RPT-1, A NEW TARGET FOR TCDD

Susanne Donat and Josef Abel

Medical Institute of Environmental Hygiene at the Heinrich-Heine University of Düsseldorf, Department of Experimental Toxicology, Auf´m Hennekamp 50, 40225 Düsseldorf, Germany

Introduction

The immune system is an important target for the toxic effects of 2,3,7,8-terachlorodibenzo-p-dioxin (TCDD). TCDD has been shown to induce thymic involution as well as suppress T and B lymphocyte function [1]. Despite intensive studies, the mechanism by which TCDD alters functions of the immune system is not exactly known. To light up mechanisms possibly involved in mediating immunotoxic effects of TCDD we performed differential display RT-PCR analysis in thymus of female C57BL/6 mice [2]. We identified regulatory protein T-lymphocyte, 1 (rpt-1) as a yet unknown TCDD responsive gene and show its upregulation in thymus and spleen of TCDD treated mice.

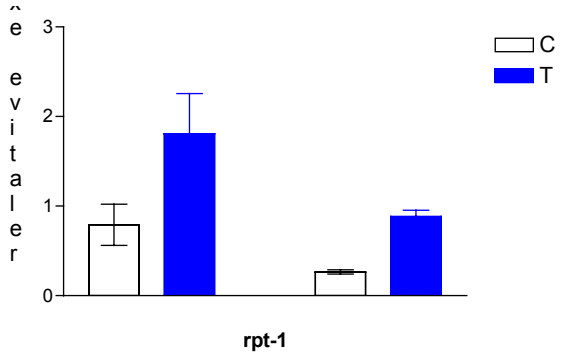
Materials and Methods

Animals and treatments: Female C57BL/6 mice at 6-8 weeks of age were i.p. injected with 10 µg TCDD/kg body weight, control animals received corn oil. Three days post treatment animals were sacrificed.
RT-PCR: RNA preparation and radioactive RT-PCR were performed as previously described [3].
Isolation of the promotor region: For isolation of the 5´-upstream region of rpt-1 the genome walker kit (Clontech) was used.
Transfection experiments: For transient transfection experiments a liposome-mediated transfer was used. Therefore HepG2 cells (2 x 10^5 cells per well) were seeded onto 6-well plates and maintained overnight. The cells were then incubated for 5 h with the DNA/transfectam mixture. Luciferase activities in cell lysates were determined with the dual luciferase reporter assay system (Promega).

Results and Discussion

To identify transcriptionally regulated genes potentially involved in mediating toxic effects of TCDD we performed DDRT-PCR analysis with mRNA from thymus of C57BL/6 mice. One of the isolated fragments prooved to be identical to rpt-1 [4]. In order to verify upregulation of rpt-1 mRNA in response to TCDD we performed RT-PCR analysis in thymus and spleen of mice treated with 10 µg TCDD/kg bw. These analysis confirmed a 2-3 fold induction of rpt-1 mRNA expression (Fig. 1A). Since rpt-1 has been shown to encode an intracellular protein that downregulates gene expression directed by the promotor of the gene encoding interleukin 2 receptor α chain (IL-2Rα), we investigated mRNA expression of IL-2Rα in thymus and spleen [4, 5]. The TCDD induced upregulation of rpt-1 mRNA expression was accompanied by downregulation of IL-2Rα mRNA expression (Fig. 1B).

A:



B:

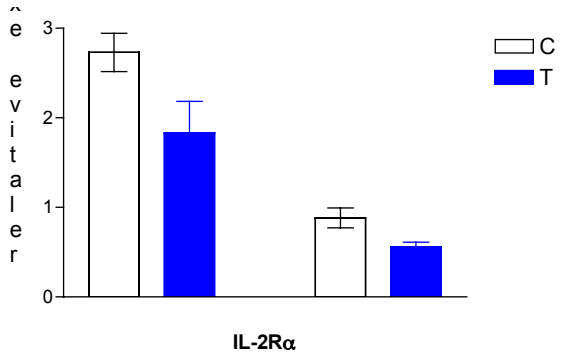


Fig. 1
A: RT-PCR analysis of rpt-1 gene expression in thymus and spleen of control (C) and TCDD-treated (T) C57BL/6 mice. Densitometric values normalized to ß-actin are given.
B. RT-PCR analysis of IL-2Rα gene expression.

The genomic structure of rpt-1 has not yet been described. In order to further elucidate the effect of TCDD on rpt-1 expression we isolated the 5´-upstream region of the rpt-1 gene. The obtained 2.5 kb genomic fragment was cloned into a luciferase reporter vector. Treatment of transiently transfected HepG2 hepatoma cells with 10 nM TCDD for 24 h led to a 2-fold induction of luciferase activity compared with untreated cells (Fig. 2). These results suggest an effect of TCDD on rpt-1 expression on a transcriptional level.

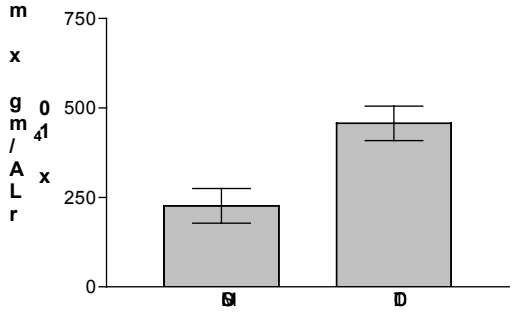


Fig. 2
Induction of rpt-1 promotor driven luciferase activity by TCDD. HepG2 cells were transiently transfected with a luciferase reporter construct of the rpt-1 promotor and treated for 24 h with 10 nM TCDD.

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