Role of CCAAT/ Enhancer Binding Protein β Activation in the Induction of Glutathione S-Transferase A2 by Toluene

Dal Woong Choi, Jong Ryeul Sohn, Kyung Whan Moon, Sang Hoon Byeon, Hi Chol Kim, Young Whan Kim

Department of Environmental Health, College of Health Sciences, Korea University, Seoul 136-703, Korea

Abstract

The expression of the glutathione S-transferase (GST), whose induction accounts for antioxidant defense system, is regulated by activation of CCAAT/enhancer binding protein β (C/EBPβ). Sick house syndrome (SHS) presents healthy damage owing to the indoor environment of a building. Toluene has been implicated in one of the important causes of SHS. The present study investigated the effects of toluene treatment on the induction of GSTA2 gene and its mechanism. H4IIE cells treated with toluene, and GSTA2 expression was determined by immunoblot analysis. The translocation of C/EBPβ was assessed by immunocytochemical assays. C/EBPβ DNA binding activity was determined by electrophoretic mobility shift assays. The role of the C/EBP binding site in the induction of the GSTA2 gene was assessed by luciferase reporter-gene activity. Toluene induced GSTA2 protein expression. In toluene-treated cells, C/EBPβ translocated to the nucleus and bound to the consensus sequence of C/EBP (TTGCGCAA). Toluene treatment increased luciferase reporter-gene activity in cells transfected with the C/EBP-containing regulatory region of the GSTA2 gene. Oxidative stress is believed to play an important role in the induction of GSTA2 gene by toluene This study shows that toluene-induced GSTA2 gene expression is dependent upon nuclear translocation and binding of C/EBPβ to the C/EBP response element in the GSTA2 gene promoter.

Introduction

The expression of the glutathione S-transferase (GST), whose induction accounts for antioxidant defense system, is regulated by activation of CCAAT/enhancer binding protein β (C/EBPβ). Sick house syndrome (SHS) presents healthy damage owing to the indoor environment of a building. Toluene has been implicated in one of the important causes of SHS. The present study investigated the effects of toluene treatment on the induction of GSTA2 gene and its mechanism.

Materials and Methods

Cell Culture : H4IIE was obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 50 U/mL penicillin, and 50μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Toluene (1 mM) was added to H4IIE cells and incubated for the indicated time period for each experiment at 37°C. Cells were then washed twice with ice-cold phosphate buffered saline (PBS) before sample preparation.

Immunoblot Analysis : SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis were performed according to previously published procedures.

Gel Shift Assay : C/EBP gel shift analysis was carried out with the labeled oligonucleotide, 5'-TGCAGATTGCGCAATCTGCA-3' that contained the C/EBP consensus sequence. The reaction mixture contained 4 μl of 5 × binding buffer (containing 20% glycerol, 5 mM $MgCl_2$, 250 mM NaCl, 2.5 mM EDTA, 2.5 mM DTT, 0.25 mg/ml poly dI-dC and 50 mM Tris-Cl [pH 7.5]), 10 μg of nuclear extract, and sterile water up to a total volume of 20 μl. The reaction mixture was preincubated without probe at room temperature for 10 min. The probe was then added and DNA-binding reactions were carried out for 30 min at room temperature. Samples were separated on 4% polyacrylamide gels at 100 V. The gels were fixed with 40% methanol /10% acetic acid, dried, and subject to scanner.

Immunocytochemistry : H4IIE cells were grown on Lab-TEK chamber slides (Nalge Nunc International Corp, Rochester, NY) and incubated in serum-free Dulbecco's modified Eagle's medium for 6 h at 37°C. Standard immunocytochemical methods were used for immunostaining of C/EBPβ as previously described (Nancy et al 1999).

Construction of GSTA2 Promoter-Luciferase Constructs and Luciferase Assay : The pGL-1651 reporter gene construct was generated by ligating the region 1.65 kb upstream of the transcription start site of the *GSTA2* gene to the firefly luciferase reporter gene coding sequences. A series of chimeric gene constructs pGL-1128, pGL-797, and pGL-197 with promoter deletions were also created. To determine the promoter activity of the segments of the GSTA2 promoter in the pGL-1128, pGL-797, and pGL-197 constructs, we used the dual-luciferase reporter assay system (Promega, Madison, WI).

Results and Discussion

H4IIE cells treated with toluene, and GSTA2 expression was determined by immunoblot analysis. Toluene induced GSTA2 protein expression.

Control Toluene

Fig. 1. Induction of GSTA2 by toluene. Immunoblot analysis of the GSTA2 protein in cytosolic extracts from H4IIE cells treated with toluene.

The translocation of C/EBPβ was assessed by immunocytochemical assays. C/EBPβ DNA binding activity was determined by electrophoretic mobility shift assays. In toluene-treated cells, C/EBPβ translocated to the nucleus and bound to the consensus sequence of C/EBP (TTGCGCAA).

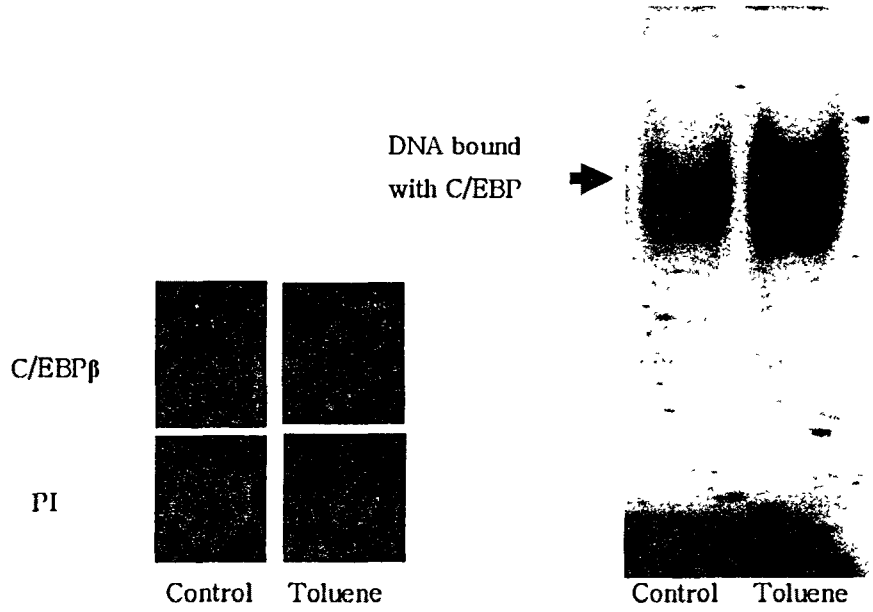

Fig. 2. Effect of toluene on activation of C/EBPβ binding to the C/EBP binding site. A)Immunocytochemical analysis of C/EBPβ. B)Gel shift analysis of protein binding to the C/EBP binding site.

The role of the C/EBP binding site in the induction of the GSTA2 gene was assessed by luciferase reporter-gene activity. Toluene treatment increased luciferase reporter-gene activity in cells transfected with the C/EBP-containing regulatory region of the GSTA2 gene.

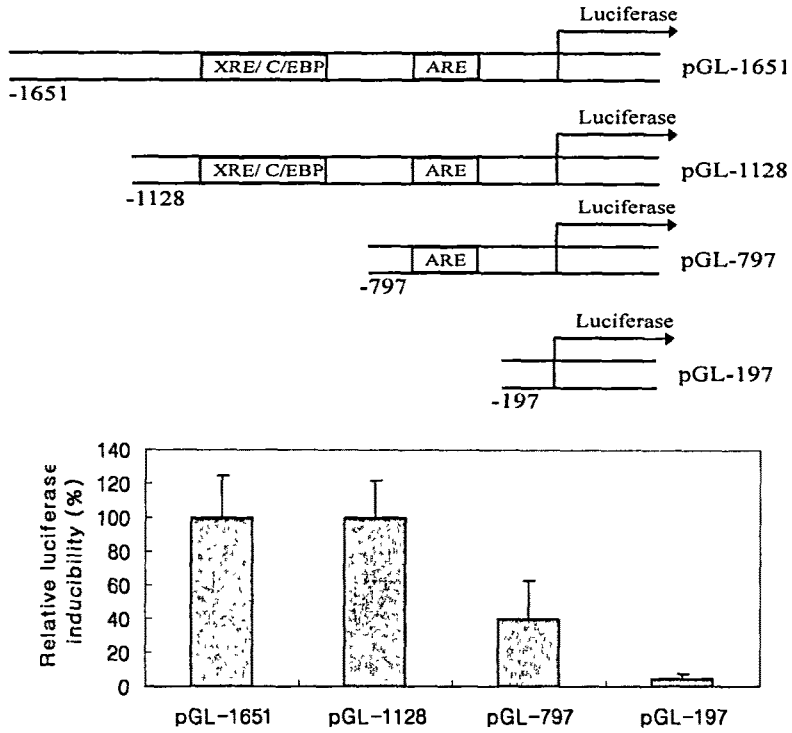

Fig. 3. Analysis of the GSTA2 promoter region in promoter-luciferase reporter gene constructs.

Conclusion

This study shows that oxidative stress is believed to play an important role in the induction of GSTA2 gene by toluene and toluene-induced GSTA2 gene expression is dependent upon nuclear translocation and binding of C/EBP to the C/EBP response element in the GSTA2 gene promoter.

Acknowledgment

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