EFFECTS OF TCDD ON THE OSTEOBLASTIC CELL LINE UMR-106

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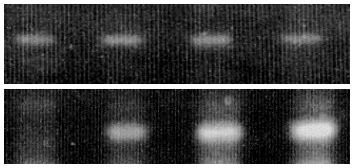
Introduction

Dioxin and dioxin-like compounds are endocrine disrupting environmental pollutants that induce skeletal abnormalities in rat pups as well as in adult animals[1,2]. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces transcription of CYP1A1, which is suggested to alter the estrogen metabolism. Bone loss and impaired bone formation are well known effects of estrogen deficiency.

Osteopontin is a non-collagenous protein involved in attachment of the bone cells to the bone surface. Thus, disturbance of the expression of osteopontin may alter the bone homeostasis.

Aim

The aim of this project was to characterize the osteoblastic cell line UMR-106 and examine if it can be used as an *in vitro* model system for studies on mechanisms behind the effects of dioxin on bone tissue.



C 10^{-9} M TCDD

Fig. 1. The expression of AhR (upper), CYP1A1 (lower) in UMR-106 cells exposed to TCDD for 24 hours.

Methods

UMR-106 cells are adherent and cultured in D-MEM, supplemented with FBS and penicillin/streptomycin.

Cells were plated in 6 well plates and exposed to different concentrations of TCDD dissolved in DMSO. Cells were exposed 6, 12 or 24h and RNA was isolated. The RNA was DNase treated and a cDNA synthesis was performed.

Reverse-transcriptase PCR with primers against AhR and CYP1A1 was performed and run on an agarose gel.

Real time PCR was performed to quantify the expression of CYP1A1 and osteopontin after exposure to TCDD.

Conclusion

Our results show that the osteoblastic cell line UMR-106 express the AhR and that the expression of CYP1A1 is induced after exposure to TCDD. Thus, this cell line may be a suitable model for studying dioxin-induced effects on osteoblasts. The decreased expression of osteopontin observed was a more rapid and sensitive response than CYP1A1 induction and may be involved in the skeletal effects caused by dioxin.

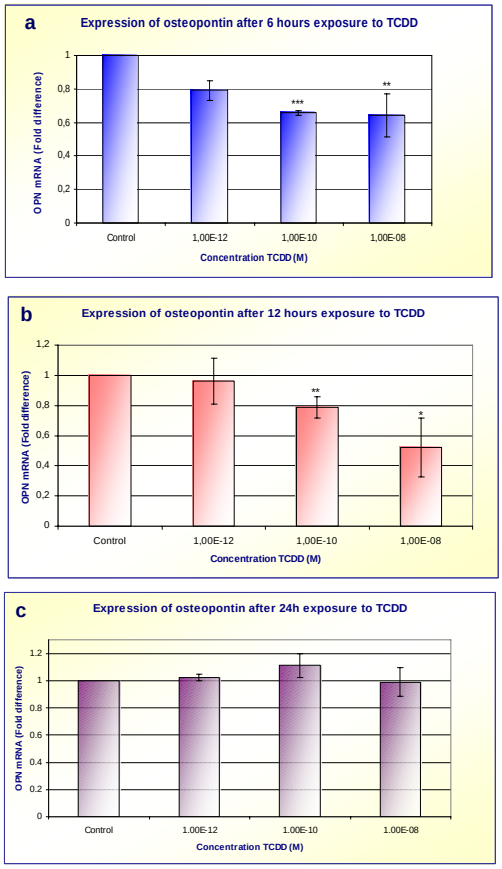


Fig. 2. The expression of osteopontin mRNA after exposure to TCDD at different concentrations for a) 6, b)12 and c) 24 hours.

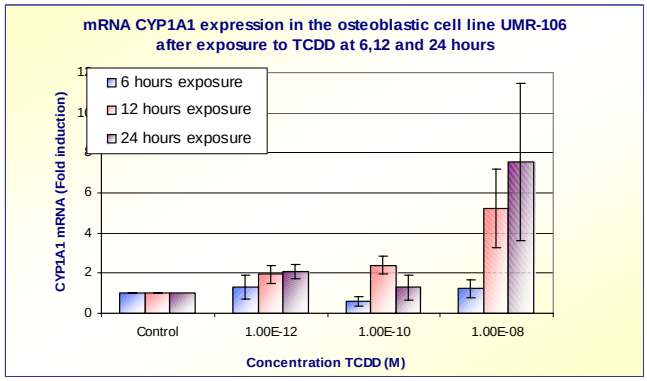


Fig. 3. Expression of CYP1A1 mRNA at different time-points and at different concentrations of TCDD.

Results

- The osteoblastic cell line UMR-106 expresses the AhR and the expression of CYP1A1 is induced after 24 hours exposure to TCDD (Fig.1).
- The expression of osteopontin was significantly decreased after 6 and 12 hours exposure to TCDD, but not after 24 hours. (Fig. 2).
- Exposure to TCDD significantly increased the expression of CYP1A1 after 12 and 24 hours (Fig. 3).

Discussion

Our results show that the expression of osteopontin is significantly decreased by TCDD during the first 12 hours of exposure. A decreased expression of osteopontin may inhibit the ability of the osteoblasts to adhere to the bone surface and thereby decrease bone formation. CYP1A1 was induced in the cells, but this effect occurred at a higher dose and later than the decreased osteopontin expression. This suggest that osteopontin is more sensitive to TCDD in these osteoblast-like cells.

References

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