Expression of glutathione S-transferase P-form by coplanar polychlorinated biphenyls in primary cultured rat liver parenchymal cells and its suppression by protein kinase inhibitors and dexamethasone

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1. Introduction

Polychlorinated biphenyl (PCB) congeners are potent carcinogens, and coplanar PCB congeners, which chlorinated at the meta and para positions, have more severe effects than non-coplanar PCBs. In order to understand tumor promotion by coplanar PCB[1,2], the specific gene expression caused by coplanar PCB exposure in primary cultured rat liver parenchymal cells was examined. 3,3',4,4',5-pentachlorobiphenyl (PenCB) induced glutathione S-transferase P-form (GST-P), which is expressed in hepatocarcinoma[3,4], while non-coplanar PCB did not[5]. GST-P mRNA was also induced by epidermal growth facter (EGF) in the cells. Protein kinase C (PK-C) inhibitors reduced GST-P expression induced either by PenCB or by EGF, but induction of GST-P expression by PenCB was not inhibited by a tyrosine kinase inhibitor. Dexamethasone (Dex, an antagonist of AP-1 transcription factor) also suppressed GST-P expression by PenCB[6]. It is suggested that AP-1 and PK-C type enzyme activity may be involved in the process of inducing GST-P.

2. Experimental procedure

Preparation of monolayer cultured rat liver parenchymal cells and their exposure to chemicals. Liver parenchymal cells, isolated from a male Wistar rat, were inoculated with modified William's E medium containing 10%(v/v) fetal bovine serum (FBS), and were cultured for 4 h in a CO_2 incubator. Following further incubation in modified William's E medium without FBS for 18-20 h, the cells were treated with either PenCB or EGF for 12 h (for northern analysis) or 23 h (for ^{35}S-labelling) with a change in the culture medium to modified William's E medium containing 6 mM nicotinamide as well as other reagents[5,6].

Labelling of newly synthesized proteins with [^{35}S]methionine and detection of ^{35}S-labelled protein by polyacrylamide gel electrophoresis (PAGE). Following exposure to chemicals, the cells were incubated for 1 h in Eagle's MEM deficient in methionine containing 100 μCi of [^{35}S]methionine/ml. After the medium was removed, the cells were lysed by adding a detergent solution (1% Nonidet P-40, 0.1% SDS and 1 mM phenylmethylsulfonyl fluoride). The lysate was subjected to

conventional SDS-PAGE and two dimensional (2D) gel electrophoresis using nonequilibrium pH gradient electrophoresis (NEPHGE) for the first dimension. ^{35}S-labelled proteins separated on the gel were detected by fluorography[5].

Immunochemical detection of GST-P and northern blot analysis of mRNA. Proteins separated on the gel were transferred to a nitrocellulose membrane by electro-blotting. The membrane was exposed to an X-ray film to detect ^{35}S-labelled proteins, and then GST-P was identified by immunochemical staining with rabbit anti-GST-P IgG and protein-A/colloidal gold[5]. The detection was enhanced by silver staining. Levels of mRNAs were determined by northern blot analysis using c-DNA probes of GST-P and cytochrome P450IA2 labelled with [^{32}P]dCTP[6].

3. Results and discussion

The expression of a protein with Mr of 25,000 (25k-protein) was markedly increased after treatment for 23 h with the coplanar PCB congeners (10-100 nM PenCB and 3,3',4,4',5,5'-hexachlorobiphenyl, hexaCB) in primary cultured rat liver parenchymal cells. However, 25k-protein expression was increased slightly by 100 nM 3,3',4,4'-tetrachlorobiphenyl, but was not elevated by 100 nM non-coplanar congeners (2,3,3',4,4'-pentachlorobiphenyl and 2,3,3',4,4',5- and 2,2',4,4',5,5'-hexaCBs), 2 mM phenobarbital and 20 μM p-aminoacetophenone.

Because the apparent Mr of GST-P (25,000) is similar to that of the 25k-protein, and it is known to be a marker for preneoplastic and neoplastic lesions caused by administration of chemical carcinogens[3,4], the relationship between the 25k-protein and GST-P was examined by 2D gel electrophoresis and immunoblotting. An autoradiogram of the membrane showed that the 25k-protein identified in a conventional SDS-PAGE separated into two proteins after 2D gel electrophoresis. Immunochemical staining indicated that one of the 25k-proteins bound the anti-GST-P antibody (Fig. 1, a right circle on panels A and B). These results suggest that GST-P is specifically induced in primary cultured rat liver parenchymal cells by coplanar PCB congeners, and that induction of this enzyme is not due to a nonspecific increase in microsomal protein synthesis caused by xenobiotics[5].

Not only PenCB but 10 ng/ml EGF was an effective inducer of GST-P. PenCB appeared to act on the EGF receptor. However, genistein (a tyrosine kinase inhibitor) had no inhibitory effect on expression of GST-P mRNA stimulated by PenCB, although the expression by EGF was reduced, suggesting that the tyrosine kinase activity of the EGF receptor is not directly related to the expression of the GST-P gene by PenCB[6].

Various protein kinases, including PK-C, are known to be activated by tyrosine kinase on the EGF receptor through the cascade system. One of these protein kinases may participate in GST-P expression by PenCB. Liver parenchymal cells were treated with PK-C inhibitors (H-7 and phloretin) in the presence of either PenCB or EGF. These inhibitors reduced expression of GST-P mRNA by both PenCB and EGF to that of the control level. However, the expression of cytochrome P450IA2 mRNA by PenCB was not inhibited by H-7 at the concentration which GST-P expression was suppressed. Since the induction of GST-P was inhibited by PK-C inhibitors, GST-P mRNA appeared to be expressed in the cells by treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA, a PK-C activator). After the cells were incubated with 10 ng/ml TPA instead of PenCB, the level of

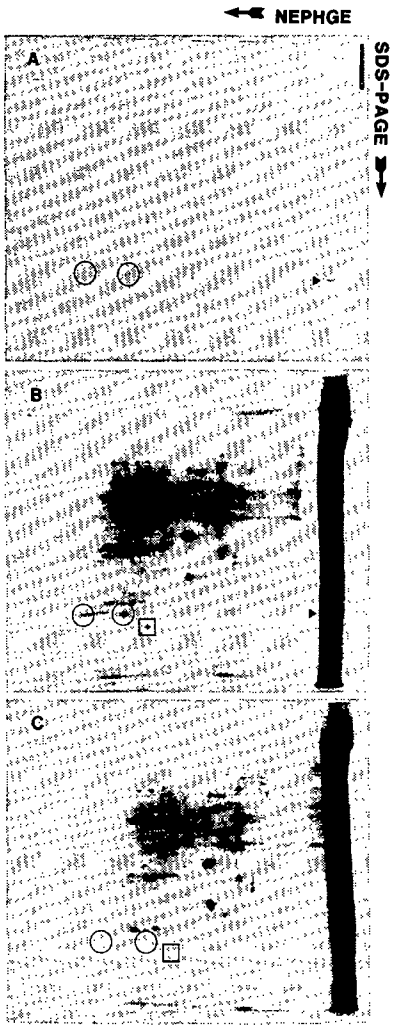

Fig. 1. Immunochemical identification of GST-P by 2D gel electrophoresis of ^{35}-labelled proteins from cells exposed to PenCB. The cells were exposed to 100 nM PenCB for 23 h. Lysates from cells exposed to PenCB and control cells were subjected to 2D gel electrophoresis using NEPHGE for the first dimension. ^{35}S-labelled proteins separated on the gel were transferred to a nitrocellulose membrane. After detection of the labelled proteins on the membrane by autoradiography, GST-P was detected immunochemically using rabbit anti-rat GST-P IgG and a protein-A colloidal gold conjugate. The detection was enhanced by silver staining. Panel A and B: the cell lysate from cells exposed to PenCB, immunochemical staining of GST-P (A), autoradiogram (B). Panel C: autoradiogram of the lysate from control cells. The cell lysates shown in panels A, B and C were subjected to SDS-PAGE shown at the right of each panel. Circles and arrow heads indicate the positions of the 25k-proteins. Squares indicate the proteins, the expression of which were increased in cells exposed to PenCB. NEPHGE and SDS-PAGE indicate the direction of the first and second dimension of electrophoresis, respectively.

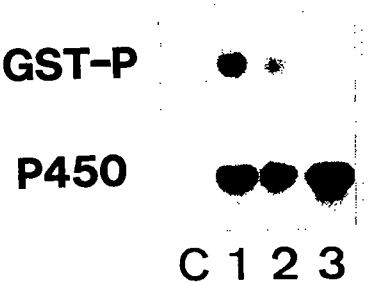

Fig. 2. Suppression of GST-P mRNA by Dex. After the cells were exposed to 100 nM PenCB in the absence (lane 1) or presence of Dex (lane 2, 10 nM; lane 3, 100nM) for 12 h, total RNAs (20 μg) extracted from the cells were subjected to northern blot analysis. Levels of GST-P and cytochrome P450IA2 (P450) mRNA were determined. C is the mRNA level in the control cells.

GST-P mRNA was elevated. These results suggest that PK-C or a related protein kinase plays a role in the induction of GST-P in the cells[6].

The 5'-upstream region of GST-P contains TPA responsive element (TRE) and related enhancer elements[7]. TRE containing genes are activated by binding AP-1 (a transcription factor) to TRE, and the binding ability of AP-1 is regulated by PK-C through the protein kinase cascade system[8]. If AP-1 is required for induction of GST-P by PenCB, this induction could be suppressed by Dex which acts as an antagonist of AP-1 by means of a mechanism by which the Dex-glucocorticoid receptor complex is bound to AP-1, abolishing its capacity to bind to TRE[9-11]. The cells were exposed to PenCB in the presence of 100 nM Dex. As expected, the expression of GST-P mRNA was completely suppressed, dropping to the level of non-exposed cells, while the expression of cytochrome P450IA2 mRNA was not affected by Dex (Fig. 2), suggesting that AP-1 or a related transcription factor is essential for expression of GST-P by coplaner PCB congeners in primary cultured rat liver parenchymal cells, as is a PK-C type enzyme[6].

Coplanar PCB congeners are well-documented inducers of cytochromes P450IA1 and IA2 and several drug metabolizing enzymes. Induction of gene expression of these enzymes is dependent on an intracellular Ah receptor and auxiliary factors (Ah receptor system) and on 5'-flanking regions of these genes which contain an Ah receptor binding site (XRE)[12]. Although an XRE is not found in the 5'-flanking sequence of GST-P, TRE and related responsive element (GPE I and II) are contained in this sequence, and these responsive elements are known to be essential for the expression of GST-P in carcinoma cells[7]. In this study, expression of cytochrome P450IA2 (a gene containing an XRE in the 5'-promotor sequence) by PenCB was not affected by PK-C inhibitirs or by Dex, although expression of GST-P was suppressed by these reagents. We propose that expression of GST-P by coplanar PCB congeners could be controlled by a novel type of regulatory system other than the Ah receptor system.

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4. References

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