ANTI-THYROID HORMONAL ACTIVITY OF THE FLAME RETARDANTS, TETRABROMOBISPHENOL A AND RELATED COMPOUNDS BY A YEAST TWO-HYBRID ASSAY

Hiroaki Kuroki[1], Satoko Sakoda[1], Hitomi Nakaoka[1], Sayuri Yonekura[1], Misato Kumate[1], Jun-ichi Nishikawa[2] and Tsutomu Nishihara[2]

[1]Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka, 815-8511, Japan
[2]Graduation School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan

Introduction

Tetrabromobisphenol A (TBBPA) is a brominated derivative of bisphenol A (BPA), known as a typical xenoestrogen, and one of the most widely used brominated flame retardant throughout the world. TBBPA has been found in river sediment (20μg/kg on a dry weight basis) in Osaka, Japan[1] and in sediment (34~270 ng/g on dry weight basis) and sewage sludge (31~56 ng/g on dry weight basis) in Sweden[2]. TBBPA was also detected in indoor air samples at an electronics recycling plant at pmol/m^3 level[3]. In addition, TBBPA was determined in human samples, e.g., plasma samples from different occupational groups in Norway[4]. In these human plasma samples TBBPA was found at ng/g lipid weight level. Therefore, TBBPA is considered to be a ubiquitous contaminant in the environment.

Recent studies from *in vitro* experiments indicate that TBBPA exhibit some biological activities. For examples, TBBPA has been shown to have a strong affinity for the transthyretin, one of the thyroid hormone-binding transport proteins in blood[5]. Quite recently, thyroid hormonal activity in rat pituitary tumor cell line GH3 and also estrogenic activity in rat pituitary tumor cell line MtT/E-2 of TBBPA were reported[6]. However, the steroid hormonal-disrupting potency of TBBPA has not yet been clarified in detail. In the preceding paper[7], we reported the estrogenic potency of hydroxylated PCBs by a yeast two-hybrid assay and also demonstrated that the assay system was useful to investigate the steroid hormonal activity of chemicals that interact with steroid hormone receptor.

In this study, therefore we examined thyroid hormonal and anti-thyroid hormonal activities of TBBPA and related compounds (Tetrachlorobisphenol A (TCBPA), Pentachlrophenol (PCP), 2,4,6-tribromophenol and 4-OH-3,3',4',5-tetrachlorobiphenyl (tetraCB)) by the yeast two-hybrid assay. We demonstrate that TBBPA, TCBPA and 4-OH-3,3',4',5-tetraCB, metabolite of CB-77, show the anti-thyroid hormonal activities.

Materials and Methods

Chemicals

TBBPA, TCBPA, PCP, 2,4,6-tribromophenol and BPA were obtained commercially. 4-OH-3,3',4',5-tetraCB was synthesized by Ullmann reaction.

Thyroid hormonal and anti-thyroid hormonal activity assay

Thyroid hormonal and anti-thyroid hormonal activities of chemicals were determined by the yeast two-hybrid assay[8] based on the ligand-dependent interaction of thyroid hormone receptor α(TRα) with coactivator, TIF2. We used the yeast transformants, which expresses two fusion proteins, GAL4 DNA binding domain-thyroid hormone receptor ligand binding domain and GAL4 activation domain -TIF2.

The yeast harbors a GAL4 binding site upstream of a *lac*Z reporter gene. Depending on the strength of interaction of the two fusion proteins, the reporter gene, ß-galactosidase is activated. Therefore, thyroid hormonal activities of chemicals are determined by measuring the activity of ß-galactosidase. The procedure of this assay is described previously by Nishikawa *et. al*[8]. In brief, after the yeasts were incubated with chemicals (final concentration:10^{-9}~10^{-4} M) for 4 hr at 30 ºC, ß-galactosidase activities were determined according to the method previously reported[8]. Anti-thyroid hormonal activity was also determined by incubating the yeast with chemicals (10^{-9}~10^{-4} M) in the presence of 5×10^{-8} M of thyroxine (T3).

Cytotoxic assay

The yeast control system (p53-SV40) was used for cytotoxic assay of chemicals. The control yeasts were incubated with chemicals (10^{-9}~10^{-4} M) under the same condition mentioned above and the ß-galactosidase activities were compared to that of DMSO.

Results and Discussion

Thyroid hormonal activity of TBBPA and related compounds

In the yeast two-hybrid assay system (TRα-TIF2), 10^{-8} M of T3 caused slightly an induction of ß-galactosidase activity and the activity increased with T3 concentration (10^{-8}~10^{-5} M). The induced ß-galactosidase activity was almost saturated at 10^{-6} M of T3. These results indicate that the yeast two-hybrid assay system is useful for determining the chemicals that interact with TRα. The thyroid hormonal activities of TBBPA and related compounds were examined in this assay system. However, all these chemicals did not exhibit the induction of ß-galactosidase activity in the range of 10^{-9}~10^{-4} M suggesting that TBBPA and related compounds do not have thyroid hormonal potency.

Anti-thyroid hormonal activity of TBBPA and related compounds

The inhibitory activities of TBBPA and related compounds against TRα were also investigated in the yeast two-hybrid assay. To determine the optimal concentration of T3 in this assay, we examined the inhibitory effect of TBBPA (10^{-5} M) on the ß-galactosidase activity induced by T3 at the concentration of 10^{-8}~5×10^{-7} M. From the results of this experiment (data not shown), we fixed the concentration of T3 to 5×10^{-8} M. So the anti-thyroid hormonal activity of chemicals were determined by co-treating the yeast with 10^{-9} ~ 10^{-4} M concentrations of chemicals and 5×10^{-8} M T3. As shown in Fig. 1, the ß-galactosidase activity induced by T3 (5×10^{-8} M) were inhibited by TBBPA, TCBPA and 4-OH-3,3',4',5-tetraCB at the dose-dependent manner (~10^{-5} or 10^{-4} M). In contrast, no inhibitory effect of BPA was observed (10^{-9} ~ 10^{-4} M). The cytotoxicities of TBBPA and related compounds were examined by the yeast control system (p53-SV40) to confirm the antagonistic activity of these compounds. TBBPA, TCBPA and 4-OH-3,3',4',5-tetraCB did not show the cytotoxicity at the concentration range of 10^{-9}~10^{-5} M or 10^{-9}~10^{-4} M (data not shown). These results indicate that TBBPA, TCBPA and 4-OH-3,3',4',5-tetraCB exhibit anti-thyroid hormonal activities at 5×10^{-6}~10^{-5} M for the former two and 5×10^{-5}~10^{-4} M for the latter, respectively (Fig. 1). PCP and 2,4,6-tribromophenol also inhibited the ß-galactosidase activity induced by T3, but cytotoxic effects of these chemicals were observed at the same concentration range. Consequently, PCP and 2,4,6-tribromophenol were not judged as antagonist for TRa. The structural requirements for anti-thyroid hormonal activity of chemicals were suggested that a 4-hydroxy group and two adjacent halogen substituents in the phenyl group were necessary factors. Antagonistic activities of TBBPA, TCBPA and 4-OH-3,3',4',5-tetraCB were 42, 35 and 42 %, respectively, when the ß-galactosidase activities at the highest concentration with no cytotoxicity were compared to that of 5×10^{-8} M T3 (100 %). The order of anti-thyroid hormonal potency was TBBPA, TCBPA > 4-OH-3,3',4',5-tetraCB.

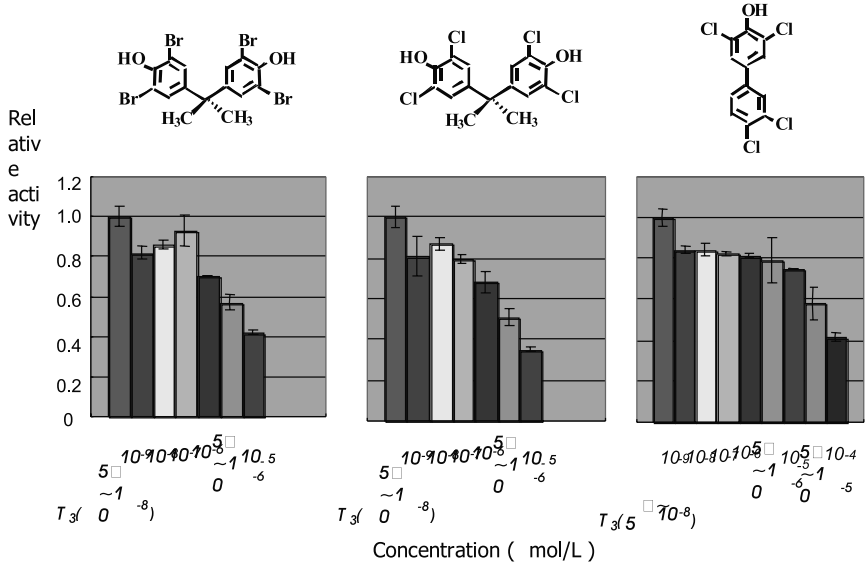

Fig.1 Assay for antagonistic activity of TBBPA, TCBPA and 4-OH-3,5,3',4'-tetraCB to thyroid hormone receptor

In conclusion, TBBPA and TCBPA exhibited anti-thyroid hormonal activity, but BPA showed no agonistic and antagonistic activity toward TRa. 4-OH-3,3',4',5-tetraCB, hydroxylated metabolite of CB-77, also showed anti-thyroid hormonal activity. On the contrary, recent study on thyroid hormonal activity of TBBPA and TCBPA was reported[6]. This discrepancy maybe due to the differences of cells used in both studies. Further studies are needed to reveal the thyroid hormonal-disrupting activity of TBBPA and related compounds.

References

1. Watanabe, I., Kashimoto, T, and Tatsukawa, R. (1983) Bull.Environ.Contam. Toxicol. 31,48-52.
2. Sellstrom, U. and Jansson, B. (1995) Chemosphere 31, 3085-3092.
3. Sjodin, A., Carlsson, H., Thuresson, K., Sjolin, S., Bergman, A. and Ostman, C. (2001) Environ. Sci. Technol. 35, 448-454.
4. Thomsen, C., Lundanes, E. and Becher, G. (2001) J. Environ. Monit. 3, 366-370.
5. Meerts, I.A.T.M., van Zanden, J.J., Luijks,E.A.C., van Leeuwen-Bol,I., Marsh,G., Jakobsson, E.,Bergman, A. and Brouwer, A. (2000) Toxicological Sciences 56, 95-104.
6. Kitamura, S., Jinno, N., Ohta, S., Kuroki, H. and Fujimoto, N. (2002) B.B.R.C. 293, 554-559.
7. Kuroki, H., Yonekura, S., Sakoda, S., Aramaki, H., Koga, N., Nishikawa, J. and Nishihara, T. (2001) Organohalogen Compounds 53, 80-83.
8. Nishikawa, J., Saito, K., Goto, J., Dakeyama, F., Matsuo, M. and Nishihara, T. (1999) 154, 76-83.