Conversion of Dieldrin to Aldrin by Intestinal Bacteria in Rats

Shigeyuki KITAMURA,* Masashi MITA, Yuri SHIMIZU, Kazumi SUGIHARA, and Shigeru OHTA

Institute of Pharmaceutical Science, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Received April 8, 1999; accepted July 21, 1999

The present study provides the evidence that dieldrin is reductively metabolized to aldrin by intestinal bacteria in rats. When dieldrin was incubated with the cecal contents of rats, aldrin, a reduced metabolite of the epoxide, was isolated from the incubation mixture. The metabolite was identified unequivocally by UV and mass spectral comparison with an authentic sample, and on the basis of its TLC and HPLC behavior. The cecal contents of rats exhibited epoxide reductase activity toward dieldrin under anaerobic conditions. However, only marginal activity was observed under aerobic conditions. Four pure strains of intestinal bacteria exhibited epoxide reductase activities to varying degrees under anaerobic conditions. The highest activity was observed in *Clostridium sporogenes*. Cell-free extracts of the intestinal bacteria in rat cecal contents showed reductase activity when supplemented with both NAD(P)H and FMN under anaerobic conditions.

Key words epoxide reduction; dieldrin; intestinal bacteria; aldrin; rat cecal contents

Dieldrin, an organochlorine pesticide used in large quantities, is extremely persistent in soil sediments.¹⁾ Dieldrin, which is carcinogenic in mice,²⁾ is also an environmental estrogen.^{3,4)} It has been reported that dieldrin induces oxidative stress in the liver and depletes monoamines in the brain.^{5,6)} Dieldrin accumulates in animal tissues because the pesticide is lipophilic and slowly metabolized in animal tissues.^{7,8)} To assess the possible risks associated with human exposure to the pesticide, it is essential to elucidate its metabolism in mammalian species.

Of the epoxide compounds, the arene oxides are known to be reduced to their corresponding arenes.^{9–11)} The participation of cytochrome P450 in microsomal arene oxide reduction was demonstrated mainly using benz[a]pyrene 4,5-oxide as a substrate.^{9,10)} The arene oxide was also reduced by aldehyde oxidase, a cytosolic enzyme.¹¹⁾ However, to the authors' knowledge the reduction of olefin oxides such as dieldrin has not been reported previously. Sugiura *et al.*⁹⁾ found that benzo[a]pyrene 7,8-dihydrodiol 9,10-oxide was not reduced by the cytochrome P450 system of rat liver microsomes. Hirao *et al.*¹¹⁾ also reported that the reduction of olefin oxides such as styrene oxide and stilbene oxide was not catalyzed by liver aldehyde oxidase.

It has been shown that aldrin is mainly metabolized to dieldrin by oxidation of the double bond in mammalian species.¹²⁾ However, no report is available concerning the reductive metabolism of dieldrin to aldrin by epoxide reduction. In the present study, the metabolism of dieldrin by the intestinal bacteria of rats was examined, focusing on epoxide reduction.

Dieldrin and aldrin were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). *Clostridium sporogenes*,

Escherichia coli, *Lactobacillus rhamnosus*, and *Streptococcus faecalis* were obtained from the Institute for Fermentation (Osaka, Japan). The cecal contents of male Wistar rats (Slc: Wistar/ST; 185–240 g) were suspended in GAM broth (Nissui, Tokyo, Japan) and gently centrifuged at 500×g for 5 min to exclude food materials. Facultative anaerobic bacteria were grown in *l*-broth at 37 °C and harvested by centrifugation. Bacteria were suspended with sterilized 0.1 M K,Na-phosphate buffer (pH 7.4). Anaerobic bacteria were grown in GAM broth using an anaerobic jar. Bacteria harvested by centrifugation were suspended in sterilized GAM broth. The concentration of the bacteria suspensions was adjusted on the basis of absorbance at 600 nm. When the absorbance was 0.1, the bacteria concentration was estimated to be 2×10⁸/ml. Cecal contents were suspended in sterilized 0.1 M K,Na-phosphate buffer and sonicated. The protein concentration was determined by the method of Lowry *et al.*¹³⁾ with bovine serum albumin as the standard protein.

For the isolation of dieldrin metabolites by cecal contents, the incubation mixture consisted of dieldrin 10 µmol and rat caecal contents in a final volume of 20 ml of 0.1 M phosphate buffer (pH 7.4). The incubation was performed at 37 °C for 3 h under a nitrogen atmosphere. The mixture was extracted with benzene, and the extract was evaporated to dryness and dissolved in methanol. An aliquot was subjected to HPLC to determine the presence of the metabolite. HPLC confirmed that the extract contained a metabolite corresponding to aldrin. The metabolite was purified from the benzene extract by HPLC.

For assay of epoxide reductase activity, an incubation was performed at 37 °C for 3 h under a nitrogen atmosphere in a Thunberg tube. A typical incubation mixture consisted of dieldrin 0.5 µmol, an electron donor 1 µmol, FMN 0.2 µmol, and an enzyme source in a final volume of 2 ml of 0.1 M phosphate buffer (pH 7.4). When cecal contents or bacteria were used, an electron donor and FMN were omitted, and 1 ml (equivalent to 20 mg) of caecal contents or 1 ml (8–13×10¹⁰ bacteria) of intestinal bacterial suspension was added. After incubation of the mixture, biphenyl 100 µg was added as an internal standard, and the mixture was extracted with benzene 5 ml. The extract was evaporated and analyzed by HPLC. HPLC was performed on a Hitachi L-6000 chromatograph (Tokyo, Japan) fitted with a 250×4.6 mm Capcell Pak C₁₈ UG 120 Å (5 µm) column (Shiseido, Tokyo, Japan). The mobile phase was acetonitrile–H₂O (7:3, v/v). The chromatograph was operated at a flow rate of 1.0 ml/min at ambient temperature and at a wavelength of 210 nm. The elution times of dieldrin, aldrin, and diphenyl (an internal standard) were 15.5, 37.5, and 9.0 min, respectively.

The cecal contents of rats had the ability to catalyze the reduction of dieldrin to aldrin under anaerobic conditions. However, little activity was observed under aerobic conditions. When the cecal contents were sonicated or boiled, the reductase activity was abolished (Fig. 1). In contrast, rat liver slices did not exhibit epoxide reductase activity toward dieldrin. Aldrin formed from dieldrin by the cecal contents of rats was identified unequivocally by comparing its mass and UV spectra with those of the authentic sample (data not shown).

The ability of four types of intestinal bacteria to reduce dieldrin was examined. These bacteria exhibited varying de-

* To whom correspondence should be addressed.

Fig. 1. Reduction of Dieldrin to Aldrin by the Cecal Contents of Rats

Each value represents the mean of three experiments. Reactions were conducted at 37 °C for 3 h with rat cecal contents under anaerobic conditions. Aldrin formation was determined by HPLC.

Fig. 2. Reduction of Dieldrin to Aldrin by Various Intestinal Bacteria

Each value represents the mean of three experiments. Reactions were conducted at 37 °C for 3 h with bacterial strains under anaerobic conditions. Aldrin formation was determined by HPLC.

degrees of reducing activity. Among the strains tested, the highest activity was observed in *Clostridium sporogenes*, and the lowest in *Lactobacillus rhamnosus* under anaerobic conditions (Fig. 2).

The ability of sonicated cell-free extracts of intestinal bacteria in the cecal contents of rats to convert dieldrin to aldrin was examined. When NADH, NADPH, or FMN was added to the incubation mixture, limited reducing activity was observed. However, the activity was enhanced by the addition of both NADH and FMN, or NADPH and FMN, under anaerobic conditions (Fig. 3). When cell-free extracts were boiled, reduction did not occur in the presence of cofactors. The reducing activity of cell-free extracts was not observed under aerobic conditions (data not shown).

Previous researchers have suggested that olefin oxides cannot be reduced in the animal body based on findings that the olefin oxides are not reduced by liver microsomes or cytosol *in vitro*.^{9,11} The present study provides the evidence that the epoxide of dieldrin is reduced by intestinal bacteria. It has been reported that dieldrin is mainly metabolized to the diol form by epoxide hydrolase.¹⁴ We have shown that the reductive metabolism is a new metabolic pathway of dieldrin. It is known that aldrin is oxidized to dieldrin by mixed function oxidase. Thus interconversion between dieldrin and aldrin occurs in the animal body (Fig. 4). The toxicity of aldrin and dieldrin does not differ significantly in animals. This interconversion may therefore be important in the maintenance of

Fig. 3. Reduction of Dieldrin to Aldrin by Cell-free Extracts of Intestinal Bacteria in Rat Cecal Contents

Each value represents the mean of three experiments. Rat cecal contents were sonicated, and the supernatants were used as cell-free extracts. The incubation mixture consisted of dieldrin 0.5 μmol, NAD(P)H 1 μmol, FMN 0.2 μmol, and cell-free extracts 0.5 ml in a final volume of 2 ml of 0.1 M phosphate buffer (pH 7.4). Reactions were conducted at 37 °C for 3 h under anaerobic conditions. Aldrin formation was determined by HPLC.

Fig. 4. Interconversion between Dieldrin and Aldrin

toxicological activity and accumulation of dieldrin in the animal body. *Clostridium sporogenes*, *Lactobacillus rhamnosus*, *Streptococcus faecalis* and *Escherichia coli*, which are usually present in human microflora, show epoxide reducing activity toward dieldrin. In humans, dieldrin is thought to be reductively metabolized to aldrin by intestinal bacteria, as shown in rats.

The importance of intestinal bacteria in the metabolism of xenobiotics has been increasingly recognized. Scheline reviewed the reduction of certain xenobiotics by intestinal bacteria.¹⁵ The involvement of intestinal bacteria in the reduction of nitro, azo, sulfoxide, hydroxamic acid, and double bond compounds, the reductive dehalogenation of α-(bromoisovaleryl)urea, and the reductive cleavage of the benzisoxazole ring have been reported.^{15–20} However, little is known about the reduction of olefin oxides by intestinal bacteria. Fukushima *et al.*²¹ have reported that an epoxysuccinic acid derivative was reduced by rat cecal contents; the corresponding olefin derivative was tentatively identified as an intermediate.

Cell-free extracts from intestinal bacteria in rat cecal contents exhibited NAD(P)H- and FMN-dependent epoxide reductase activity toward dieldrin. Recently, we reported that a similar flavin-dependent enzyme system in cell-free extracts of intestinal bacteria functions in the reduction of 1-nitropyrene, zonisamide, and α-(bromoisovaleryl) urea.^{19,20,22} The reduction of these compounds by intestinal bacteria appears to be catalyzed by a flavin-dependent bacterial reductase with reduced flavin as an electron donor. A detailed investigation of the reduction mechanism of xenobiotics by intestinal bacteria is under way.

REFERENCES

- 1) Santerre C. R., Blazer V. S., Khanna N., Reinert R. E., Barrows F. T., *Bull. Environ. Contam. Toxicol.*, **58**, 334—340 (1997).
- 2) Thorpe E., Walker A. I., *Food Cosmet. Toxicol.*, **11**, 433—442 (1973).
- 3) Gellert R. J., *Environ. Res.*, **16**, 131—138 (1978).
- 4) Wade M. G., Desaulniers D., Leingartner K., Foster W. G., *Reprod. Toxicol.*, **11**, 791—798 (1997).
- 5) Bachowski S., Xu Y., Stevenson D. E., Walborg E. F. Jr., Klaunig J. E., *Toxicol. Appl. Pharmacol.*, **150**, 301—309 (1998).
- 6) Sanchez-Ramon J., Facca A., Basit A., Song S., *Exp. Neurol.*, **150**, 263—271 (1998).
- 7) Gilroy D. J., Carpenter H. M., Siddens L. K., Curtis L. R., *Fund. Appl. Toxicol.*, **20**, 295—301 (1993).
- 8) Ejobi F., Kanji L. W., Kyule M. N., Müller P., Krüger J., Latigo A. A. R., *Bull. Environ. Contam. Toxicol.*, **56**, 873—880 (1996).
- 9) Sugiura M., Yamazoe Y., Kamataki T., Kato R., *Cancer Res.*, **40**, 2910—2914 (1980).
- 10) Wrighton S. A., Fahl W. E., Shinnick F. L. Jr., Jefcoate C. R., *Chem.-Biol. Inter.*, **40**, 345—356 (1982).
- 11) Hirao Y., Kitamura S., Tatsumi K., *Carcinogenesis*, **15**, 739—743 (1994).
- 12) Nakatsugawa T., Ishida M., Dahm P. A., *Biochem. Pharmacol.*, **14**, 1853—1865 (1965).
- 13) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265—275 (1951).
- 14) Matthews H. B., Matsumura F., *J. Agr. Food Chem.*, **17**, 845—852 (1969).
- 15) Scheline R. R., *Pharmacol. Rev.*, **25**, 451—523 (1973).
- 16) Gingell R., Bridges J. W., Williams R. T., *Xenobiotica*, **1**, 143—156 (1971).
- 17) Ishida M., Kitamura S., Tatsumi K., *Biochem. Mol. Biol. Int.*, **39**, 1157—1165 (1996).
- 18) Williams J. R., Grantham P. H., Marsh H. H., Weisburger J. H., *Biochem. Pharmacol.*, **19**, 173—188 (1970).
- 19) Kitamura S., Kuwasako M., Ohta S., Tatsumi K., *J. Pharm. Pharmacol.*, **51**, 79—84 (1999).
- 20) Kitamura S., Sugihara K., Kuwasako M., Tatsumi K., *J. Pharm. Pharmacol.*, **49**, 253—256 (1997).
- 21) Fukushima K., Arai M., Suwa T., Satoh T., *Drug Metab. Dispos.*, **18**, 264—266 (1990).
- 22) Narai N., Kitamura S., Tatsumi K., *J. Pharm. Dyn.*, **7**, 407—413 (1984).