The Promotion of Malignant Transformation of Mouse Fibroblasts *in vitro* by 2,3,7,8-TCDD Is Associated with Changes in the Arachidonic Acid Metabolism.

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INTRODUCTION

2,3,7,8-TCDD, a prototype of many halogenated aromatic hydrocarbons, is one of the most powerful tumor promoters in rodent bioassays[1]. Nevertheless, only a limited number of *in vitro*-models exist for the study of biochemical events at a cellular and molecular level associated with tumor promotion by TCDD and for characterizing factors that potentiate or inhibit its promoting activity. Such *in vitro*-models used for TCDD include two stage (initiation-promotion) transformation-systems, *e.g.*, the transformation of mouse fibroblasts such as C3H 10T1/2[2] and C3H/M2[3] or rat tracheal epithelial cells[4] initiated with N-methyl-N´-nitro-N-nitrosoguanidine (MNNG) and methylcholanthrene (MCA).

The role of arachidonic acid (AA) metabolites as modulators in the multi-step process of carcinogenesis, particularly in tumor promotion, has often been postulated with supportive evidence from epidemiological[5], *in vivo*- and *in vitro*-studies[6]. The role of AA metabolism in tumor promotion is strongly suggested by the following observations: (i) inhibitors of AA metabolism reduce the promoting activity of various tumor promoters *in vivo* and *in vitro*; (ii) the levels of specific AA metabolites are enhanced in tumors.

Evidence supporting a role of AA metabolites in the C3H/M2 mouse fibroblast transformation assay is the inhibition of the promoting effect of TCDD by hydrocortisone, an inhibitor of phospholipase A, indomethacin, an inhibitor of cyclooxygenase, and caffeic acid, an inhibitor of lipoxygenase[7]. In the present investigation, the enhancement of the prostaglandin (PG)E_2 and 6-keto-$PGF_{1\alpha}$ levels after TCDD-treatment of MNNG- or MCA-initiated fibroblasts was studied.

MATERIALS AND METHODS

TCDD was obtained from Ökometric (Bayreuth, Germany) and was > 99% pure. The following compounds were purchased from the indicated companies: MNNG (Sigma, Munich, Germany); MCA (Eastman Organic Chemicals, Rochester, NY); basal Eagle's medium and fetal calf serum were obtained from Gibco (Eggenstein, Germany).

C3H/M2 mouse fibroblasts *in vitro* were cultured as described previously for the transformation assay[3, 8]. Briefly, cells harvested from logarithmically growing stock cultures were plated on day 0 in basal Eagle's medium supplemented with 10% fetal calf serum. After 24 h, the cultures were treated for 24 h with initiating agents, *i.e.*, MNNG (0.1 mg/ml), MCA (1 mg/ml), or solvent control, dimethylsulfoxide (0.5%). Thereafter, the medium was renewed and the cells were allowed to grow in fresh medium. Beginning on day 5 until the end of the experiment, with each of the (twice-weekly) medium renewals TCDD or solvent were added. After 8 weeks medium samples were taken for PG analysis and the cells were fixed and stained to determine the transformation rate.

Elution profiles of AA metabolites from the culture medium were obtained by HPLC[9]; PGs were quantitatively determined by ELISA kits (Immunotech, Hamburg, Germany).

RESULTS

C3H/M2 fibroblasts initiated with MNNG or MCA were labelled with ^{3}H-AA at the end of the promoting treatment with TCDD. Thereafter, ^{3}H-AA release into the medium was measured in the presence of the calcium ionophor A23187: In TCDD-treated cultures the AA-release was only slightly enhanced. The AA metabolites from the culture medium were further analyzed by HPLC. No qualitative changes in the elution profiles of MNNG-, MCA-initiated and control cultures were observed after treatment with TCDD (1.5×10^{-12} M). The major peaks were attributed to 6-keto-$PGF_{1\alpha}$ and PGE_2.

A quantitative determination of the accumulation of these prostaglandins was obtained by ELISA. Higher PG levels were found in cultures of malignantly transformed cells as compared to normal fibroblasts. After short-term (24h) treatment of untransformed cells with TCDD, no significant differences in PG concentrations were detected in the culture medium. However, after long-term treatment, TCDD markedly enhanced the levels of 6-keto-$PGF_{1\alpha}$ (preliminary results) and PGE_2 in MCA-initiated but not in control cultures (Table 1). This effect of TCDD was significant after 8 days and was observed at very low TCDD concentrations (10^{-13} M to 10^{-12} M) which were also maximally effective in promoting the malignant transformation.

Addition of indomethacin (20 µg/ml) to the cultures completely blocked the enhancement of transformation by TCDD and reduced the PG levels to about 10 % of those in control cultures.

Table 1: Enhancement of PGE_2 accumulation after long-term treatment with TCDD

Cultures were pretreated with solvent (DMSO) or with the initiating agent MCA. Thereafter, cells were treated with TCDD for 7 weeks. 24 h after the last medium renewal, samples were taken for ELISA analysis of PGs; the cells were fixed and the transformed foci per treated dishes were counted.

	DMSO	TCDD	
		10^{-13} M	10^{-12} M
A) PGE_2 accumulation		(% of controls)[a]	
DMSO	100 ± 20[b]	158 ± 52	125 ± 21
MCA	77 ± 35	192 ± 32*	170 ± 82*
B) Transformation	(number of transformed foci/ dishes treated)		
DMSO	0 / 34	0 / 23	0 / 35
MCA	3 / 37 (0.08)[c]	7 / 26 (0.27)	11 / 44 (0.25)

a) PGE_2 concentrations from cultures without MCA-treatment were set to 100%.
b) Data represent the mean ± SD (3 independent experiments).
c) Numerical value of the ratio.
*) $P<0.05$; compared to MCA-initiated cultures without TCDD.

DISCUSSION AND CONCLUSIONS

The results of the present study indicate that in carcinogen-initiated C3H/M2 mouse fibroblast cultures the promotion of malignant transformation by TCDD is associated with higher levels of 6-keto-$PGF_{1\alpha}$ and PGE_2 which are the major AA metabolites (HPLC analysis of the culture medium). Both TCDD effects, promotion and enhancement of PG accumulation, are inhibited by indomethacin. Moreover, not only inhibitors of cyclooxygenases but also inhibitors of lipoxygenases, *e.g.* caffeic acid or nordihydroguaiaretic acid, have been found to abolish the TCDD-mediated enhancement of malignant transformation (mouse fibroblasts) and of mitotic rates (primary rat hepatocytes)[7]. Thus, stimulation of growth and tumor promotion may be due to a more complex pattern of AA metabolites[10] which may act at different stages of the carcinogenic process[11]. Reports on the expression of cyclooxygenase-2[12] and 12-lipoxygenase[13] in various cell lines and tumors support the suggestion that these enzymes play a role in growth regulation and tumor promotion. In conclusion, both enzymes, cyclooxygenase and lipoxygenase, may be critical targets in the mechanisms of tumor promotion.

REFERENCES

1. Pitot, H.C., Goldsworthy, T., Campbell, H.A. and Poland, A. (1980): Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res. 40, 3616-3620.
2. Abernethy, D.J. and Boreiko, C.J. (1987) Promotion of C3H/10T1/2 morphological transformation by polychlorinated dibenzo-p-dioxin isomers. Carcinogenesis 8, 1485-1490.
3. Wölfle, D. and Marquardt, H. (1996) Antioxidants inhibit the enhancement of malignant cell transformation induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Carcinognesis 17, in press.
4. Tanaka, N., Nettesheim, P., Gray, T., Nelson, K. and Barrett, J.C. (1989) 2,3,7,8-Tetrachlorodibenzo-p-dioxin enhancement of N-methyl-N'-nitro-N-nitrosoguanidine-induced transformation of rat tracheal epithelial cells in culture. Cancer Res. 49, 2703-2708.
5. Giovannucci, E., Egan, K.M. and Hunter, D.J. (1995) Aspirin and the risk of colorectal cancer in women. N.Engl.J. Med. 333, 609-614.
6. Marnett, L.J. (1992) Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res. 52, 5575-5589.
7. Wölfle, D., Marotzki, S., Hamel, U., and Marquardt, H. (1994) Stimulation of growth and enhancement of malignant cell transformation by tumor promoting polychlorinated hydrocarbons: Involvement of arachidonic acid metabolism. Proc.Amer.Assoc.Cancer Res. 35, 1007.
8. Marquardt, H., Philips, F.S. and Sternberg, S.S. (1976) Tumorigenicity in vivo and induction of malignant transformation and mutagenicity in cell cultures by adriamycin and daunomycin. Cancer Res. 36, 2065-2069.
9. Schäfer, W, Strandberg, C. Gaillard, T., Zahradnik, H.P. (1988) On-line sample clean-up and HPLC analysis of prostaglandins in urine, amniotic fluid, and plasma using a column-switching technique. Eiconanoids 1, 101-106.
10. Fischer, S.M. Baldwin, J.K., Jasheway, D.W., Patrick, K.E., and Cameron, G.S. (1988) Phorbol ester induction of 8-lipoxygenase in inbred SENCAR (SSIN) but not C57BL/6J mice correlated with hyperplasia, edema, and oxidant generation but not ornithine devarboxylase induction. Cancer Res. 4,: 658-664.
11. Fürstenberger, G., Gross, M., and Marks, F. (1989) eicosanoids and multispage carcinogenesis in NMRI mouse skin; role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). Carcinogenesis 10, 91-96.
12. Sano H. Kawahito, Y. Wilder, R.L., Hashiramoto, A., Mukai, S., Asai, K. Kimura, S., Kato, H. Kondo, M. and Hla, T. (1995) Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res. 55, 3785-3789.
13. Krieg, P., Kinzig, A., Ress-Löschke, M., Vogel, S., Vanlandingham, B., Stephan, M. Lehmann, W.-D., Marks, F., and Fürstenberger, G. (1995) 12-Lipoxygenase isoenzymes in mouse skin tumor development. Mol.Carcinog. 14, 118-129.