red 3	12	11 (91)$^{b,\beta)}$	2 (16)	0	5 (41)$^{x,A,b)}$	2 (16)	0
2	+	−	Red 3	19	10 (52)$^{\beta)}$	2 (10)	0	1 (5)	4 (21)$^{a,a)}$	1 (5)
3	+	+	Red 105	14	14 (100)$^{l,A,b,\beta)}$	1 (7)	0	2 (14)	3 (21)	2 (14)
4	+	−	Red 105	20	11 (55)$^{\beta)}$	1 (5)	0	1 (5)	1 (5)	1 (5)
5	+	+	—	11	7 (63)	0	0	0	1 (9)	0
6	+	−	—	20	7 (35)	1 (5)	0	0	0	2 (10)
7	−	−	Red 3	19	0$^{C,b)}$	0	0	0	0	0
8	−	−	Red 105	20	0$^{C,b)}$	0	0	0	0	0
9	−	−	—	20	0$^{C,b)}$	0	0	0	0	0

DHPN, N-bis(2-hydroxypropyl)nitrosamine; TD, thyroidectomy; F, follicular; P, papillary; S, solid.
Analysis of significance of differences by χ^2-test: *x*) $P<0.05$ (compared with group 2); *l*) $P<0.05$ (compared with group 4); *A*) $P<0.05$, *C*) $P<0.001$ (compared with group 5); *a*) $P<0.05$, *b*) $P<0.01$ (compared with group 6); α) $P<0.05$, β) $P<0.01$, γ) $P<0.001$ (compared with group 9).

The mean thyroid weights in groups 1, 2, 3, 4, 6 and 7 were higher than that in group 9. The weights in groups 1 and 3 were significantly higher than in group 5. The weights in groups 2 and 4 were lower than in group 6, but not significantly. The weight in group 7 was significantly higher than that in group 9 and the weight in group 8 was significantly lower than that in group 9.

The ratios of the mean liver weight to the mean body weight in groups 1 and 3 were significantly lower than that in group 5. That in group 4 was significantly lower than that in group 6, but the ratio in group 2 not significantly lower. In groups 4 and 8 the ratios were lower than that in group 9, and in group 4 the ratio was significantly lower than that in group 9.

Table III. Numbers of Thyroid Tumors in Thyroidectomized and Non Thyroidectomized Rats Treated with DHPN, Red 3 and Red 105

Group	Treatment			No. of rats	Mean number of tumors per rat (total number)					
					Adenoma			Carcinoma		
	DHPN	TD	Red 3 or 105		F	P	S	F	P	S
1	+	+	Red 3	12	6.42 (77)$^{x,l,A,a)}$	0.25(3)	0	0.50(6)	0.17(2)	0
2	+	−	Red 3	19	0.42 (8)	0.10(2)	0.05(1)	0.05(1)	0.10(2)	0.10(2)
3	+	+	Red 105	14	7.43(104)$^{x,l,A,a)}$	0.07(1)	0	0.14(2)	0.29(4)	0.14(2)
4	+	−	Red 105	20	0.35 (7)	0.10(2)	0	0.05(1)	0.05(1)	0.05(1)
5	+	+	—	11	1.45 (16)	0	0	0	0.09(1)	0
6	+	−	—	20	0.35 (7)	0.10(2)	0.05(1)	0	0	0.10(2)
7	−	−	Red 3	19	0	0	0	0	0	0
8	−	−	Red 105	20	0	0	0	0	0	0
9	−	−	—	20	0	0	0	0	0	0

DHPN, N-bis(2-hydroxypropyl)nitrosamine; TD, thyroidectomy; F, follicular; P, papillary; S, solid.
Analysis of significance of differences by *t*-test: *x*) $P<0.05$ (compared with group 2); *l*) $P<0.05$ (compared with group 4); *A*) $P<0.05$ (compared with group 5); *a*) $P<0.05$ (compared with group 6).

Table IV. Serum TSH, T4 and T3 in Thyroidectomized and Non Thyroidectomized Rats Treated with DHPN, Red 3 and Red 105

Group	Treatment			No. of rats	TSH (ng/ml)	T4 (μg/dl)	T3 (ng/ml)
	DHPN	TD	Red 3 or 105				
1	+	+	Red 3	12	5.5±3.1$^{z,C,b,\beta)}$	4.4±1.5$^{z,A)}$	83.4±9.4$^{z,a,\gamma)}$
2	+	−	Red 3	19	1.4±0.9	6.7±0.9$^{C,c,\gamma)}$	108.2±24.4$^{C,a)}$
3	+	+	Red 105	14	2.1±2.2	3.3±1.0$^{n)}$	77.4±12.6$^{m,C,\gamma)}$
4	+	−	Red 105	20	1.2±0.6$^{b,\beta)}$	5.4±0.5$^{C,c,\gamma)}$	89.6±4.8
5	+	+	—	11	1.5±0.5	3.4±0.5	83.8±9.8
6	+	−	—	20	1.6±0.2	3.5±0.6	94.8±14.2$^{A)}$
7	−	−	Red 3	19	2.0±0.5$^{A,b)}$	6.9±2.1$^{C,c,\gamma)}$	110.2±14.9$^{C,b,a)}$
8	−	−	Red 105	20	1.0±0.1$^{B,c,\gamma)}$	5.4±0.2$^{C,c,\gamma)}$	95.4±0.9$^{B)}$
9	−	−	—	20	1.9±0.7	3.5±0.8	98.2±20.7$^{A)}$

DHPN, N-bis(2-hydroxypropyl)nitrosamine; TD, thyroidectomy.
Analysis of significance of differences by *t*-test: *z*) $P<0.001$ (compared with group 2); *m*) $P<0.01$, *n*) $P<0.001$ (compared with group 4); *A*) $P<0.05$, *B*) $P<0.01$, *C*) $P<0.001$ (compared with group 5); *a*) $P<0.05$, *b*) $P<0.01$, *c*) $P<0.001$ (compared with group 6); α) $P<0.05$, β) $P<0.01$, γ) $P<0.001$ (compared with group 9).

Incidence of Thyroid Tumors The incidences of thyroid follicular adenomas in groups 1, 2, 3, 4 and 5 were higher than that in group 6 (Table II). The incidence in group 3 was significantly higher than those in groups 5 and 6. The incidence in group 1 was higher than those in groups 5 and 6 and the difference between group 1 and group 6 was significant. The incidence of thyroid cancers was significantly higher in group 1 than in groups 5 and 6.

The mean number of thyroid tumors in each group is summarized in Table III. There were significantly more follicular adenomas in groups 1 and 3 than in groups 5 and 6.

Serum TSH The serum TSH levels were higher in groups 1 and 3 than in group 5 (Table IV) and the difference between group 1 and group 5 was significant. The level was significantly lower in group 4 than in group 6. Serum TSH level was significantly lower in group 8 than in group 9.

Serum T4 The concentrations of serum T4 in groups 2 and 4 were significantly higher than that in group 6 (Table IV), and the levels in groups 7 and 8 were significantly higher than that in group 9. The level in group 1 was significantly different from that in group 5, but the level in group 3 was not.

Serum T3 The concentrations of serum T3 in groups 1 and 3 were lower than that in group 5, but there was no significant difference between groups 1 and 5. The concentration of serum T3 in group 2 was significantly higher than that in group 6. It was lower in group 4 than in group 6 (but not significantly different). It was significantly higher in group 7 than in group 9 and lower in group 8 than in group 9.

Discussion

The present studies indicate that Red 3 and Red 105 promoted the development of thyroid tumors in partially thyroidectomized male Wistar rats treated with DHPN, but not in non-thyroidectomized rats.

There are various reports on promoters of thyroid tumors such as PTU,[3] AT,[4] Pb,[5,6] DDPM[7] and DAAS.[8] PTU[3] and AT[4] induce enlarged thyroids. Red 3 and Red 105 induce significantly enlarged thyroids in partially thyroidectomized rats but not in non-thyroidectomized rats. PTU inhibits iodine oxidation, iodination and coupling,[15] decreasing serum T4 and T3. TSH secretion is stimulated by decreased serum levels of T4 and T3. The increase in serum TSH then induces goiter with small follicles.[3] Pb, which did not induce small follicles, induced an enlarged liver but Red 3 and Red 105 did not. Pb stimulates drug-metabolizing enzymes.[16] Therefore, turnover of T4 and T3 might be accelerated by the stimulation of drug-metabolizing enzymes induced by Pb. The effect of Red 3 or Red 105 might involve a different mechanism from that of PTU or Pb.

An increase in T4, decreases in T3 and thyroid peroxidase activity, and no significant increase of TSH were observed in rats treated with Red 3 for 2 weeks.[17] However, KI inhibits the secretion of T4, and T3 has a variable inhibitory effect on it.[18] Red 3 and Red 105 contain iodine (I) within the molecules. Therefore Red 3 and Red 105 might inhibit secretion of T4 and T3 as KI does. The serum TSH level may be influenced by Red 3 or Red 105 through the fluctuation of T4 and T3 levels thus induced, although Red 3 and Red 105 did not induce goiter or small follicles, as KI does not.[9]

The effects of Red 3 and Red 105 on serum TSH, T4 and T3 were not as consistent and not as strong as that of KI. KI promoted the development of thyroid tumors in rats treated with DHPN.[9] Red 3 and Red 105 promoted the development of thyroid tumors in thyroidectomized rats treated with DHPN, but did not in non-thyroidectomized rats treated with DHPN.

Partial thyroidectomy increases serum TSH and induces thyroid regeneration.[19,20] Red 3 and Red 105, having a KI-like effect presumably due to their iodine atoms, may thus promote the development of thyroid tumors in DHPN-treated rats by increasing serum TSH synergistically with partial thyroidectomy.

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