Do 5L Rat Hepatoma Cells Form Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in a Peroxidase-catalyzed Reaction?

Clkryt, P.
Institute of Toxicology, University of Würzburg, Versbacher Str. 9, D-8700 Würzburg, Fed. Rep. of Germany

ABSTRACT

The formation of polychlorinated dibenzo-p-dioxins and dibenzofurans in a peroxidase-catalyzed reaction was studied in 5L rat hepatoma cells which are sensitive to the toxic action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The detection of dioxins was performed with a bioassay which determines the proliferation-inhibition caused by TCDD and related compounds in 5L cells. The first coincubation experiments with chlorophenols and peroxides in micromolar concentrations did not produce clear-cut results. Therefore, it is necessary to increase the sensitivity of the assay system used and to employ other biological tests for the detection of dioxins to determine whether cultured cells are competent to form dioxins from suitable precursors.

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) are ubiquitous contaminants of the environment. The main sources of PCDDs/PCDFs are diverse processes of combustion, but recently their biological formation has been described[1,2]. Incubation of 2,4,5-trichlorophenol (TCP) or of other chlorophenols with various peroxidases in the presence of hydrogen peroxide leads to the formation of PCDDs/PCDFs, including the highly toxic 2,3,7,8-substituted congeners. Many mammalian cells contain a substantial levels of peroxidase activity and peroxides are formed continously during normal cellular metabolism. Thus, in principle, production of dioxins in the presence of chlorophenols seems possible in living cells. In the current study, we have used the 5L rat hepatoma cell line as a model and to stimulate peroxidative metabolism, we added peroxides to the culture medium. The detection of the biological formation of PCDDs/PCDFs was carried out with a bioassay that takes advantage of a characteristic of the 5L cells: cell proliferation is inhibited on exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds[3]. The proliferation-inhibitory effect of TCDD causes a reduction in cellular DNA content compared to that of untreated cells. 5L rat hepatoma cells, dedifferentiated descendents of H4IIEC3 Reuber cells, differ with respect to their TCDD-sensitivity from most other cultured cells, which are exceptionally resistant to the toxic effect of TCDD[4] despite its high toxicity to all animal species.

MATERIALS AND METHODS

TCP was from Riedel-de Haen (Seelze), TCDD and pentachlorophenol (PCP) were from Supelco (Bad Homburg, all F.R.G.). Dedifferentiated 5L Reuber hepatoma cells were cultured using standard techniques. The amount of DNA was determined as described in[3] and cell numbers were counted using a Coulter counter.

RESULTS AND DISCUSSION

Effect of TCDD-treatment on cell number and DNA content in 5L cells. Treatment of 5L cells with TCDD leads to a dose-dependent decrease in the cell number as well as in the DNA content of the culture compared to that of untreated cells (Fig. 1). The two curves are nearly parallel so that the DNA content of the culture can be taken at least as a qualitative marker of the presence of TCDD or related compounds in the culture.

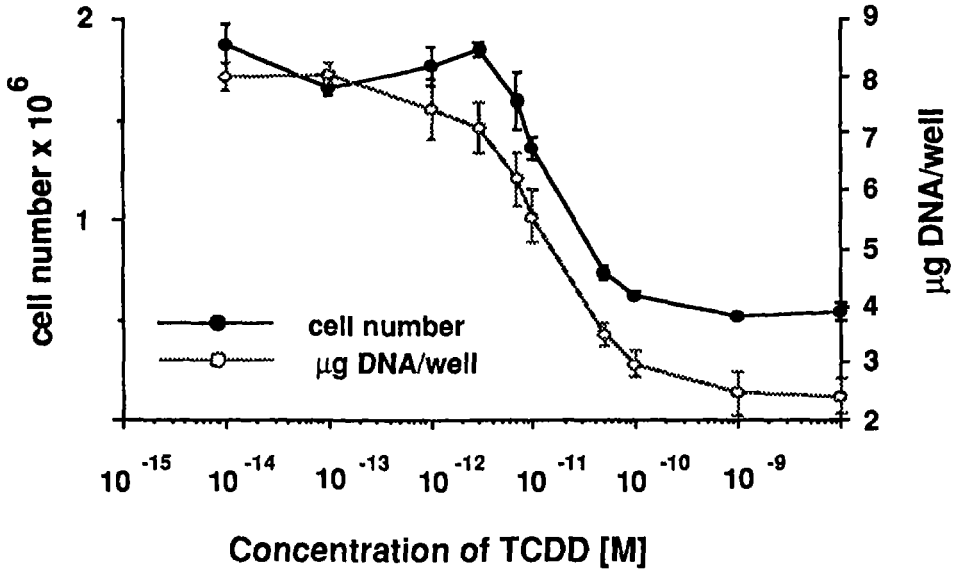

Figure 1: Effect of TCDD-treatment on DNA content and on cell number in 5L cells.

Toxicity of chlorophenols and peroxides to 5L cells. The biological formation of PCDDs/PCDFs by a peroxidase-catalyzed mechanism is dependent on the concentrations both of the chlorophenol and of the peroxide. Before coincubation of the reactants, the toxicity of the chlorophenols and of the peroxides to 5L cells were determined. The two chlorophenols TCP and PCP and the two peroxides H_2O_2 and t-butylhydroperoxide (t-BHP) were tested. The effect of TCP and of H_2O_2 on cell viability is shown in Fig. 2. Concentrations of TCP greater than 10 µM are cytotoxic to 5L cells, whereas the cytotoxic action of H_2O_2 starts at a 10-fold higher concentration. The sensitivity of 5L cells to PCP is comparable with their sensitivity to TCP, but t-BHP is 10-fold more toxic than H_2O_2. According to the cytotoxicity of

the components, the following maximal concentrations were used in this study: H_2O_2 = 100 µM, t-BHP = 10 µM; TCP = 10 µM and PCP = 10 µM.

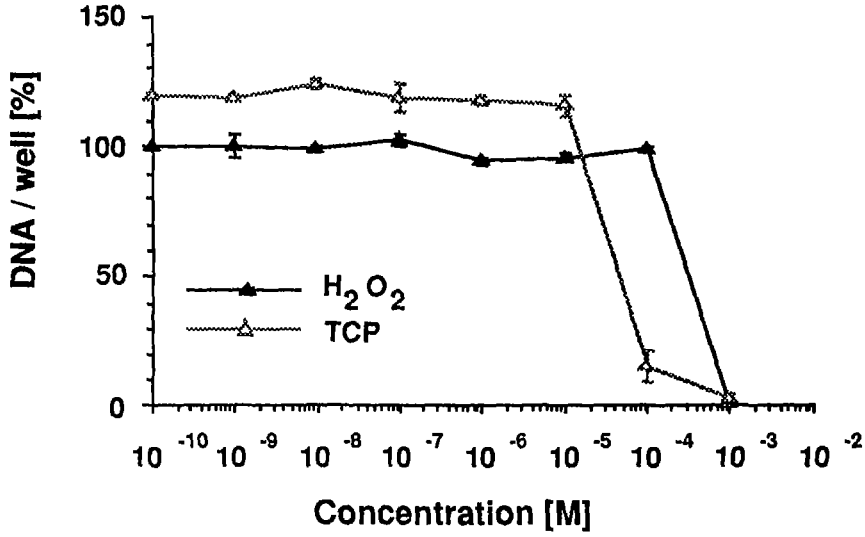

Figure 2: Dose-dependent effect of TCP- or H_2O_2-treatment on cell viability in 5L cells.

Effect of coincubation of chlorophenols and peroxides on cell proliferation of 5L cells. Fig. 3 presents first results of our study which addresses the question of whether cultured cells are able to form PCDDs/PCDFs in a peroxidase-catalyzed reaction. In summary, the following conclusions can be drawn from the preliminary data of Fig. 3 and of analogous experiments with PCP and the organic peroxide t-BHP (data not shown):
(1) There is very little, if any dioxin formation by a peroxidase-catalyzed mechanism in cultured cells and it could not be demonstrated with certainty.
(2) As shown in Fig. 3, the formation of PCDDs/PCDFs in the culture depends strongly on the concentration of the two reactants, the chlorophenol and the peroxide. This relationship has to be studied in more detail.
(3) Because of the lack of effect, it is necessary to study additional markers of dioxin production in culture, e.g. enzyme induction of the cytochrome CYP1A1 which is a very sensitive measure of the presence of dioxins in inducible cultured cell lines.
(4) In addition, the study should include cells with a high peroxidase activity, e.g. cells derived from the genital tract.
The data of Fig.3 do not exclude the possibility of dioxin formation in living cells, but on the other hand they do not demonstrate it. These first experiments were performed in the presence of 10 % fetal calf serum (FCS) in the culture medium. 5L cells are more sensitive to the proliferation-inhibition by TCDD at low FCS levels. Thus, we are now repeating the experiments with low FCS levels in the culture medium in order to study more closely the question of dioxin formation from reactive precursors in 5L cells.

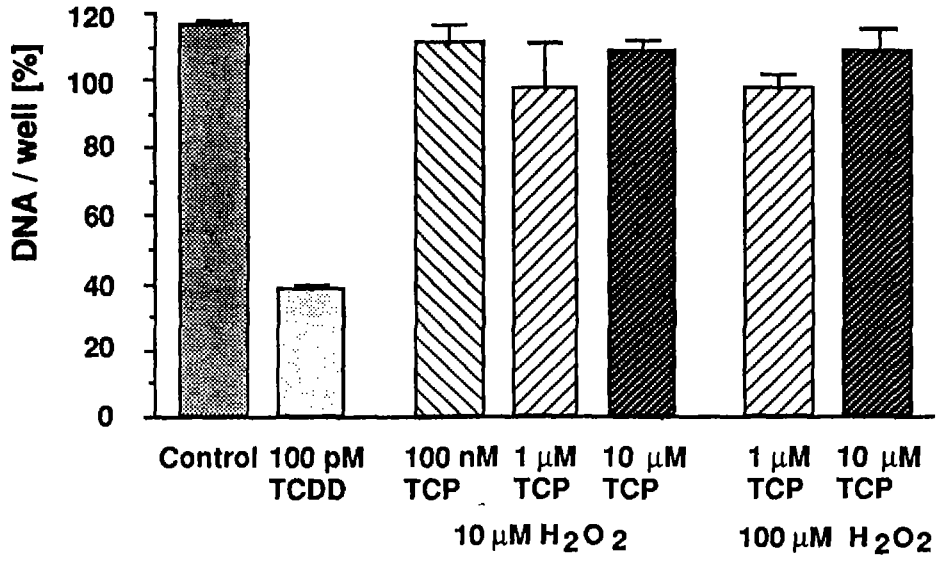

Figure 3: Effect of coincubation of TCP and H_2O_2 on cell proliferation in 5L cells.

ACKNOWLEDGEMENTS

I thank Dr. A. Dunlop for critical reading of the manuscript. The expert technical assistance of Mrs Eva Holzer is gratefully acknowledged. The study was supported by a grant (07DIX10) from the Federal Ministry for Research and Technology (BMFT).

REFERENCES

1 Öberg LG, Glas B, Swanson SE, Rappe C, Paul KG. Peroxidase-catalyzed oxidation of chlorophenols to polychlorinated dibenzo-p-dioxins and dibenzofurans. *Arch Environ Contam Toxicol* 1990; 19:930-8.

2 Öberg LG, Glas B, Rappe C. Biochemical Formation of PCDDs and PCDFs. IN: *Abstractbook of 11th International Symposium on Chlorinated Dioxins and Related Compounds, Dioxin'91*. Research Triangle Park, NC, USA, 1991:19.

3 Göttlicher M, Cikryt P, Wiebel FJ. Inhibition of growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin in 5L rat hepatoma cells is associated with the presence of the Ah receptor. *Carcinogenesis* 1990; 11:2205-10.

4 Knutson JC, Poland A. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: failure to demonstrate toxicity in twenty-three cultured cell types. *Toxicol Appl Pharmacol* 1980; 54:377-83.