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Research Note

Inhibition of Respiratory Activities of *Giardia lamblia* by Halogenated Bisphenols

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Previous studies on halogenated bisphenols in our laboratory disclosed that bithionol, dichlorophene and hexachlorophene were able to sterilize *Entamoeba histolytica, Trichomonas vaginalis* and *Giardia lamblia* at the concentrations of 0.15 to 0.6 mM in cultures (Kawasaki and Takeuchi, 1984; Takeuchi *et al.*, 1984; 1985). Since these bisphenolic derivatives also inhibited the aerobic metabolism of *E. histolytica* at comparable concentrations (Kawasaki and Takeuchi, 1984; Takeuchi *et al.*, 1984), we suggested that such inhibitory effects may be responsible, at least partially, for killing this parasite *in vitro*, although physiological significance of its aerobic metabolism is not known. The present study was designed to investigate effects of these bisphenolic derivatives on the aerobic metabolism of another bisphenolic derivatives-sensitive protozoon, *G. lamblia*.

Trophozoites of *G. lamblia* (Portland I strain) were grown in BI-S-33 medium (Diamond *et al.*, 1978) as modified by Keister (1983). After cultivating for 72 hr at 35.5°C, the parasites were harvested and washed as described previously (Takeuchi *et al.*, 1985) except that only 50 mM Tris-HCl buffer, pH 7.4 containing 0.25 M sucrose was employed for washing the parasites. Finally, the parasites were suspended in the buffered sucrose in a final protein concentration of 5 to 8 mg/ml.

The large granule fraction, i.e., 15,000 g × 20 min pellet, was prepared in the same manner as isolation of the subcellular fractions of *T. vaginalis* (Takeuchi and Kobayashi, 1982) except that trophozoites of *G. lamblia* suspended as above were disrupted by homogenization for 10 min. The resulting pellet was washed once and finally suspended in the buffered sucrose to yield 1.1 to 2.3 mg protein/ml.

Respiratory activities, i.e., endogenous respiration, NADH oxidase and NADPH oxidase activities, were determined polarographically at 30°C according to Weinbach and Diamond (1974) with a Clark type oxygen electrode (Rank Bros., Bottisham, England, UK). Endogenous respiration and the activities of oxidases were assayed using the intact trophozoites and the large granule fraction respectively, since our preliminary study, performed on the basis of Weinbach *et al.* (1980), demonstrated the oxidase activities in the large granule fraction and the 110,000g × 120 min supernatant fluid, the former of which showed the significantly higher enzymatic activities. Halogenated bisphenols, i.e., bithionol [2,2′-thiobis (4,6-dichlorophenol)], dichlorophene [2,2′-methylenebis (4-chlorophenol)], hexachlorophene [2,2′-methylenebis (3,4,6-trichlorophenol)], tetrach-

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lorobisphenol A [2,2-bis (3,5-dichloro-4-hydroxyphenyl) propane] and tetrabromobisphenol A [2,2-bis (3,5-dibromo-4-hydroxyphenyl) propane], were supplied by Tokyo Kasei Inc. (Tokyo, Japan). All chemicals were of the highest purity commercially available. Preparation of the bisphenolic solutions and consequent control experiments were done as described previously (Takeuchi *et al.*, 1984). Protein concentrations were estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Table 1 summarizes concentrations of these bisphenolic derivatives needed for 50% inhibition of endogenous respiration, and the activities of NADH and NADPH oxidase. Among the derivatives tested, tetrachlorobisphenol A and tetrabromobisphenol A seemed more inhibitory, while bithionol, dichlorophene and hexachlorophene also inhibited the respiratory activities at the concentrations comparable to those required for inhibiting the endogenous and 2-propanol-supported respiration of *E. histolytica* (Takeuchi *et al.*, 1984). Although addition of 0.5 mM menadione (methanol solution) increased the activities of NADH- and NADPH-supported respiration in the large granule fraction and far more pronouncedly in the 110,000g supernatant fluid, suggesting the presence of diaphorase activities which seemed compatible with Weinbach *et al.* (1980), the rates of inhibition by halogenated bisphenolic derivatives of these menadione-linked NADH and NADPH-supported respiration were much lower than those of the oxidase activities. For instance, more than 1 mM bithionol was required for 50% inhibition of menadione-linked NADPH-supported respiration in the supernatant fluid. Monophenolic derivatives like 2,4,6-tribromo-3-hydroxytoluene, 4,6-dichlororesorcinol and 2,4-dichlorophenoxyacetate were also barely inhibitory at more than 1 mM, which appears to be well in accord with the data on the respiration of *E. histolytica* (Takeuchi *et al.*, 1984).

Table 1 Inhibition of endogenous respiration, and the activities of NADH and NADPH oxidase in *Giardia lamblia* by bisphenolic derivatives

Bisphenols added	Concentrations needed for 50% inhibition (mM)		
	Endogenous respiration	NADH oxidase	NADPH oxidase
Bithionol	0.45	0.40	0.21
Dichlorophene	0.75	0.82	0.48
Hexachlorophene	0.47	0.50	0.20
Tetrachlorobisphenol A	0.23	0.13	0.11
Tetrabromobisphenol A	0.30	0.15	0.15

Note: Data are average values of at least three independent determinations. The assay mixture for NADH oxidase contained the large granule fraction of *G. lamblia* (1.1 to 2.3 mg protein), 0.5 mM NADH and 50 mM Tris-HCl buffer, pH 7.4 containing 0.25 M sucrose in a final volume of 2 ml. NADPH oxidase activity was determined using the same assay mixture except that NADH was replaced by 0.5 mM NADPH. Endogenous respiration was assayed with the same assay mixture as above, except that higher amounts of the intact trophozoites of *G. lamblia* (5.3 to 6.9 mg protein) were employed and no substrates were added. The bisphenolic derivatives were added before or after the respiratory reactions were measured for approximately 0.5 to 1 min. Under the present experimental conditions, the respiratory activities proceeded linearly for at least 2 min.

These observations suggest that the bisphenolic derivatives are able to inhibit respiratory activities of *G. lamblia* at the concentrations needed for sterilizing trophozoites of this parasite in the modified BI-S-33 medium (Takeuchi *et al.*, 1985). It seems possible, therefore, that the inhibition of the respiratory activities in *G. lamblia* may be responsible, at least partially, for *in vitro* killing of this parasite as suggested on *E. histolytica* (Takeuchi *et al.*, 1984), although physiological significance of these respiratory activities is not known. The relatively low susceptibility of menadione-linked NADH and NADPH-supported respiration to the bisphenolic derivatives was also found in *E. histolytica* (Takeuchi *et al.*, unpublished observation); however, the reason is not known at present.

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ランブル鞭毛虫の呼吸活性に対するハロゲン化ビスフェノール誘導体の阻害作用

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ランブル鞭毛虫の *in vitro* での増殖がハロゲン化ビスフェノール誘導体によって強く阻害されるので，呼吸活性（内因性呼吸，NADH oxidase, NADPH oxidase）に対する効果を検討した．その結果，ビチオノール，ジクロロフェン，ヘキサクロロフェンの呼吸活性に対する50%阻害濃度は *in vitro* での殺虫濃度とほぼ同程度(0.2～0.8mM) であった．一方，テトラクロロビスフェノールA，テトラブロモビスフェノールAの50%阻害濃度はやや低いように思われた．これらのデータは呼吸活性の阻害がハロゲン化ビスフェノール誘導体の *in vitro* の殺虫作用のメカニズムの1つである可能性を示唆する．