EFFECT OF DRUG-METABOLIZING ENZYME ACTIVITY INDUCED BY POLYCHLORINATED BIPHENYL ON THE DURATION OF OXYTETRACYCLINE IN CARP

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ABSTRACT

A study was made of the induction of drug-metabolizing enzyme activity and the effect of the induced activity on the duration of oxytetracycline (OTC) in carp, *Cyprinus carpio*. Carp were fed a diet containing polychlorinated biphenyl (PCB)(0,1 mg/100 g body weight/day) for 2 week to induce drug-metabolizing enzymes. After pretreatment, OTC was administered in feed to PCB-treated and control carp as a single dose of 50mg/kg body weight.The result demonstrate that PCB highly induses benzo (a) pyrene hydroxylase among the enzymes of carp, but the induced hydroxylase does not react to OTC, resulting in no change in their tissue levels and duration time.

Key words: drug-metabolizing enzyme activity, polychorinated biphenyl, oxytetracycine, induction, carp.

INTRODUCTION

Although Brodie and Maickel (1962) reported that both freshwater and marine fishes were incapable of microsomal oxidation, it has been generally accepted that a cytochrome P-450 – dependent mixed function oxygenase (MFO) takes the initial enzymatic step in the detoxication pathway in fish (Bend *et al*., 1976). When exposed to a wide variety of compounds, including drugs, polycyclic hydrocarbons, herbicides, fungicides, insecticides, methyl cholantherene and polychlorinated biphenyl (PCB), it was not suprising that fish has developed biochemical transformation pathways for their rapid elimination (Conney, 1977; Hodgson and Kulkarmi, 1984)

Polychlorinated biphenyl (PCB) is a potent inducer of cytochrome P-450 and MFO enzyme activity in carp (Lumban Batu, 2001a). Induction of enzyme activity may protect the fish from carcinogenesis by increasing detoxication rates and elimination (Lumban Batu, 2001 a). Several environmental contaminants absorbed by fish are generally oxidized, reduced, hydrolyzed or conjugated by drug-metabolizing enzymes mostly localized in liver and then excreted into bile, urine or surrounding water via gills (Lumban Batu, 2001 b).

In our previous paper (Lumban Batu, 2002 c) the study was performed on the absorption and duration of oxytetracycline (OTC) in yellowtail, red sea bream, ayu, carp and rainbow trout. Among all the tested fishes, rainbow trout took the longest period for absorption and depuration of OTC. The OTC in the intestines of tested fishes except rainbow trout disappeared after 24 hr post dosing, in shorter periods than in their other tissues. Therefore, the time depuration of OTC administrated to fish may be influenced by drug-metabolizing enzyme activity.

The present study was performed to elucidate the effect of drug-metabolizing enzyme activity on the tissue levels and duration of OTC in PCB treated and control carp, in which the activity of drug-metabolizing enzymes were induced by administration of PCB.

MATERIALS AND METHODS

Special chemicals

In this experiment radioactive [^{14}C] benzo (a)pyrene [60 Ci/µmol] and ACS-II aqueous scintillator were purchased from Radiochemical Centre, Amersham, and [^{35}S] H_2SO_4 (carrier free) from Japan Atomic Energy Research Institut, Glucose- 6-phosphate dehydrogenase, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate, reduced NADP, bovine serum albumin and UDP glucuronic acid were purchased from Sigma Chemical Co. Polychorinated biphenyl were obtained from Tokyo Kasei Kogyo Co., and Oxytetracycline [4- (dimethyl amino] – 1,4, 4X, 5, 5X, 6,11, 12a – octahydro – 3,5,6,10,12,12a-hexahydroxy – 6- methyl – 1,11 –dioxo – 2-naphthacenecarboxamide, OTC] was offered from Kyowa Hakko Kogyo Co, Ltd. Acetonitrile was HPLC grade and all other chemical were the highest grade commercially available.

Fish

Carp (ca. 82 g each) were purchased from a fishfarm and aclimatized in aquarium at least for one week prior to use for the experiment.

Induction of drug-metabolizing enzyme activity by PCB

Two hundred and forty carps were selected and divided randomly into two groups and placed in the respective 1,000 liters aquarium. In order to determine the effect of drug-metabolizing enzyme activity on the tissue levels and duration of OTC in carp, one group was subjected to the inducation of drug-metabolizing enzyme by oral administration of PCB which is a potent inducer of microsomal mixed function oxygenase (MFO). To prepare the test diets containing PCB a commercially diet was immersed in the respective acetone solutions and dried by an air blower. The fish of the induction group were fed with PCB diet at a dose 0.1 mg PCB/100 g body weight once a day for 2 weeks. A second group of fish was fed with PCB-free diet as a control.

Determination of drug-metabolizing enzyme activity

After 2 weeks administration, fifthteen fish of both group of PCB treated and control were dissected after exsanguination by decapitation. The liver obtained from fifteen fish of each group were pooled, frozen by immersion in liquid nitrogen and strored at 80^{o}C. The pooled livers were homogenized with 0.25 M sucrose 10 mM Tris-HCI buffer (pH 7.5) containing trypsin inhibitor (1 mg/ml), and subjected to cell fractionation, using an ultracentrifuge at 0 ^{o}C. These microsomes and cytosol fraction were subjected to the determination of drug-metabolizing enzyme activities.

The microsomal fraction was subjected to the determination of the cytochrome P-450 content and the activities of aryl hydrocarbon hydroxylase and glucuronic transferase following the methods of Omura and Sato (1964); Cantfort *et al* ., (1977) and Illing and Benford (1976). The cytosol fraction was subjected to the determination of phenol sulfate transferase and glutathione transferase activity toward 1- chloro – 2,4 – dinitrobenzene – glutathione transferase following the methods of Kobayashi *et al.*, (1987) and Habig *et al.*, (1974). The protein content of each fraction was also determined by the method of Lowry *et al.*, (1951) using bovine albumin as a standard.

Administration of OTC

All test fish were starved for one day before oral administration of OTC. The PCB treated and non-treated fish were divided into three groups of 30 each and anaesthetized with 50 ppm MS-222 water for 10 min. Then, the diets containing adequate amount of OTC was orally administered to carp at one dose of 50 mg in 3 g diet/kg body weight, by oral gavage using a plastic syringe (Combitips, 0.5 ml, Eppendrof Co.,Ltd). Then the fishes were immediately transfered to the respective aquarium.

Determination of OTC in tissues

At 2,4,8,12,26,50,74 and 98 hr after OTC administration, the three fish of each group were taken out from aquarium and cut at the hind brain with scissors. Blood was collected by cutting the tail off behind the anal fin. The blood, liver, muscle and kidney collected from each three fish at each interval were pooled, frozen in liquid nitrogen and then stored in a freezer -20 ^{o}C.

The extraction of OTC from fish tissues was carried out as shown in the previous report (Lumban Batu, 2002c). After removal of interfering substances, the concentration of OTC in the tissues were determined by reversed phase high-performance liquid chromatography (HPLC), under the condition as follows: Instrument, TOSOH HPLC 8010 system; Column. TOSOH TSK – GEL ODS 80 Tm (250 mm x 4,6 mm); mobil phase, methanol – acetonitrile – 0.2 M oxalic acid (1: 1: 4,4, pH 2,0); Flow rate 0,5 ml/min; Temperature, 35^{0}C; Detector, UV 360 nm (TOSOH UV 8010); Sensitivity, 0.1 AUFS ; Sample volume, 20 µℓ; Data Analysis, TOSOH Super System Controller (Lumban Batu, 2002c).

RESULTS AND DISCUSSIONS

Many xenobiotics in fish are not directly conjugated but must first be metabolically transformed to yield a group suitable for combination with the conjugating agent. This reaction was carried out primarily by microsomal cytochrome P-450-dependent reaction. In several species of fish the existense of liver microsomal cytochrome P-450 capable of metabolizing lipid-soluble xenobiotics and can increase the activities of several MFO activities (Chambers and Yarbrough, 1979; Bend and James, 1979).

In this present experiment, the cytochrome P-450 content in the microsomes of carp liver was induced approximately 3,18 times that in control by the administration of PCB-diet for 2 weeks (Table 1), the electron transfer system have been studied in detail in carp, and the metabolism of compounds such as biphenyl, benzo(a)pyrene and 2,5-diphenyl-oxazole by carp liver microsomes have been found to require oxygen and NADPH generating sytem. The metabolism of these compounds by carp liver microsomal enzyme system was inhibited strongly by carbon monooxide (Lumban Batu, 2001b). This information and the fact that cytochrome P-450, as well as NADPH cytochrome c reductase system are present in carp, suggest strongly that fish have a cytochrome P-450 mediated monooxygenase system which is very similar to that described in mammals (Lumban Batu, 2001b). This experiment shows induction of cytochrome P-450 in carp by PCB. These findings were utilized to investigate the inducibility of the carp MFO system. System are prensent in carp, suggest strongly that fish have a cytochrome P-450 mediated monooxygenase system which is very similar to that described in mammals (Lumban

Batu, 2001 b). This experiment shows induction of cytochrome P-450 in carp by PCB. There finsdings were utilized to investigate the inducibility of the MFO system.

Table 1. Cytochrome P-450 content and the activites of drug-metabolizing enzymes in PCB- treated and control carp liver

Treatment	P-450 content [a)]	Benzo(a)pyrene-hydroxylase [b)]	Glucuronyl-transferase [b)]	Glutathione-transfererase [c)]
PCB-Treated	0.34	0.70	0.052	73.1
Control	0.11	0.04	0.067	30.7

The values are expressed as the average of fifteen samples pooled with fifteen fish livers each. [a)] nmol/mg microsomal protein, [b)] nmol/min/mg microsolmal protein,[c)] µmol/min/ g liver.

The activity of benzo (a) pyrene hydroxylase in PCB-treated group increased considerably, corresponding to 17,5 times that in control, whereas the increase in the activity of glutathione transferase toward 1-choloro-2,4-dinitro benzene, was smaller compared to that of hydroxylase. Glucoronyl transferase activity toward p-nitrophenol was not induced by PCB (Table 1). This would suggest that the nature of cytochrome P-450 involved resembles enzymatically control cytochrome P-450.

Nebert *et al.*, (1987) has defined cytochrome (s) P_1-450 as all forms of membrane bound hemoprotein associated with NADPH-dependent MFO activities and capable of binding carbon monooxide (CO) when reduced. Cytochrome p_1-450 was defined as the polycyclic aromatic hydrocarbon-inducible from (s) of cytochrome P-450 associated with induced aryl hydrocarbon hydroxylase (AHH) activity; and cytochrome P-448 was defined as the polycyclic aromatic hydrocarbon-inducible from of cytochrome (s) P-450 associated with the greatest hypsochromic (blue) shift in the χmax. of the Soret peak of the reduced hemoprotein-CO complex. From these defenitions it would appear that cytochrome P-450 and P-448 metabolize certain compounds quite differently. Therefore, the results presented here showed that the glucuronyl transferase activity of PCB-treated was relative low compared to that of control may be related to the different types of cytochrome p-450 in the liver microsome of carp, with resultant differences in the metabolism and induction capability of PCB to MFO.

The PCB administrated to carp are biotransformed by drug metabolizing enzymes, such as: benzo(a)pyrene hdroxylase, glucuronyl transferase and glutathion transferase and the cytochrome P-450 was probably involved in the clearance of OTC from the tissues of carp.

The Table 2 shows the maximum concentration and biological half-life of OTC in the liver and kidney of PCB-treated and control carp. The maximum concentration of OTC in the liver of PCB-treated slightly different to that of control, and in the kidney approximately 1,5 times to that of control. The maximum OTC concentrations in the liver and kidney of carp were attained respectively at 12 hr and 26 -12 hr. The time required to attain to the respective maximum level and biological half-life of OTC in the liver of PCB-treated was at reported in carp (Lumban Batu, 2002 c). Whereas in the kidney of PCB-treated was at 26 hr.

Table 2. Maximum concentration and biological half-life of oxytetracycline in the liver and kidney of PCB treated and control carp

Tissue	Treatment	Maximum concentration (ppm)	Biological half-life (hr)
Liver	PCB	2.51	12
	Control	2.77	12
Kidney	PCB	0.83	26
	Control	0.55	12

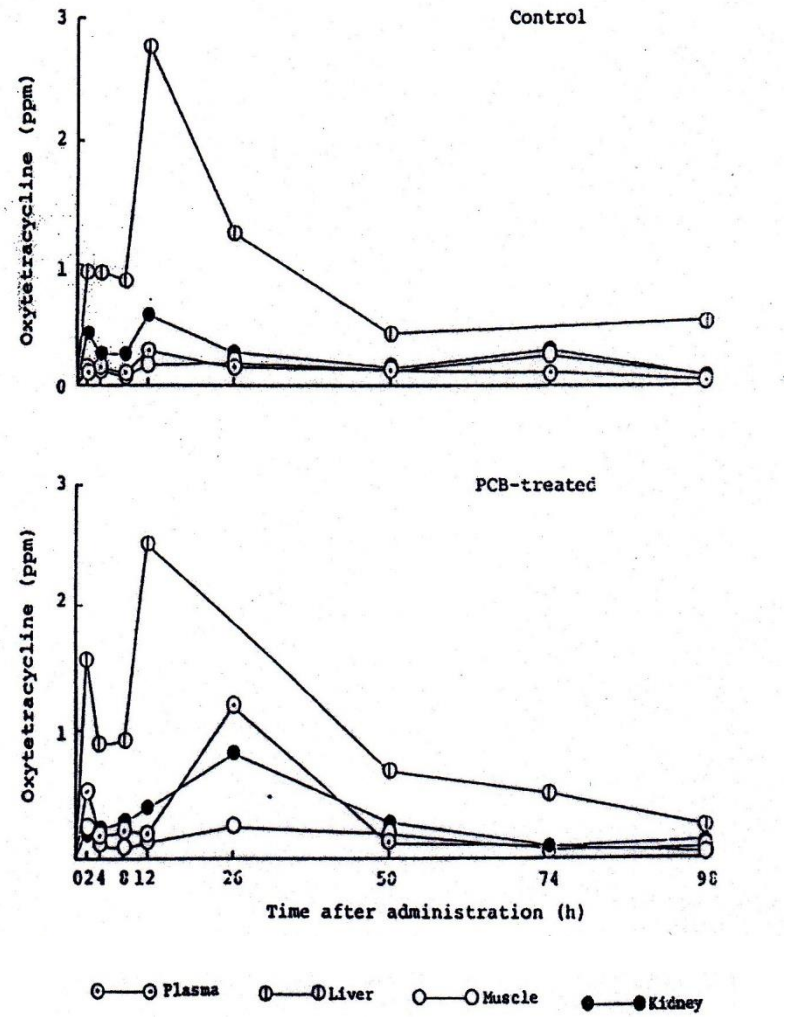

Fig. 1. Changes in the concentration of oxytetracycline in the tissues of control and PCB-trated carp, after oral administration at a dose of 50 mg/kg body weight

Fig.1. show the change in the concentration of OTC in the various tissues of carp in PCB-treated and control groups. In Fig. 1. no apparent difference was observed in the tissues between the PCB-treated and control groups.

These results demonstrated eventhough that PCB highly induses arly hydrocarbon hydroxylase (AHH) among the enzymes involved in cytochrome p-450 in the hepatic microsomes of carp, but the induced hydroxylase does not react to OTC, resulting in no change in

their tissue levels (up to 3 fold) in benzo(a)pyrene hydroxylase activity after pre-treatment of carp, cannot be explained in terms of increase in the total of cytochrome P-450 content. Hence it is possible that a novel enzyme with different substrat specificity were induced by PCB in carp, and one prescription may either inhibit or induce a certain cytochrome P-450 involved in the clearance of OTC. In our previous report (Lumban Batu, 2002 d) have been demonstrated *in vitro* that the drug-metabolizing enzyme induced by PCB react to oxolinic acid (OA), resulting in the reduction of the maximum OA levels in the tissues and also in the shortening of its duration time in the tissues. However, the benzo(a)pyrene hydroxylase did not react to OTC. The result suggest that differences in the activity of drug-metabolizing enzymes, cytochrome P-450 content, substrat and enzymatic reactions to OTC promotes differences in the metabolic pathway, elimnation and duration of OTC in carp. Then the relation between the enzymatic mechanism with the absorption and depuration time of OTC in the tissues of carp may be the area of future interest on the regulation of individual P-450 genes, with the availability of chiremic genes (Guengerich, 1988; Lumban Batu, 1996). Thus, these results demonstrated that the pattern of absorption and excretion of OTC did not show any significant changes by PCB treatment.

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