Regulation of Prostaglandin Endoperoxide H Synthase-2 Induction by TCDD in Rat Hepatocytes

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Introduction

Prostaglandin endoperoxide H synthase-2 (PGHS-2) is a key enzyme in the production of prostaglandins playing an important function in different physiological and pathophysiological processes. Recently, we have shown that TCDD led to increased PGHS-2 mRNA expression in vitro and in vivo (1,2). However, the molecular mechanisms for TCDD-stimulated PGHS-2 mRNA elevation has not been elucidated. Recently Matsumura's group has reported that TCDD can activate the tyrosin kinase c-Src (3) which is known to regulate PGHS-2 gene expression (4). Here, we describe the possible role of c-Src in the pathway of the TCDD-induced PGHS-2 expression in primary cultured rat hepatocytes.

Materials and Methods

Cell culture conditions. Rat hepatocytes (female Sprague-Dawley rats) were prepared as previously described (5). Murine Hepa1c1c7 cells were grown in D'MEM-alpha medium containing 10% FCS.

RT-PCR. RT-PCR analysis were performed according to (2). The respective bands were scanned, and the integrated band intensities were normalized to β-Actin.

PGE_2 production. The PGE_2 level in culture medium was quantitated by enzyme immunoassay according to manufacture's instructions (Cayman Chemical).

Luciferase assay. Hepa1c1c7 cells were transiently transfected with a luciferase reporter construct of the murine PGHS-2 promoter containing 891 bp of the 5'-untranslated region.

Electromobility-shift-analysis: EMSA were performed as described previously (6). Double-stranded oligonucleotides containing consensus sequences for the C/EBP or DRE binding site of the rat PGHS-2 promoter were used for band shift analysis with nuclear extracts from control and TCDD-treated Hepa1c1c7 cells.

Results and Discussion

Dose-dependent induction of PGHS-2 and CYP1A1 mRNA expression by TCDD.
For dose-response studies rat hepatocytes were treated with various doses of TCDD (0.01-10 nM) and the PGHS-2 and CYP1A1 mRNA content was analysed after 24h. The results in Figure 1A showed that TCDD increased PGHS-2 and CYP1A1 expression in a dose-dependent manner. Significant induction of PGHS-2 and CYP1A1 expression was found at 0.1 and 0.01 nM TCDD, respectively. The maximal activation of PGHS-2 (3 to 5-fold) in hepatocytes occured at concentrations of 10 nM TCDD whereas CYP1A1 mRNA expression was increased about 12-fold.

PGE_2 production of rat hepatocytes
To evaluate whether the increased PGHS-2 mRNA after TCDD is associated with an enhanced PGE_2 level we measured PGE_2 concentrations in the cell supernatants. The results of time course studies revealed that TCDD elevated PGE_2 synthesis in rat hepatocytes (Figure 1B). TCDD-treated and control cells showed a biphasic increase of PGE_2 levels. Maximal PGE_2 production of TCDD-treated hepatocytes occured after 2.5 h and 6 h with a 4-fold increase above PGE_2 level in supernatant of control cells.

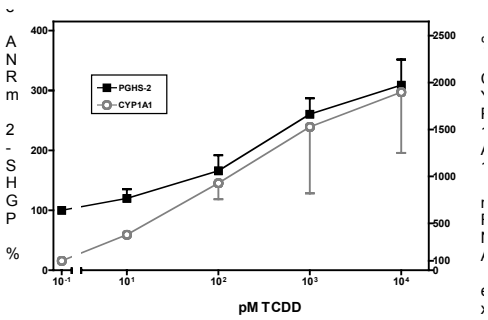

Fig. 1A Dose-dependent induction of PGHS-2 and CYP1A1 mRNA by TCDD.

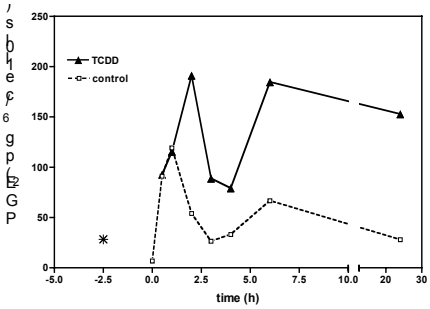

Fig. 1B Time course of PGE_2 production after TCDD treatment.

Effect of TCDD on a luciferase reporter construct of the murine PGHS-2 promoter
TCDD led to an enhanced transcription (2-3 fold) of a luciferase reporter gene under control of the murine PGHS-2 promoter (Figure 3), indicating that TCDD-mediated increase of PGHS-2 mRNA is transcriptionally controled.

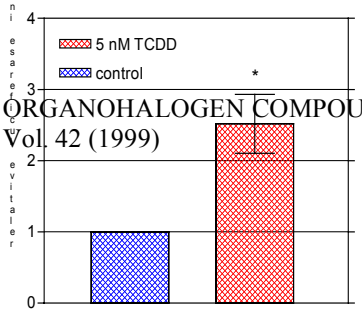

Fig. 3 Hepa 1c1c7 cells were transiently transfected with the luciferase reporter construct pTIS10L (-961 to +70 bp) of the murine PGHS-2 promoter, and treated with 5 nM TCDD for 16 h. Luminescence activity is given as relative induction compared to control cells.
* significantly different vs control $p < 0.05$.

Effect of Herbimycin A and Geldanamycin on TCDD-mediated PGHS-2 and CYP1A1 mRNA induction

To investigate whether PGHS-2 induction is mediated by the tyrosine kinase c-Src we analysed the effect of TCDD on PGHS-2 mRNA expression in presence of specific c-Src inhibitors such as Herbimycin A and Geldanamycin. Cells were preincubated with 0.5 µM Herbimycin A or 3 µM Geldanamycin for 10 min and then cotreated with 10 nM TCDD for 12 h. As shown in Fig. 2A Herbimycin A and Geldanamycin suppressed both constitutive and TCDD-induced PGHS-2 mRNA expression whereas CYP1A1 induction was not affected by Herbimycin A or Geldanamycin (Fig. 2B). These results indicate an involvement of c-Src in TCDD mediated PGHS-2 induction.

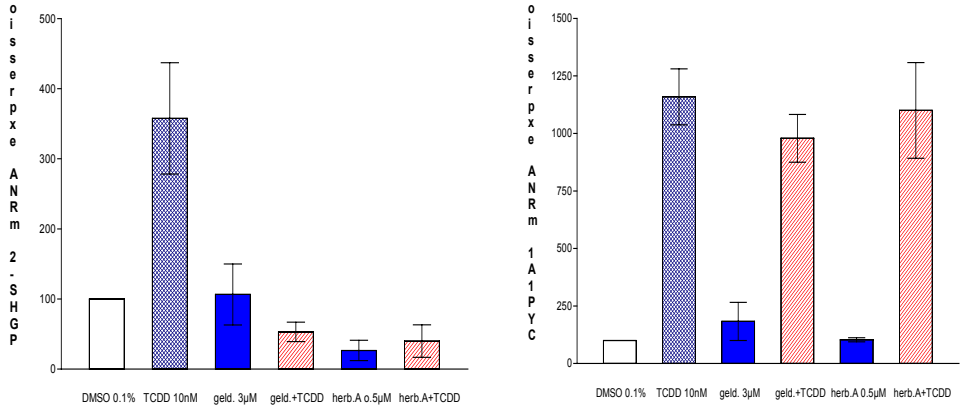

Fig. 2 Effect of Herbimycin A and Geldanamycin on PGHS-2 (A) and CYP1A1 mRNA (B) induction. Cells were preincubated with c-Src inhibitors for 10 min and cotreated with 10 nM TCDD

Effect of TCDD on DNA-binding activity of the C/EBP response element

Recently it was shown that TCDD can enhance DNA binding activity of trans-acting factor C/EBPβ (7). Since a C/EBP binding site is located within the PGHS-2 promoter we performed EMSA analysis. Figure 4 shows that TCDD enhanced DNA binding activity of C/EBP 2-fold above control. Supershift analysis with specific antibodies revealed that C/EBP is the major component of the DNA-protein complex. From the results we conclude that c-Src mediated activation of C/EBPβ is an important pathway for PGHS-2 induction by TCDD.

Fig. 6 Detection of C/EBP binding complexes by EMSA. Nuclear protein extracts of Hepa1c1c7 cells were incubated with a ^{32}P-endlabeled oligonucleotide containing the C/EBP binding site of the PGHS-2 promoter. Cells were treated with 0.1% DMSO or 10 nM TCDD as indicated. Super shift assays were performed using an anti-C/EBP̄ or anti C/EBP̄ antibody. Specificity of C/EBP binding was controlled by adding a 200-fold excess of unlabeled C/EBP oligo (last lane).

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