EFFECT OF DIFFERENT PCBs ON THE CYP3A3/4 REGULATION IN MONKEY HEPATOCYTES

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial compounds that have been detected as contaminants in the biotic and abiotic environment[1]. The widespread industrial utilization of PCBs is due to their chemical stability, physical properties, inflamability and dielectric properties. For example, PCBs were used as flame retardants, plasticizers, organic diluents and dielectric fluids for capacitors and transformers.
However, PCBs also tend to accumulate in the food chain because of their lipophilic nature and cause a complex spectrum of biological and toxicological effects.
The 209 possible congeners can be divided in three groups, with different biochemical and toxic responses[2].
One group of PCBs are the so called "non dioxin-like" PCBs, with two or more *ortho* chlorines. These PCBs have an enzyme induction pattern resembling phenobarbital to some extent. These *"ortho"* substituted PCBs are highly abundant in the environment, but useful bioparameters for the human risk assessment are presently not available. Some biochemical or toxic responses are: 1) ability to induce cytochrome P450 2B1/2 in rats (phenobarbital-type induction)[3,4], 2) promotor effects in the presence of some genotoxic compounds[5], 3) effects on the thyroid hormones and vitamin A metabolism[6], 4) interaction on vitamin K metabolism[7] and 5) neurotoxic effects[8].
There is no orthologous form of the rat cytochrome P450 2B1/2 found in humans. Therefore riskassessment of these PCBs for humans based on the induction of cytochrome P450 in rodents is questionable.
Cytochrome P450 3A3/4, orthologous to rat cytochrome P450 3A1/2, is the most abundant P450 isoenzyme in human liver. It is also inducible by phenobarbital and glucocorticoids and involved in metabolism and activation of numerous drugs and many carcinogens.
The goal of the study presented in this paper was to determine differences in induction of cytochrome P450 3A3/4 by phenobarbital, PCB#153 (2,2',4,4',5,5' hexachlorobiphenyl) and PCB#187 (2,2',3,4',5,5',6 heptachlorobiphenyl) in hepatocytes from the Cynomolgus monkeys (*Macaca fascicularis*). These monkeys express a range of cytochrome P450 proteins which is comparable with human[9].

METHODS

Chemicals

PCB#153 (2,2',4,4',5,5' hexachlorobiphenyl) and PCB#187 (2,2',3,4',5,5',6 heptachlorobiphenyl) were synthesized and purified to >99% as determined on a GC-ECD. Bovine serum albumine (BSA), gentamicin, insulin, hydrocortisone, L-glutamine, Hanks' balanced salt solution (H.B.S.S), ethylene glycol-bis-(ß-amino ethyl ether)N,N,N',N' tetra acetic acid (EGTA), 11ß hydoxytestosterone were all purchased from Sigma, St. Louis, MO, USA. Fetal clone serum was from Greiner, Alphen a.d. Rijn, The Netherlands. Collagenase was obtained from Boehringer Mannheim, Mannheim, Germany. CYP 3A4 cDNA (Beaune *et al*)[10] was a gift from Dr. D. Pompon (Gif-sur-Yvette, France). Monoclonal antibodies towards cytochrome P450 3A1/2 were kind gifts from Dr. P.J. Kremers, Université de Liège, Belgium. Secondary antibodies were provided by ITK Diagnostics (Uithoorn, The Netherlands).

Animals, cell isolation and cell culture

Cynomolgus monkeys (*Macaca fascicularis*) were bred at the National Institute of Public Health and Environmental Protection (RIVM, Bilthoven, The Netherlands) and served as donors for kidney cells which are used for the production of the poliomyelitis vaccine. Hepatocytes were isolated as described by Mennes *et al.*[11]

The cells were plated on 9 cm tissue dishes (Greiner) at a density of $8*10^6$ cells/dish in 10 ml Williams' E medium, supplemented with 5% (v/v) fetalclone serum and 1 µM insulin, 10 µM hydrocortisone and 50 mg/l gentamycin. Cells were incubated in a humified atmosphere of air (95%) and CO_2 (5%) at 37°C. During the first 4 hours, 4 mM $CaCl_2$ and 4 mM $MgCl_2$ were added to the medium. After 4 hours, the medium was replaced and the cells were preincubated for 14 hours. Next, the medium was replaced by a medium containing either phenobarbital, or the individual PCBs (#153 or #187) or DMSO (control, 0.1% v/v) and cells were exposed for 24, 48 or 72 hours.

Isolation and analysis of RNA

Cells were harvested in ice-cold PBS using a rubber policeman. RNA was isolated using Trizol reagent (GibcoBRL, Breda, The Netherlands), and bound to a Hybond N^+-membrane. The mRNA expression on the dot blots was determined using radiolabeled CYP3A3/4 probes. The membranes were exposed to a x-ray film and the intensity of the spots was used to quantify the amount of expressed mRNA.

Western blotting

Microsomal proteins of the hepatocytes were isolated as described by Rutten *et al*[12]. The proteins were separated on a SDS-PAGE gel and blotted on polyvinylidene difluoride sheets. Antibodies raised to rat P450 3A1/2 were used for immunochemical staining.

Cytochrome P450 enzyme activity

Testosterone hydroxylating activity was measured directly in the hepatocyte monolayers using HPLC as described earlier[13].

RESULTS AND DISCUSSION

The effect of phenobarbital, PCB#187 and PCB#153 on the induction of CYP3A3/4 mRNA is shown in figure 1A, 1B and 1C. An increase in CYP3A3/4 level was reached after treatment with all inducers at 24 and 48 hours. Between 24 to 72 hours, control levels of CYP3A3/4 mRNA decreased 10 times compared to the levels before the experiment (t=0). This time-dependent decrease in CYP3A3/4 mRNA levels shown in control and exposed cells, is also observed by others, eg. Wortelboer *et al*[4], with regard to CYP3A

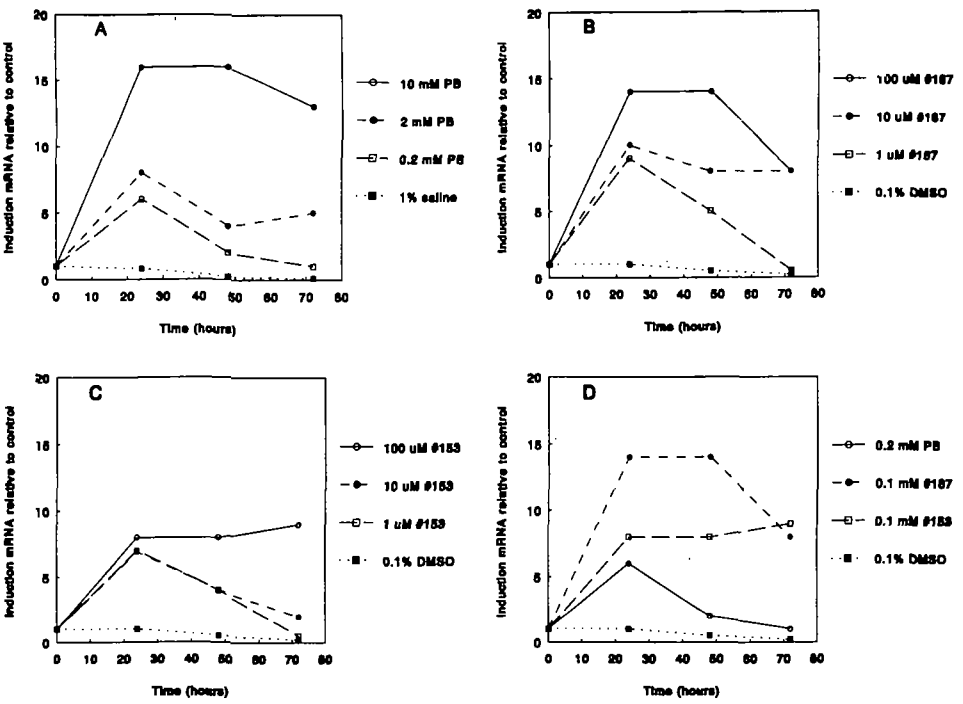

Figure 1. Induction CYP 3A3/4 mRNA relative to CYP 3A3/4 mRNA levels at t=0 in monkey hepatocytes at 24, 48 and 72 hours after treatment with:
A. 10 mM, 2 mM, or 0.2 mM phenobarbital in saline
B. 100 µM, 10 µM, 1 µM PCB#187 in DMSO (0.1% v/v)
C. 100 µM, 10 µM, 1 µM PCB#153 in DMSO (0.1% v/v)
D. 0.2 mM PB, 0.1 mM PCB#187 or 0.1 mM PCB#187.

protein(activity) in hepatocytes. 10 mM phenobarbital induced the CYP3A3/4 mRNA 15 times at 24 hours after treatment. 100 µM PCB#187 or PCB#153 increased the mRNA level respectively, 12 or 8 times. Based on these studies with monkey hepatocytes PCB#187 is a more potent inducer of CYP3A3/4 than PCB#153. This is shown in figure 1D. PCB#187 is a *tri-ortho* substituted congener while PCB#153 is a *di-ortho* substituted congener. Recently, it has been reported that PCB#187 is also a potent inducer of cytochrome P450 2B1/2 in the liver of the rat[15]. These results are in good agreement with those obtained in our monkey hepatocyte study.

In addition, it can also be concluded that in monkey hepatocytes PCB#187 is a more potent inducer of CYP3A3/4 mRNA than phenobarbital.

Despite the increase in CYP3A3/4 mRNA levels, western blots indicated (not shown) that CYP3A protein levels did not increase after treatment with these different inducers. In addition, the 6ß-testosterone hydroxylating activity of the hepatocytes, indicating CYP3A3/4 enzyme activity, was not influenced and remained at a constant level between 9-15 $nmol*min^{-1}mg\ p^{-1}$.

Based on our experiments with monkey hepatocytes it can be concluded that some *ortho* substituted PCBs can be potent inducers of CYP3A3/4 mRNA. However, this increase in mRNA levels did not concur with *de novo* protein synthesis or increased enzymatic activity of CYP3A3/4 in monkey hepatocytes.
Whether or not this lack in induction of CYP3A protein and protein-activity in monkey hepatocytes is due to the system (eg. lack of available heme) or a regulatory mechanism needs to be studied.

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