AH-RECEPTOR-MEDIATED INDUCTION OF DRUG-METABOLIZING ENZYMES BY INDIRUBIN AND INDIGO

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Introduction

Indigo and indirubin have been used to dye cloth for denims and blue jeans[1)]. Indigo-producing plants have also been used in traditional Chinese medicine[2)] and indirubin was identified as an active ingredient against leukemias[3)]. Pathways for the endogenous production of indirubin and indigo in the human body were postulated by Gillam *et al.*[4)] (Fig. 1). The formation of indoxyl and isatin, which are intermediates in the formation of indirubin or indigo from indole, is catalyzed by human cytochrome P450s. Recently, Adachi *et al.*[5)] identified the endogenous ligands of AhR in human urine as indirubin and indigo, using recombinant yeast assay[6)]. The ligands were extracted from acid-treated human urine and FBS (fetal bovine serum), and their levels in the body were high enough to activate the AhR, 0.2 nM and 0.07 nM, respectively. Aryl hydrocarbon receptor (AhR) is a ligand-binding transcription factor which was isolated as a TCDD receptor in the cell, but it remains an orphan receptor. The AhR ligand activity of indigo was about the same as that of TCDD, and the potency of indirubin was 50 times that of TCDD[5)]. Thus, they are expected to have an important physiological role *in vivo*. In this study, we examined the physiological effect of indirubin and indigo in mice *in vivo*.

Materials and Methods

Animals and treatment

Male C57BL/6J:Jcl mice (5-6 weeks old) from CLEA Japan, Inc. (Tokyo) were housed in the cages at 22 °C with a 12-hr light/dark cycle, with free access to tap water and a standard pellet diet. *Ahr*-deficient mice (*Ahr* $^{-/-}$) were maintained in the Research Facilities for Laboratory Animal Science, Hiroshima University. Male C57BL/6J:Jcl mice were given indirubin or indigo (1-50 mg / kg body weight) dissolved in Panacete 810^{TM} (5 mL / kg) by gavage or by intraperitoneal injection for three days. Vehicle control mice were given the same volume of Panacete 810^{TM}. Male *Ahr*$^{-/-}$ mice (5-6 weeks old) were treated with indirubin or indigo (5 mg/kg body weight) in the same manner as *Ahr*$^{+/+}$ (wild : C57BL/6J:Jcl) mice.

Preparation of liver microsomes and enzyme assays

One day after the last dose, mice were killed and the livers were quickly removed. Microsomes were prepared according to usual methods. The ethoxyresorufin-*O*-dealkylase (EROD), methoxyresorufin-*O*-dealkylase (MROD) and pentoxyresorufin-*O*-dealkylase (PROD) activities in liver microsomes were assayed by a fluorophotometric method[7)].

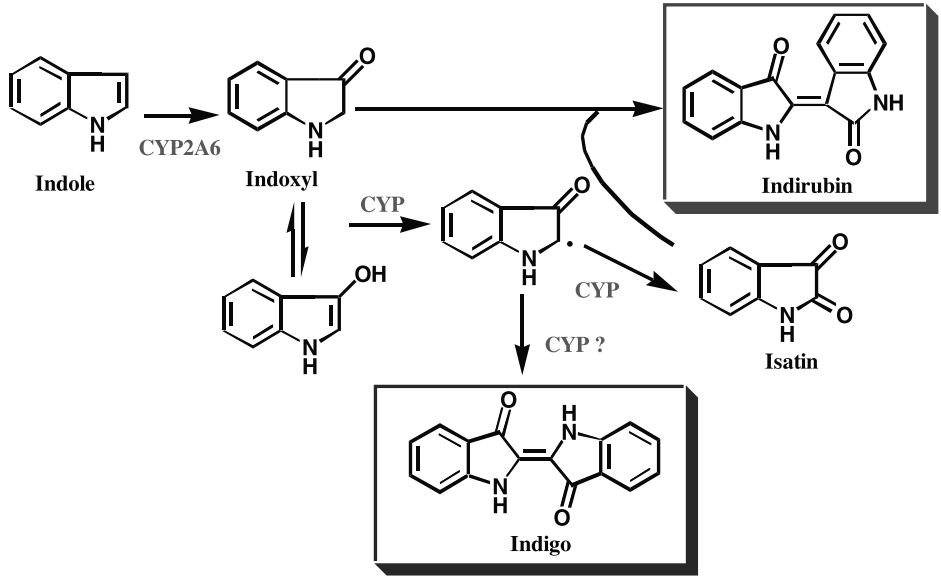


Figure 1. Postulated scheme for the formation of indigo and indirubin

Histochemical analysis of mouse liver

Liver sections were stained with hematoxylin-eosin or Sudan black.

Results and Discussion

Effects of indirubin and indigo on liver microsomal enzyme activities

The microsomal alkyoxyresorufin-*O*-dealkylase activities, EROD, MROD and PROD, of male C57BL/6J:Jcl mice after treatment with indirubin or indigo (1, 5, 10 and 50 mg / kg body weight) by gavage for three days were examined. The EROD and MROD activities were increased dose-dependently compared with that of the control mice. Indigo induced the EROD and MROD activities by 1.3- and 1.4-fold, respectively, at 5 mg /kg b.w. which was the most effective dose.

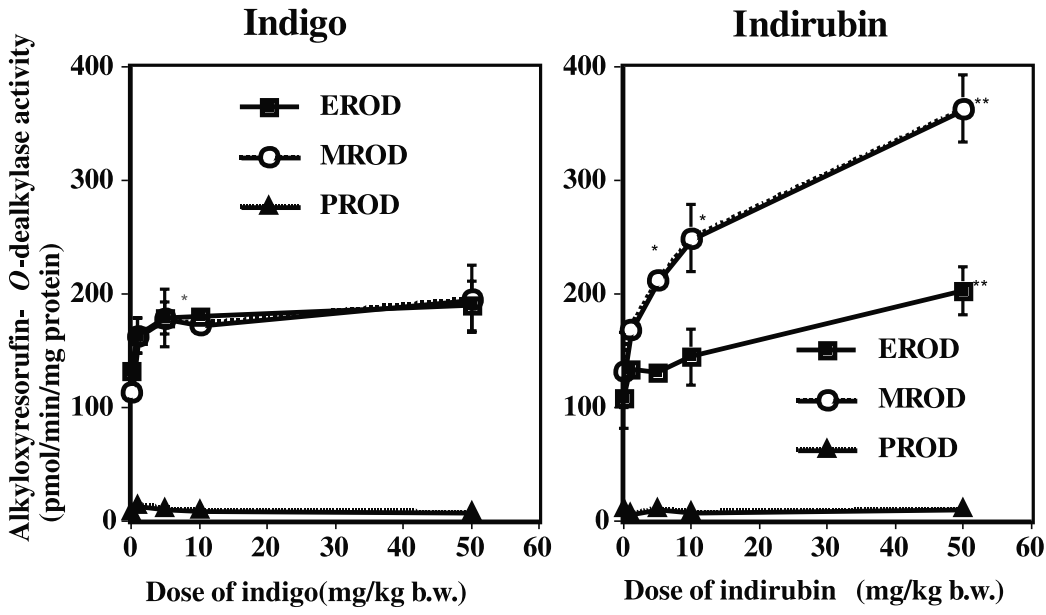
Indirubin induced the EROD and MROD activities by 1.9- and 2.7-fold, respectively. However, the PROD activity, which is due to phenobarbital-inducible cytochrome P450, was not induced by indirubin or indigo (Fig. 2).

Inductive effect of indirubin and indigo on liver enzyme activities of $AhR^{-/-}$ mice

When indirubin or indigo was applied to $Ahr^{-/-}$ mice, which lack AhR, no inductive effect on EROD or MROD was observed (data not shown). Thus, the induction of the EROD and MROD activities by indirubin and indigo is suggested to be mediated by AhR.

Histochemical analysis of mouse liver

Livers were obtained from mice after indirubin or indigo treatment, 50 mg / kg b.w., and vehicle control mice, The liver sections were stained with hematoxylin-eosin or Sudan black. Indirubin and indigo caused no significant damage to the liver, and lipid droplets, which are usually observed after TCDD treatment, were not increased.



*p<0.05 , **p<0.01 vs vehicle control.

Figure 2. Dose-dependent induction of EROD, MROD and PROD in mice by indirubin and indigo

In this study, we demonstrated that indirubin and indigo induce EROD and MROD activities in mice. The induction was mediated by AhR. Although their AhR ligand activities in recombinant yeast assay were the same as or stronger than that of TCDD, the inductive activities for drug-metabolizing enzymes in mice were less than that of TCDD and higher doses of indirubin and indigo were required for the induction. Our preliminary experiments suggest that some hydrophobic compounds as metabolites of indirubin formed by liver microsomes. These compounds should be excreted earlier than TCDD, which persists in the body. In histochemical analysis, no toxicological changes were observed in liver sections from high dose-treated mice compared to vehicle-treated mice. The results suggest that endogenous indirubin and indigo might be physiological ligands for AhR.

Acknowledgements

This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health, Labour and Welfare of Japan and Grants-in-Aid for Scientific Research on Priority Areas (13027256, 14042240) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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