PRELIMINARY ASSESSMENT OF THE POTENCY OF 2,3,7,8-TETRA (BROMO/CHLORO)DIBENZO-P-DIOXINS TO INDUCE EROD ACTIVITY IN PRIMARY CELL CULTURES OF RAT HEPATOCYTES

Gudrun Blankenburg, Otto Hutzinger*, and Diether Neubert

Institut für Toxikologie und Embryopharmakologie
Freie Universität Berlin, Garystr. 5, D-1000 Berlin 33

*Institut für Ökologische Chemie und Geochemie
Universität Bayreuth, Universitätsstraße 30, D-8580 Bayreuth

ABSTRACT

Primary hepatocyte cultures generally rapidly lose their capacity to catalyze cytochrome-P450-dependent monooxygenase reactions. We found that activity of ethoxyresorufin O-deethylase (EROD) may be maintained or restored by adding 2,3,7,8-TCDD to the culture medium.

This ability of primary rat hepatocyte cultures was used *in vitro* to comparatively assess the ability for EROD induction by some mixed-halogenated 2,3,7,8-substituted dibenzo-*p*-dioxins, containing chlorine as well as bromine.

The mixed brominated-chlorinated congeners studied included:
1B3CDD : 2-monobromo-3,7,8-trichloro-dibenzo-*p*-dioxin,
2B2CDD : 2,3-dibromo-7,8-dichloro-dibenzo-*p*-dioxin,
3B1CDD : 2,3,7-tribromo-8-monochloro-dibenzo-*p*-dioxin.
These substances were compared with 2,3,7,8-TCDD (4CDD) and 2,3,7,8-TBrDD (4BDD).

While the *in vitro* ability to induce EROD activity was about the same for 1B3CDD and 2B2CDD when compared with 2,3,7,8-TCDD, the preliminary studies suggest that the potencies of B4DD and 3B1CDD appear to be lower on a molar basis.

KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-Tetrabromodibenzo-*p*-dioxin; 2,3,7,8-Tetra(bromo/chloro) dibenzo-*p*-dioxins; Primary hepatocyte cultures; Wistar rats; Ethoxyresorufin O-deethylase = EROD

ABBREVIATIONS

TCDD or 4C = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
TBDD or 4B = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
1B3CDD, 2B2CDD, 3B1CDD = 2,3,7,8-Tetra(bromo/chloro)dibenzo-*p*-dioxins;
EROD = Ethoxyresorufin O-deethylase;
DMSO = Dimethylsulfoxide;
PCDDs = Polychlorinated dibenzo-*p*-dioxins; PCDFs = Polychlorinated dibenzofurans.

INTRODUCTION

There have been several reports on the ability of PCDDs/PCDFs to induce cytochrome P450-dependent monooxygenase activities in established cell lines, mostly hepatoma, *in vitro* (e.g. NIWA et al. 1975; BRADLAW and CASTERLINE 1979; BRADLAW et al. 1980; HUDSON et al. 1983; SAWYER and SAFE 1985). Since the metabolic capacity of these cells is limited it was of interest to test whether typical effects caused by TCDD, e.g. inductions of typical monooxygenase reactions *in vivo* (e.g. KITCHIN and WOODS 1979; ABRAHAM et al. 1988) may also be achieved in primary hepatocyte cultures of rodents and non-human primates. These cells may be expected to exhibit a greater similarity with hepatocytes *in vivo*, regardless of their loss in monooxygenase activity.

In this communication we present data on the induction of ethoxyresorufin O-deethylase activity in primary hepatocyte cultures of rats by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). After establishing the test system we assessed the potency of several mixed-2,3,7,8-tetrahalogenated dibenzo-*p*-dioxins for their ability to induce EROD activity in this *in vitro* system. This potency was compared with that of the corresponding tetra-chlorinated or tetra-brominated substances.

MATERIAL AND METHODS

Primary rat hepatocyte cultures
Livers of female Wistar rats (Bor: Wisw/spf, TNO, Winkelmann, Borchen, FRG) weighing 200 to 230 g were perfused with collagenase-solution as described by MADLE et al. (1987), and hepatocytes were isolated.

These hepatocytes were cultivated in collagen-coated culture flasks with Williams E medium (WEM) plus 10% fetal calf serum (FCS). Per 25 cm^2 culture flask 1.5 x 10^6 cells were cultured in 5 ml medium. The medium was changed first after 1.5 hrs.

Measurement of EROD activity
The monolayer of hepatocytes was removed from the bottom of the flasks and the cells were homogenized in a calium phosphate buffer medium, using a Potter-Elvehjem homogenizer. EROD activity was measured spectrofluometrically in the homogenates according to the method of BURKE et al. (1985). Protein contents were determined with the biuret-method using an automated bichromatic analyser (ABA 100, Abbott, Wiesbaden, FRG) and Preciset^R protein standards (Boehringer Mannheim, FRG).

Statistical evaluation
For statistical evaluations we used a standard software program (Minitab, Penn State Univ., 1987).

RESULTS AND DISCUSSION

EROD activity in untreated cultured rat hepatocytes

The freshly isolated rat hepatocytes used in our studies showed an EROD activity of 20 to 70 pmoles resorufin formed x mg $protein^{-1}$ x min^{-1}. This monooxygenase activity was found reduced to about 65% of the initial value within the first 2 hrs of incubation, and after 24 hrs of incubation only 20% of the initial EROD activity was measurable (Fig. 1). This behaviour is well-known from the literature.

Ability of TCDD to maintain or induce EROD activity in culture

When 50 pg TCDD/ml was added to the initial culture medium the decline in EROD activity could be largely prevented (Fig. 2), and an activity similar to the one found in the freshly attached hepatocytes (1.5 hrs after initiating the culture) was observed for at least 72 hrs.

It was more interesting to check whether EROD activity could be restored after the considerable decline within the first 24 hrs in culture. For these experiments 50 pg TCDD/ml (final concentration) were added to the medium 24 hrs after initiating the cultures. 24 hrs later the EROD activity was greatly increased and stayed at this level at least up to 72 hrs of the culture period (Fig. 3). This reproducible ability to induce EROD activity subsequent to the initial decline suggested that this system may be suitable for comparative testing of the inducing potential of various PCDDs/PCDFs.

Potency of mixed-tetrahalogenated dibenzo-*p*-dioxins to induce EROD activity

Since we were interested in comparing the biological activity of a series of 2,3,7,8-tetrahalogenated dibenzo-*p*-dioxins, containing chlorine as well as bromine in the same molecule, we used the system mentioned for this purpose.
All congeners were tested at a concentration equivalent to 50 pg TCDD/ml on a molar basis. This corresponds to 1.6 x 10^{-10} M. The results obtained are compiled in Figure 4.

It is obvious from these exploratory data that TCDD clearly induces the EROD activity, and that 1B3CDD and 2B2CDD exhibit about the same potency. On the other hand, the congeners with a high degree of bromination (4BDD and 3B1CDD) apparently show a lower potency to induce EROD activity.

CONCLUSIONS

Our data indicate that rat hepatocyte that have lost most of their EROD activity during primary culturing may serve as a test system for analysing the inducing effects of PCDDs/PCDFs. A well-measurable induction is obtained with concentrations of TCDD lower than 1 x 10^{-10} M. Thus, the system shows a sufficient sensitivity for such studies.

There seem to be differences in the potency of 2,3,7,8-tetra(bomo/chloro)dibenzo-*p*-dioxins to induce EROD activity in this *in vitro* system. However, these data have to be confirmed by a closer analysis of the dose-response relationships. Furthermore, these first *in vitro* data neither allow a prediction of the extent of such an effect *in vivo* nor do they establish "TCDD-toxic equivalency" factors, since it is well-known that an *in vitro/in vivo* comparison and risk assessments are meaningless unless kinetic data are available and taken into consideration (Neubert 1988).

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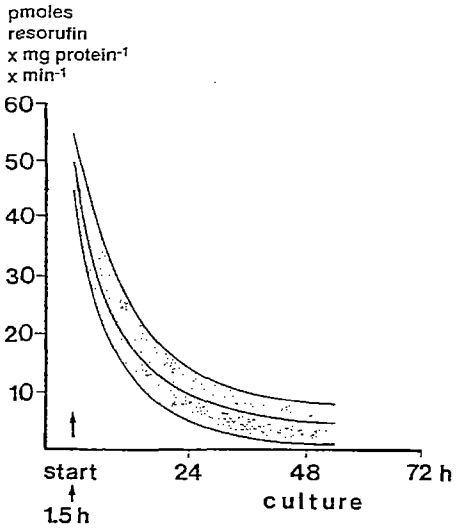

Figure 1:
Time course of EROD activity during primary culturing of isolated rat hepatocytes. Enzyme activity was measured in the homogenates. The monooxygenase activity steadily declined during the culturing.

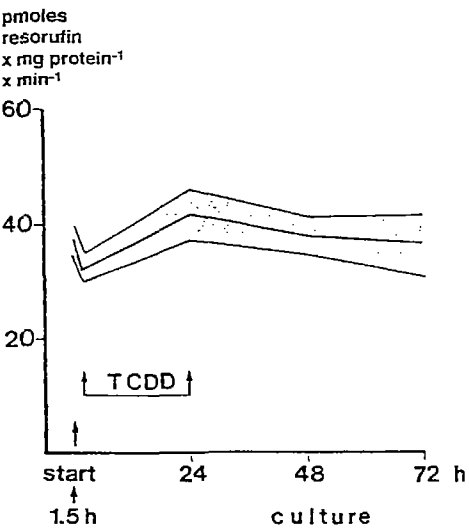

Figure 2:
Stabilization of EROD activity by TCDD during primary culturing of isolated rat hepatocytes. 156 pM TCDD was present in the medium from the initiation of the monolayer culture.

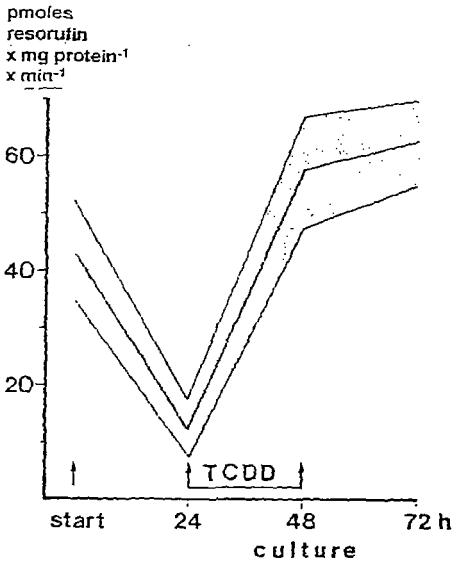

Figure 3:
Induction of EROD activity by TCDD subsequent to the spontaneous decline of this activity in rat hepatocytes during primary culturing. 156 pM TCDD was added to the medium 24 hrs after initiation of the monolayer culture.

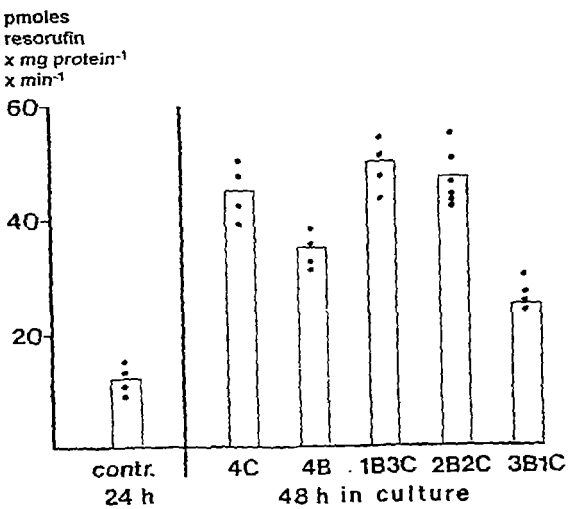

Figure 4:
Induction of EROD activity by 156 pM of various 2,3,7,8-tetra (bromo/chloro)dibenzo-*p*-dioxins subsequent to the spontaneous decline of this activity in rat hepatocytes during primary culturing. The congeners were added to the medium 24 hrs after initiating the monolayer cultures. The effect was evaluated 48 hrs after addition of the inducers. The white bars give the median values.
4C = 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin;
4B = 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin;
1B3CDD = monobromo-trichloro-dibenzo-*p*-dioxin;
2B2CDD = dichloro-dibromo-dibenzo-*p*-dioxin;
3B1CDD = trichloro-monobromo-dibenzo-*p*-dioxins.