INHIBITION OF APOPTOSIS IN RAT HEPATOCYTES TREATED WITH POLYCHLORINATED BIPHENYLS (PCBs)

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Introduction

In experimental animals, PCBs result, e.g., in neurotoxicity, endocrine disturbances, and tumor promotion[1,2]. For practical reasons, PCBs were subdivided into ‚dioxin-like' and ‚non-dioxin-like' congeners[3]. This principle is based on the fact that a number of PCBs exert biological effects similar to those of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) the most toxic dioxin congener. In particular, these PCBs bind to the dioxin or aryl hydrocarbon receptor (AhR) and lead to characteristic effects on the expression of AhR-regulated genes including cytochrome P450 (CYP)1A1[1]. In contrast, a number of ‚non-dioxinlike' PCBs are inactive or almost inactive as AhR-agonists but induce a battery of drug-metabolizing enzymes including CYP2B1/2B2 known as phenobarbital-inducible genes[4]. Therefore, these PCBs sometimes are categorized as ‚phenobarbital-like' inducers.

A current hypothesis suggests that the inhibition of the intrinsically enhanced rate of apoptosis in preneoplastic clones (foci) may play a central role in the mechanism of tumor promotion [5]. In fact, inhibition of apoptosis in preneoplastic foci has been demonstrated for the liver tumor promoters TCDD[6] or phenobarbital[7]. In rat hepatocytes in primary culture, anti-apoptotic effects were also described for both compounds[8].

This study was designed to investigate a possible relationship between 'phenobarbital-type' or 'mixed-type' induction of CYP isozymes/activities, and the effects on apoptosis in rat hepatocytes treated with phenobarbital or certain PCBs.

Materials and Methods

Chemicals

Bovine serum albumin, collagenase type IV, and phenobarbital were from Sigma (Steinheim, Germany), Dulbecco's modified Eagles's medium (DMEM) from Seromed (Berlin, Germany), and Waymouth's medium MD 705/1 and fetal calf serum from Gibco/BRL (Heidelberg, Germany), ITS and ITS$^+$ from Becton Dickinson (Heidelberg, Germany), and the PCBs IUPAC number 28 (2,4,4'-trichlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), and 187 (2,2',3,4',5,5',6-heptachlorobiphenyl) from Promochem (Wesel, Germany). All other chemicals were at the highest purity commercially available.

Hepatocyte Culture and Treatment
Male Wistar rats were obtained from Charles River (Kisslegg, Germany) and were kept under standard conditions. Adult animals at a body weight of 150-180 g were anesthetized, and hepatocytes were isolated as described [8]. The cells were cultured using the rat collagen sandwich procedure. The cells were seeded at a density of 100,000/cm^2 on collagen-coated 60 mm Petri dishes, and were then covered with collagen. PCBs were added dissolved in DMSO, phenobarbital dissolved in sterile aqua bidest. Controls were treated with DMSO or aqua bidest. only. The cultures were washed, harvested, and homogenized after 48 h, and 7-ethoxyresorufine O-deethylase (EROD) and pentoxyresorufine O-dealkylase (PROD) activities were determined.

Inhibition of apoptosis
Hepatocytes were seeded at a density of 60,000/cm^2, and pulse-treated with UV light as described[8]. Phenobarbital or PCBs were added 90 min after irradiation. For the counting of apoptotic nuclei, the cells were fixed, washed and air-dried 12 h after tretament with the tumor promoters, and were stained with an aqueous 8 µM solution of 4',6-diamidino-2-phenylindole (DAPI) and 10 µM sulphorhodamine 101. The microscopic analysis of the encoded slides was carried out using a Zeiss fluorescence microscope (Axioskop) equipped with a BP 450-490 excitation filter and a LP 520 emission filter. All experiments were carried out in double, and 3 x 1000 nuclei were examined on each slide. Condensed, half moon-shaped, and scattered nuclei were summarized as apoptotic nuclei.

Statistical analysis
Means and standard deviations were calculated from independent experiments. For (multiple) comparisons of means of treated cultures with untreated controls Dunnett's test for independent samples was used.

Results and Discussion
In rat hepatocytes cultured between two layers of collagen (‚sandwich culture') UV pulse-irradiation at a specific intensity of 90 J/m^2 almost doubled the number of apoptotic nuclei after 12 h. Addition of 2 mM phenobarbital 90 min after UV irradiation completely suppressed the increase in apoptosis. For further experiments with the PCBs 28, 101, 138 and 187 standard conditions were used (single UV irradiation with 90 J/m^2, determination of apoptotic nuclei after 12 h). It was found that phenobarbital and the four PCBs tested inhibited the increase in apoptosis in a concentration-dependent manner. This effect reached a level of at least 90% (at least 90 % inhibition of additional, UV-induced apoptosis) with phenobarbital at 10^{-7} M (preliminary data), with PCB 28 at 10^{-9} M, with PCB 101 at 10^{-7} M (Fig. 1), with PCB 138 at 10^{-8} M and with PCB 187 at 10^{-6} M, i.e, the range of ‚spontaneous' apoptosis found without UV irradiation was achieved.
All PCBs tested and phenobarbital resulted in a concentration-dependent induction of CYP 2B1/2B2-catalysed PROD activity in cell homogenates. With phenobarbital a complete induction curve was obtained for EROD activity, whereas the PCBs were inactive or almost inactive, with the exception of PCB 138, a 'mixed-type' inducer. Fitting of a sigmoidal dose-response using a log-

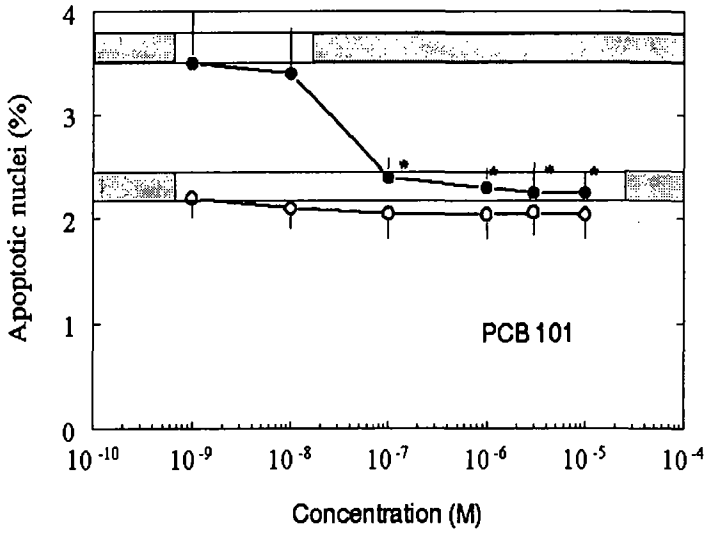

Fig. 1. Effect of PCB 101 on apoptosis in rat hepatocytes in primary culture 12 h after irradiation with UV-light (90 J/m^2). Symbols and error bars represent means and S.D. from three independent experiments. *Significantly different ($p \leq 0.05$) from results obtained with DMSO-treated cultures, represented by two bands showing the standard deviation range of apoptosis in UV-treated (upper band) and untreated (lower band) cultures.

Tab. 1. EC_{50} values for induction of PROD- and EROD-activities in rat heptocytes in primary culture, 48 h after treatment with phenobarbital, PCB 28, 101, 138 or 187.

Inducer	PROD induction EC_{50} ±S.D. (M)	EROD induction EC_{50} ±S.D. (M)
Phenobarbital	$2.4 \times 10^{-5} \pm 1.7 \times 10^{-6}$	$1.8 \times 10^{-5} \pm 12 \times 10^{-6}$
PCB28	$3.3 \times 10^{-6} \pm 3.4 \times 10^{-7}$	-
PCB 101	$5.7 \times 10^{-6} \pm 9.7 \times 10^{-7}$	-
PCB138	$3.2 \times 10^{-7} \pm 8.5 \times 10^{-8}$	$5.8 \times 10^{-6} \pm 8.5 \times 10^{-7}$
PCB187	$1.2 \times 10^{-7} \pm 2.0 \times 10^{-8}$	-

probit procedure allowed the calculation of EC_{50}-values and 95% confidence intervals (Tab. 1). The PROD-inducing potencies of the PCBs followed the rank order PCB 187>PCB138>PCB28>PCB101. Our results indicate that UV light-induced apoptosis in rat hepatocytes is completely suppressed with phenobarbital, the three ‚non-dioxinlike PCBs 28, 101, and 187, and the 'mixed-type' inducer PCB 138. A similar result was previously obtained with TCDD[8]. However, TCDD and most PCBs used in this study could not inhibit ‚spontaneous' apoptosis (not shown) arguing for two distinct types of apoptosis. One type is elicited by UV-irradiation which may lead to DNA damage or another type of cellular stress. Analysis of CYP activities revealed no relationship between inhibition of UV-induced apoptosis and induction of EROD or PROD activity. This result suggests that mesurements of CYP induction as a parameter for a tumor-promoting potency of PCBs in the liver may be misleading. Second, it can be speculated that the signalling events leading to induction of CYP isozymes are distinct from those operative in inhibition of apoptosis.

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References

1. Safe, S.H. (1994) Crit. Rev. Toxicol. 24, 87.
2. Hemming, H., Flodström, Wärngård, L., Bergman, Ä, Kronevi, T., Nordgren, L. and Åhlborg, U.G. (1993) Eur. J. Pharmacol. Environ. Toxicol. 248, 163.
3. Giesy, J.P. and Kannan, K. (1998) Crit. Rev. Toxicol. 28, 511.
4. Connor, K., Safe, S., Jefcoate, C.R. and Larsen M. (1995) Biochem. Pharmacol. 50, 1913.
5. Bursch, W., Lauer, B., Timmermann-Trosiener, L., Barthel, G., Schuppler, J. and Schulte-Hermann, R. (1984) Carcinogenesis 5, 453.
6. Stinchcombe, Buchmann, A., Bock, K.W. and Schwarz, M. (1995). Carcinogenesis 16, 1271.
7. Plant, N.J., Horley, N.J., Dickins, M., Hasmall, Elcombe, C.R. and Bell, D.R. (1998) Carcinogenesis 19, 1521.
8. Wörner, W. and Schrenk, D. (1996) Cancer Res. 56, 1272.