ALTERED EXPRESSION OF GROWTH FACTORS IN THE HUMAN BREAST CANCER CELL LINE MCF-7 BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

Ch. Vogel and J. Abel. Medical Institute of Environmental Hygiene, Dep. of Toxicology, Auf'm Hennekamp 50, 4000 Düsseldorf, Germany.

Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds exhibit a broad spectrum of antiestrogenic activities in the estrogen receptor postive MCF-7 cell line. For example, TCDD inhibits the estradiol-induced cell proliferation and the secretion of a 52 kDa-protein[1].

Several studies have shown that TCDD and tamixofen, a triphenylethylene-derived antiestrogen, exhibit comparable effects in animals as well as in MCF-7 cells[2]. Tamoxifen was found to stimulate the secretion of transforming growth factor-ß (TGF-ß) which is thought to act as a hormonally regulated negative growth factor on MCF-7 cells[3]. In a previous study, we found an increased secretion of TGF-ß after treatment of MCF-7 cells with 3,3',4,4'-tetrachlorobiphenyl[4].

Since the mechanisms by which TCB or TCDD can stimulate the secretion of TGF-ß are unknown, this preliminary study is focused on the TCDD-induced changes of different growth factor expression with special regards to TGF-ß.

Materials and Methods

Nearly confluent monolayers of MCF-7 cells were cultured in serum-free HAM's F-10 medium and treated with 100 nM TCDD (dissolved in DMSO, 0.1% final concentration) for periods of 20 and 48 h, respectively. Control cells were incubated with the solvent vehicle. At the indicated times the cells were harvested and total RNA was isolated by the cesium chloride method[5]. The expression of different growth factors in MCF-7 cells was demonstrated by the polymerase chain reaction (PCR) method[6]. The gene sequences used to construct the different oligonucleotide primers were from published sources: ß-actin[7], TGF-ß$_1$[8], TGF-ß$_2$[9], TGF-ß$_3$[10], TGF-α[6], IL-1ß[11] and TNF-α[11]. Optimal annealing temperature was defined for each primer pair. For semi-quantitative analysis of the PCR reaction radioactive-labeled

dCTP (1 μCi [α^{32}P]-dCTP) was included in the PCR reaction mixture. The PCR products were analysed on a 10% polyacrylamide gel and visualized by autoradiography. The respective bands were compared to the predicted fragment size, cut out and counted for radioactivity. Since the PCR is only proportional to the amount of cDNA in the exponential reaction phase[12], three reactions with increasing cycle numbers were performed for one cDNA concentration. Each PCR-amplified DNA product was corrected by the expression of ß-actin. The values of changes of the different mRNA transcripts are given relative to the controls.

Results

Results of the PCR analyses show that mRNA transcripts of all tested growth factors could be detected in MCF-7 cells.

The effect of TCDD on the expression of different growth factors are summarized in Table 1. With the exception of TGF $ß_1$ an increase of the mRNA-transcripts of studied growth factors was observed after TCDD treatment. Incubation of MCF-7 cells with 100 nM TCDD for 20 or 48h led to a time-dependent increase of TGF-$ß_2$ and TGF-$ß_3$ mRNA. The effect was more pronounced for TGF-$ß_3$ than for TGF-$ß_2$. After an incubation of 20 or 48h the TGF-$ß_3$ mRNA increased 3.4- and 5.9-fold above the control values, whereas the TGF-$ß_2$ mRNA was enhanced only by a factor of 1.8 and 2.3, respectively. The corresponding factors for the increase of IL-1ß were 5.7 and 5.8, for TNF-α 5.6 and 5.5 and for TGF-α 2.9 and 3.2. These results may indicate that an incubation of MCF-7 cells with 100 nM TCDD for a period of 20h led to a maximal induction of IL-1ß, TNF-α and TGF-α.

Table 1. Relative changes of growth factor expression in MCF-7 cells after treatment with 100 nM TCDD for a period of 20 and 48h

treatment	TGF-$ß_1$	TGF-$ß_2$	TGF-$ß_3$	TGF-α	IL-1ß	TNF-α
20h	1.1	1.8	3.4	2.9	5.7	5.6
48h	1.4	2.3	5.9	3.2	5.8	5.5

Conclusions

Our results confirm previous findings that TCDD can change the expression of several growth factors such as TGF-ß, TGF-α, and IL-1ß[13,14,15]. However, in contrast to the studies in a human keratinocyte cell line, which showed a decrease of TGF-$ß_2$ mRNA

after TCDD exposure[16], we observed a marked increase of TGF-ß mRNA in TCDD treated MCF-7 cells. The expression of TGF-$ß_3$ was enhanced 5.9-fold above control. Recently, an increase of TGF-$ß_1$ and TGF-$ß_2$ mRNA was reported in the palatal shelves of TCDD treated mouse embryos, and it has been suggested that changes of the pattern of growth factors may be involved in the teratogenic action of TCDD[13]. TGF-ß has been shown to be important in the regulation of cell growth and differentiation. TGF-ß regulates the synthesis of extracellular matrix proteins and is also functionally in the immune system[17]. TGF-ß can inhibit the growth of various normal and malignant cell lines "in vitro"[17]. Thus, the growth inhibitory effect of TCDD on MCF-7 cells may be partly mediated by the increased expression of TGF-ß.

In conclusion, TCDD seems to exert a cell specific alteration of growth factor pattern which may be one of the causes for the pleiotropic action of this compound.

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