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Induction of cytochrome P450 1A1 gene expression, oxidative stress, and genotoxicity by carbaryl and thiabendazole in transfected human HepG2 and lymphoblastoid cells

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Abstract

Carbaryl and thiabendazole, two widely used pesticides, have been shown to induce cytochrome P450 1A1 (CYP1A1) expression, but neither compound is capable of displacing [^{3}H] 2,3,7,8-tetrachlorodibenzo-*P*-dioxin from its aryl hydrocarbon receptor binding site. In the present study, we investigated the transcriptional regulation of *CYP1A1* as well as other genes in various human hepatoma HepG2 cell lines stably transfected with the chloramphenicol acetyl transferase (CAT) reporter gene and cloned under the control of each of 14 promoters or response elements from relevant stress genes. Carbaryl and thiabendazole were found to activate CYP1A1 at the level of transcription, as demonstrated by the dose-dependent increase in reporter CAT and CYP1A1 mRNAs. Moreover, this effect appeared to be mediated via the xenobiotic responsive element (XRE), because both pesticides specifically activated various fusion constructs containing XRE sequences (CYP1A, glutathione *S*-transferase, and XRE). Carbaryl and to a lesser extent thiabendazole also activated other stress genes such as *c-fos* and *NF-κBRE*, *HSP70* and *GRP78*, and *GADD153* at a transcriptional level. These data suggest that these molecules induce early alert genes, including those known to be sensitive to oxidative stress. This led us to examine the genotoxic effect of carbaryl and thiabendazole by an *in vitro* DNA repair solid-phase assay. Both compounds provoked a strong DNA-damaging activity in the human lymphoblastoid cell line that constitutively expresses human CYP1A1 cDNA, but not in the parental line, indicating that CYP1A1 is chiefly implicated in carbaryl and thiabendazole genotoxicity. This effect was confirmed on HepG2 cells. These observations support the notion that intracellular signals leading to CYP1A1 induction, oxidative stress, and genotoxicity are intimately related.

Keywords: Carbaryl; Thiabendazole; CYP1A1; Oxidative stress; Genotoxicity

1. Introduction

In recent years, studies on the potentially adverse effects of pesticides have increasingly focused on chronic health effects including cancer, not only for occupationally exposed groups but also for the general population. Indeed, their toxicity is well documented in rodents [1–3], where several pesticides were shown to be genotoxic [4–7] or tumor promoters [8–11], but not in humans. Therefore, in order to evaluate other potential health hazards, especially for pesticides ingested as residues in food, information concerning their effects on liver is important, as they must pass this key organ (skin and lungs are also major routes of entry for such chemicals) before entering the systemic circulation.

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Abbreviations: AhR, aryl hydrocarbon receptor; CAT, chloramphenicol acetyl transferase; CYP1A1, cytochrome P450 1A1; FBS, fetal bovine serum; GADD, growth arrest and DNA damage; GRP78, 78-kD glucose-regulated protein; GST, glutathione *S*-transferase; HMTII, human metallothionein II; HSP, heat shock protein; 3-MC, 3-methylcholanthrene; NF-κBRE, nuclear factor of κ enhancer responsive element; RARE, retinoid acid responsive element; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; UDS, unscheduled DNA synthesis; and XRE, xenobiotic responsive element.

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In mammalian liver, the CYP system constitutes a superfamily of enzymes that play a critical role in the oxidative metabolism of a wide variety of both endogenous and exogenous compounds. A property of several forms of CYPs is their inducibility by xenobiotics such as polycyclic and polyhalogenated hydrocarbons, drugs [12], and pesticides [13–17]. One of the most studied is the CYP1A1 enzyme, which metabolizes a large number of xenobiotics [12] to cytotoxic and/or mutagenic derivatives. Induction of CYP1A1 is mediated by the AhR through the following cascade of events: *i*) the lipophilic ligand goes through the membrane into the cytoplasm and attaches to the Ah receptor–heat shock protein 90 (hsp90) complex; *ii*) this fixation induces a conformational change, provoking hsp90 dissociation and the liberation of the ligand–receptor complex; *iii*) the latter migrates into the nucleus and heterodimerizes with the Arnt protein (aryl hydrocarbon receptor nuclear translocator); *iv*) finally, this heterodimer behaves as a transcriptional factor which binds to the XRE and induces the transcription of the *CYP1A1* gene [18,19].

Many pesticides have been shown to induce or inhibit CYPs [13–17]. Among them, thiabendazole provoked an increase in ethoxyresorufin-*O*-deethylase activity and CYP1A1 mRNA steady state, although it did not displace [^{3}H]TCDD from its AhR binding site [20,21]. Recently, we made the same observations for another pesticide, carbaryl, which also induced CYP1A1 [22] but was not capable of displacing [^{3}H]TCDD from AhR in competitive binding studies using 9S-enriched fractions of human cytosol. However, the CYP1A1 induction was inhibited by the AhR antagonist α-naphthoflavone [23–25] and by 8-methoxypsoralen, which blocks the binding of the activated AhR to DNA [26], suggesting that induction involves the participation of the AhR and the XRE, but does not seem to be mediated by a direct carbaryl–receptor interaction.

In this study, we investigated the molecular changes induced by carbaryl and thiabendazole by using various HepG2 cell lines stably transfected with the CAT reporter gene, cloned under the control of promoters and response elements of 14 relevant stress genes. We also assessed their genotoxic effect by an *in vitro* DNA repair assay, using either HepG2 cells or parental and stably CYP1A1-transfected human lymphoblastoid cell lines.

We also demonstrated that carbaryl and thiabendazole were able to activate CYP1A1 at the level of transcription as demonstrated by the dose-dependent increase in CYP1A1 mRNAs in HepG2.241c.1-transfected cells. This effect appeared to be mediated via the AhR pathway, because both molecules specifically activated the CAT reported gene under the control of XRE sequences. Carbaryl and to a lesser extent thiabendazole induced other alert genes which were activated by oxidative stress and agents, provoking protein perturbations and DNA damage, which suggested that these pesticides could generate oxidative signals. These results led us to assess their genotoxic effect by an *in vitro* DNA repair solid-phase assay. Although no intrinsic effect was observed, a strong DNA-damaging activity was demonstrated in a human lymphoblastoid cell line that constitutively expresses human CYP1A1 cDNA, indicating that CYP1A1 is chiefly implicated in carbaryl and thiabendazole genotoxicity. This effect was confirmed on HepG2 cells, but at a much lower dose, suggesting variable tissue-specific sensitivities.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), minimum Eagle's medium (MEM), penicillin–streptomycin, L-glutamine, Eagle's non-essential amino acids, sodium pyruvate, and FBS were from Boehringer Ingelheim Bioproducts. RPMI-1640 medium, horse serum, L-histidinol, DMSO, and 3-MC were from Sigma. Geneticin (G418) was from GIBCO. Carbaryl and thiabendazole were from Cluzeau Info. Lab. TCDF was from Chemsyn Science Laboratories.

2.2. Cell cultures and treatments

2.2.1. HepG2

HepG2 cells were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL), sodium pyruvate (1 mM), non-essential amino acids (0.1 mM), and L-glutamine (2 mM). The cultures were incubated at 37° in a humidified atmosphere containing 5% CO_2. Cell cultures were subcultured every 5 days at a 1:2 ratio.

2.2.2. HepG2.241c.1

This human hepatoma HepG2 cell line was stably co-transfected with plasmid DNAs pRNH241C (a construct containing sequences from −1140 to +59 of the human *CYP1A1* gene directing the transcription of the bacterial CAT reporter gene) and pRSVneo (an expression vector for neomycin resistance gene). Cells were dispersed in Eagle's MEM + 10% FBS, 50 μg/mL of streptomycin, 50 U/mL of penicillin, and 0.4 mg/mL of G418. Approximately 10^6 cells in 10 mL of media were plated on poly-L-lysine-treated 100-mm Corning culture dishes. Two days after plating, medium was changed to eliminate G418 prior to conducting experiments.

2.2.3. Xenometrix cell lines

For details of the constructs, plasmids and cell lines, see Ref. 27. The HepG2 cell line was stably co-transfected with plasmid DNAs pSVneo (an expression vector for neomycin resistance gene) and 14 plasmids containing CAT reporter gene under the control of different promoters or response elements. The different plasmids were: 1) p1646CAT (cytochrome P4501A1 or CYP1A1 promoter from −1646 to +57); 2) pGSTYaCAT3 (glutathione *S*-transferase *Ya* subunit or GSTYa promoter from −96 to +49); 3) pXRECAT2

(2 xenobiotic responsive elements or XRE); 4) pHMTIIA (human metallothionein-IIA or MTIIA promoter from −892 to +11); 5) pFOSCAT3 (human c-fos promoter from −404 to +4); 6) pSP-CAT (4 nuclear factor κ enhancers (B site) or NF-κBRE); 7) pXHFCAT3 (human collagenase promoter from −520 to +6); 8) pHP-CAT (2.8-kb human heat shock protein or HSP70 promoter); 9), pCRECAT2 (2 cyclic adenosine monophosphate response elements or CRE) 10) pP53RECAT2 (5 p53 response elements or p53RE); 11) pRARECAT2 (4 retinoic acid responsive elements or RARE); 12) pGADD153Ha-CAT (hamster 153-kD growth arrest and DNA damage promoter or GADD153 promoter from −778 to +21) 13) pGADD45sCAT (human 45-kD growth arrest and DNA damage promoter or GADD45 promoter from −909 to +144) (these two promoters are completely different and do not answer to the same structures); 14) pGRP78PRCAT (rat 78-kb glucose-regulated protein or GRP78 promoter). Cell lines were cultured in MEM containing 10% FBS, 50 μg/mL of streptomycin, 50 U/mL of penicillin, 0.1 mM MEM non-essential amino acids, 2 mM L-glutamine, and 1 mM sodium pyruvate.

2.2.4. h1A1v2 and cHol

h1A1v2 and cHol were obtained from Gentest Corporation. The h1A1v2 cell line was developed to constitutively express transfected human CYP1A1 cDNA. The vector conferred selection by L-histidinol in a medium depleted in L-histidine and supplemented in L-histidinol. The transfected control cell line, cHol, contained the vector without a cDNA insert [28]. Cells were grown in suspension culture in RPMI-1640 medium supplemented to 9% with horse serum, and 2 mM L-histidinol. Cells were routinely passaged at 37° as suspensions containing 2–6. 10^5 cells/mL in 50 mL of medium in 160-mL flasks in an atmosphere of 95% air and 5% CO_2. To help support heme synthesis, 30 μg/mL of 5-aminolevulinic acid was added to the medium. Cells were seeded on 6-well microtiter plates for unscheduled DNA synthesis (UDS)-like assays, and grown to near confluence. Then, 3-MC, TCDF, or pesticides dissolved in DMSO (final concentration 0.5%) were added to the cultures for 24 or 72 hr, with a change of chemical-containing medium every 24 hr.

2.3. Preparation of total RNA

After treatment, cells were washed twice with cold PBS and lysed by addition of TRIzol Reagent (Life Technologies). Genomic DNA in the cell lysate was sheared by passage through a 25-gauge needle. Total RNA was prepared according to the manufacturer's protocol, which is a modification of the method of Chomczynski and Sacchi [29]. Total RNA yield was determined by UV spectroscopy at 260 nm.

2.4. Ribonuclease protection assays

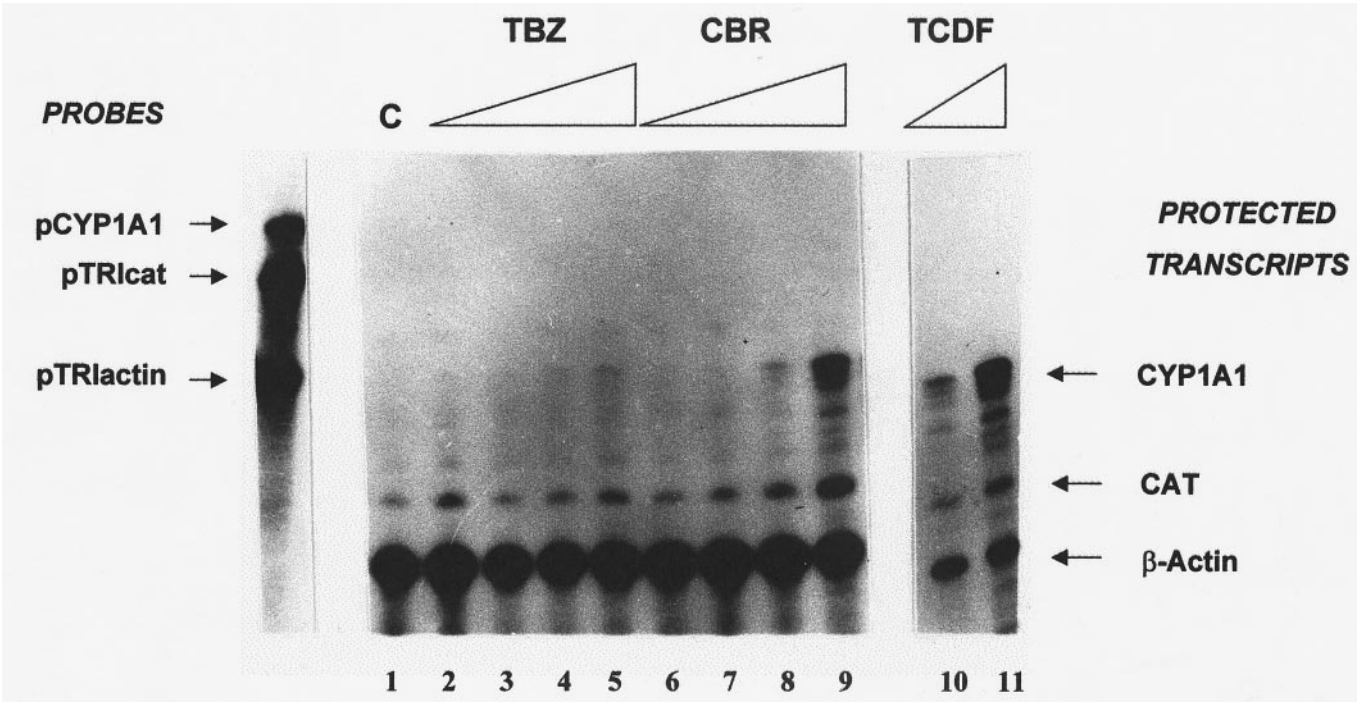
RPAs were conducted using the reagents and basic protocols provided by Ambion Inc. The probes used for detection of the CAT and β-actin mRNAs were supplied by Ambion. The CYP1A1 (pRNH433) probe corresponds to the DNA sequence from the 5′ end of exon 7 (position +5909 to +5830) cloned into pTRIPLEscript vector (Ambion). The *in vitro* transcription SP6 MAXIscript Kit (Ambion) was used to synthesize radioactive transcripts. The SP6 RNA promoter was used to transcribe probes in the presence of [^{32}P]UTP (DuPont NEN). Different amounts of unlabeled UTP were added to adjust the specific activity of the probes. In general, both the CAT and CYP1A1 probes were labeled to the same specific activity, while the β-actin probe was labeled at a 5-fold lower specific activity to ensure saturation of the high levels of actin mRNA. The sizes of the full-length transcripts were 341nt, 271nt, and 200nt for the CYP1A1, CAT, and β-actin probes, respectively. Upon hybridization with total RNA and subsequent digestion with ribonucleases A and T1, protected probe fragments migrated at 222nt, 152nt, and 120nt, which represented the levels of the respective CYP1A1, CAT, and β-actin mRNA present. Free probes were run as markers at a 1:125 dilution to the amount of probe present in the hybridization cocktail. Probes were routinely monitored to ensure that they were in excess of their intended target transcript.

2.5. CAT assay

After the chemical incubation period, the cells were washed twice and lysed with a detergent-based buffer to release total cellular protein. An aliquot was used to measure the amount of protein by the technique of Bradford [30]. The remaining cellular protein was transferred to 96-well plates containing polyclonal anti-CAT antibodies. A standard sandwich ELISA was performed. In the final step, horseradish peroxidase catalyzes a color change reaction that can be measured at O.D. 405 [27].

2.6. Chemiluminescent detection of DNA damage by a DNA repair synthesis assay (3D)

Following treatments, the chemical-containing media were removed from the plate at the end of the different incubation periods. When measuring the extent of DNA damage, genomic DNA was prepared from the treated cells by a lysis buffer [31], the purified genomic DNA was recovered, and DNA lesions were then detected and quantified by the "3D" assay. The repair synthesis assay was performed essentially as described previously [31], with minor modifications as follows. Briefly, aliquots (50 μL) of genomic lysis DNA samples either exposed to agents or untreated were adsorbed on sensitized 96-well-microtiter plates. The standard repair reaction was carried out in 50 μL (final volume/per well) and typically contained protein extract in reaction buffer with 0.4 μM each of dGTP, dCTP, dATP, and biotin-21-dUTP. After repair incubation, the wells were washed and the biotin-21-dUTP incorporated



	1	2	3	4	5	6	7	8	9	10	11
CAT / actin x 10^{-3}	26	28	17	27	35	21	19	26	96	179	231
CYP1A1 / actin x 10^{-3}	6	12	14	18	30	-	6	40	249	1172	3528

Fig. 1. CYP1A1 transcriptional activation by thiabendazole (TBZ) and carbaryl (CBR) in HepG2.241c.1 cells. (Upper panel) Ribonuclease protection assay. HepG2.241c.1 cells were treated with pesticides or TCDF at concentrations of 1, 2.5, 5, or 10 μg/mL indicated by triangles over appropriate lanes. As controls, cultures were treated with solvent vehicules, ie. either ethanol (final concentration of 0.01%) or DMSO (final concentration of 0.02%). The AhR agonist TCDF was used as positive control at 1 and 10 nM concentrations. Approximately 25 μg of total RNAs was hybridized with antisense transcripts to detect messenger RNA levels of CYP1A1, CAT, and β-actin. The TCDF-positive control was exposed to film for one tenth of the time used for TBZ and CBR. Due to the A: T rich nature of the target sequences, CYP1A1 mRNA levels were detected as triplets. (Lower panel) Quantitation of mRNA levels was done by using AMBIS radiolytic imaging.

into DNA was detected by incubation with ExtrAvidin conjugated to peroxidase, after which the chemiluminescent substrate mixture was added. Emitted light was measured with a luminometer (Lumax 2, SFRI) and expressed as a relative light unit (RLU). The DNA repair synthesis activity was expressed as the ratio of RLU in treated versus untreated genomic DNA.

3. Results

3.1. Effect of carbaryl and thiabendazole on CYP1A1 gene expression

It has previously been shown that carbaryl and thiabendazole significantly induced the ethoxyresorufin-*O*-deethylase activity and increased the steady-state concentration of CYP1A1 transcripts in HaCaT and HepG2 cells [22] and in rabbit hepatocytes [20,21]. In order to further confirm these results, a ribonuclease protection assay was used to analyze CYP1A1 induction by carbaryl and thiabendazole in a stably transfected HepG2-derived cell line, HepG2.241c.1. When this cell line was challenged by either TCDF, carbaryl, or thiabendazole, a dose-dependent increase in CAT and CYP1A1 mRNA levels was observed, although to a lesser extent than with the positive control TCDF (Fig. 1). Thiabendazole was always less effective than carbaryl. As expected, no effect was obtained on β-actin mRNAs.

3.2. Induction by carbaryl and thiabendazole of CAT levels in cell lines stably transfected by constructs containing an XRE sequence

First, the effect of both molecules was estimated by using a 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based cellular viability assay on the parental HepG2 cell line. No appreciable cytotoxicity was observed over the range of concentrations tested (3.13–50 μg/mL or 250 μM) (data not shown).

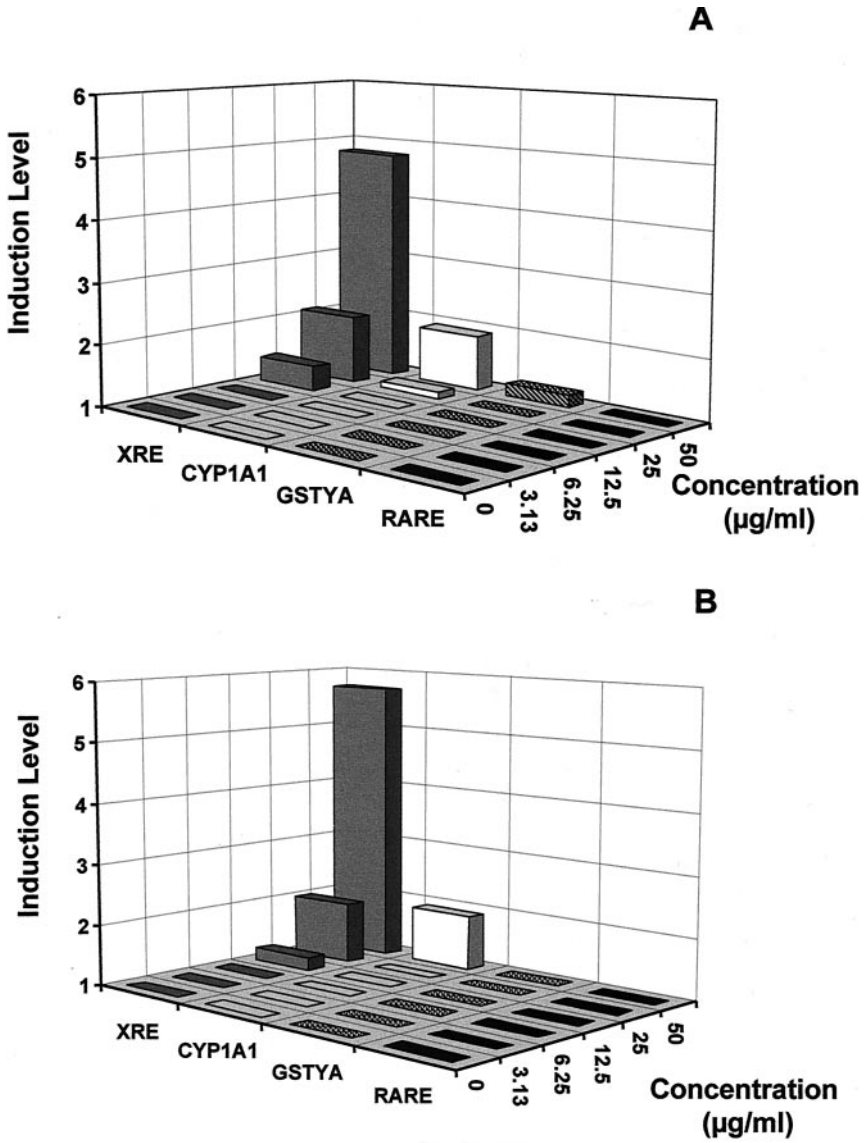


Fig. 2. Dose-dependent induction of CAT levels by carbaryl (A) and thiabendazole (B) in 4 different cell lines stably transfected by the CAT reporter gene cloned under control of *CYP1A1* and *GSTYa* promoters or *XRE* and *RARE* responsive elements. In this assay, activation by compounds had to reach inductions greater than the 2.0-fold level to be considered as statistically significant.

To investigate whether these inductions were mediated via the classical Ah-dependent cascade of events and not through another signaling pathway, we studied the comparative effects of carbaryl and thiabendazole on 4 different transfected HepG2 cell lines. Three of these had stably integrated constructs composed of the CAT reporter gene under the control of different promoters or response elements containing an XRE sequence, i.e. *CYP1A1*-, *GSTYa*-, and synthetic *XRE–CAT* fusion constructs [27]. The fourth cell line used contained a synthetic *RARE–CAT* fusion construct, since it has been demonstrated that retinoic acid was capable of inducing CYP1A1 via the binding of RARs to the *RARE* response element [32,33].

As expected, 3-MC (10 μM), the positive control for the Ah-dependent signaling pathway, significantly and dose-dependently induced all the constructs containing an XRE: e.g. *CYP1A1*, *XRE*, and *GSTYa* (39-, 14-, and 5.6-fold over control, respectively) (data not shown, see [27] for details). Carbaryl and thiabendazole significantly activated the *XRE* construct (4.8- and 5.7-fold, respectively at 50 μg/mL or 248 μM) in a dose-dependent manner (Fig. 2A and B). However, they were much less effective on the *CYP1A1* and *GSTYa* constructs ($\leq$2-fold over control) (Fig. 2A and B). Finally, none of the three tested compounds exerted any effect on the *RARE* construct (Fig. 2A and B).

3.3. Induction by carbaryl and thiabendazole of other stress genes at the transcriptional level

In addition to these four stably transfected cell lines, the two pesticides were also tested in 10 other recombinant human liver cell lines generated by creating stable transfec-

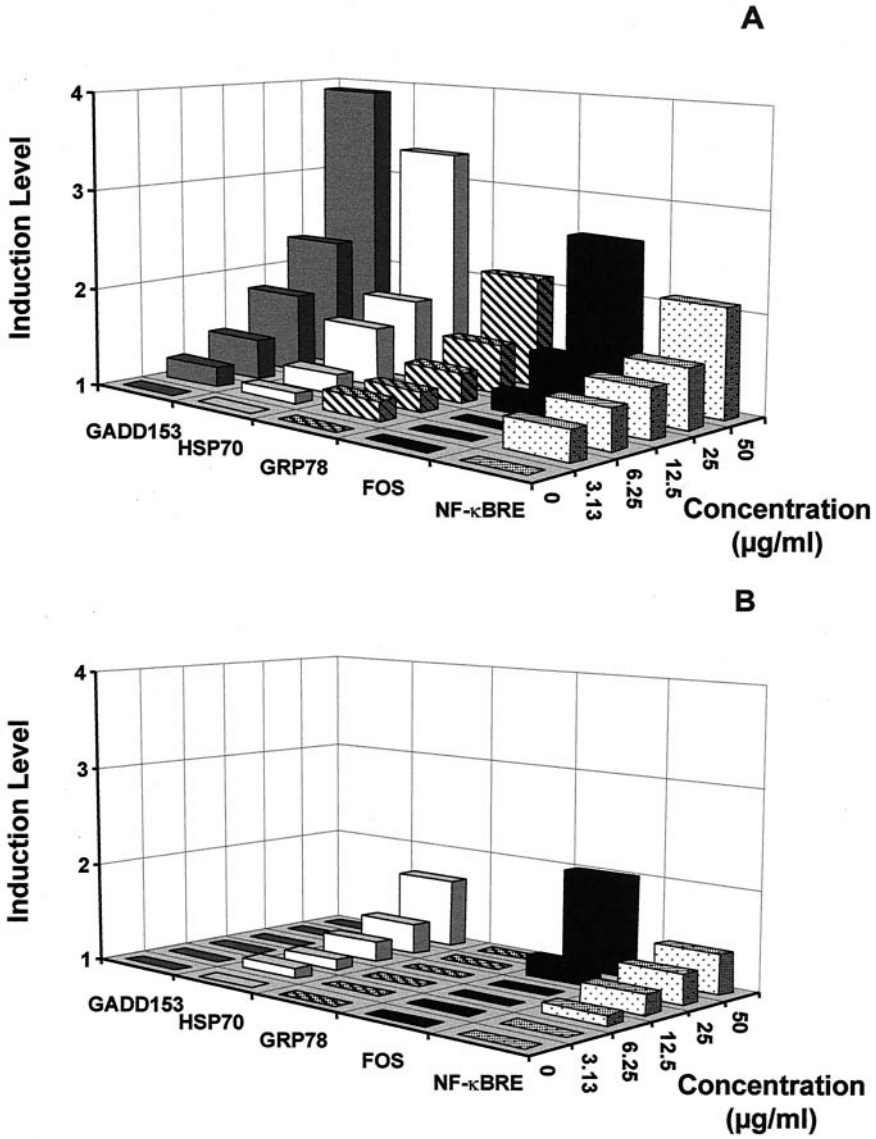


Fig. 3. Dose-dependent induction of CAT levels by carbaryl (A) and thiabendazole (B) in 5 different cell lines stably transfected by the CAT reporter gene cloned under control of *c-fos* and *NF-κBRE*, indicative of an oxidative component, *HSP70* and *GRP78*, representative of protein pertubations, and *GADD153*, indicative of DNA damage. In this assay, activation by compounds had to reach inductions greater than the 2.0-fold level to be considered as statistically significant.

tants that comprise a broad range of promoter– or responsive element–CAT gene fusion constructs responsive to DNA damage, heavy metal ions, protein denaturants, and changes in intracellular cyclic AMP levels (for details of the genes implicated in a stress response, see [27]).

Among them, five constructs showed statistically significant ($P \leq 0.05$) transcriptional gene activation by carbaryl (Fig. 3A). All of these reached inductions greater than the 2.0-fold level and could therefore be considered as having putative biological impact. The combination of both the *c-fos* and the *NF-κBRE* constructs showing inductions in a dose–response manner is indicative of an oxidative component. The dose-dependent induction of *HSP70* (to 3.3-fold at 50 μg/mL) and *GRP78* (to 2.1-fold at 50 μg/mL) represents protein perturbations. *GADD153*, induced to 3.9-fold at 50 μg/mL, indicates a low level of DNA damage. Both DNA damage and protein perturbation are likely consequences of an oxidative stress component exposure.

Thiabendazole also induced three of these constructs (Fig. 3B), namely *c-fos*, *NF-κBRE*, and *HSP70*. Induction was, however, marginal since it never reached the 2-fold threshold. Moreover, thiabendazole did not exert any effect on the *GRP78* and *GADD153* constructs. These results are indicative of a weaker oxidative stress, provoking fewer protein and DNA perturbations than carbaryl, in this stress gene assay. Thiabendazole also induced an additional and weak *HMTIIA* response (data not shown), which was observed previously in the presence of oxidants and which may indicate interactions with thiols in this metalloprotein [27].

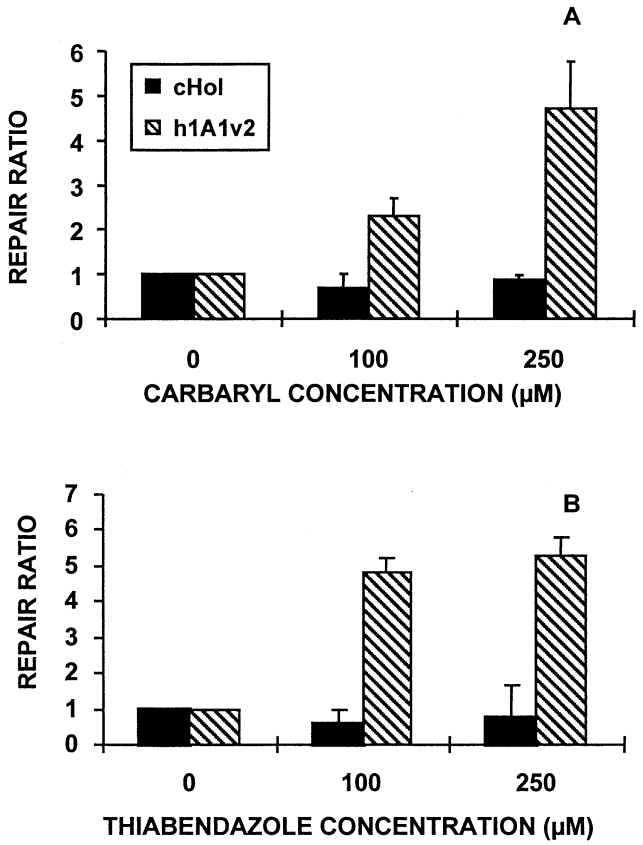


Fig. 4. Effect of carbaryl (A) and thiabendazole (B) on DNA repair in two human lymphoblastoid cell lines, i.e. the parental line cHol and its CYP1A1 stably transfected counterpart, h1A1v2. Cells were treated with DMSO (0.5%), carbaryl or thiabendazole (100 and 250 μM) for 72 hr with a change of chemical-containing medium every 24 hr. DNA damage was monitored by unscheduled DNA synthesis (DNA) 3D assay. The data are expressed as means ± SD from three determinations.

3.4. Genotoxic effect of carbaryl and thiabendazole

Since carbaryl and to a lesser extent thiabendazole provoked CYP1A1 activation but also oxidative stress, protein perturbations, and DNA damage, we studied their capability to provoke genotoxic effects. These studies were conducted using an *in vitro* DNA repair solid-phase assay based on cell DNA absorption on sensitized microplate wells, incorporation of digoxigenilated deoxynucleotides during the repair synthesis step, and detection by chemoluminescence in an ELISA-like reaction.

First, two human lymphoblastoid cell lines were used: the parental line, cHol, which possesses a very low basal CYP1A1 activity (0.7 pmol/mg/min), and its CYP1A1 stably transfected counterpart h1A1v2, in which this enzyme is expressed at a high level (91 pmol/mg/min) [28]. Three successive treatments of 24 hr with carbaryl or thiabendazole did not induce any DNA repair in the parental lymphoblastoid cell line cHol (Fig. 4A and B). By contrast, carbaryl and thiabendazole induced 500% DNA repair in the transfected lymphoblastoid cell line h1A1v2 (Fig. 4A and B). These results suggest that increased CYP1A1 expression (h1A1v2) correlates with a stronger genotoxic effect, which would mean that a reactive metabolite would be produced by CYP1A1.

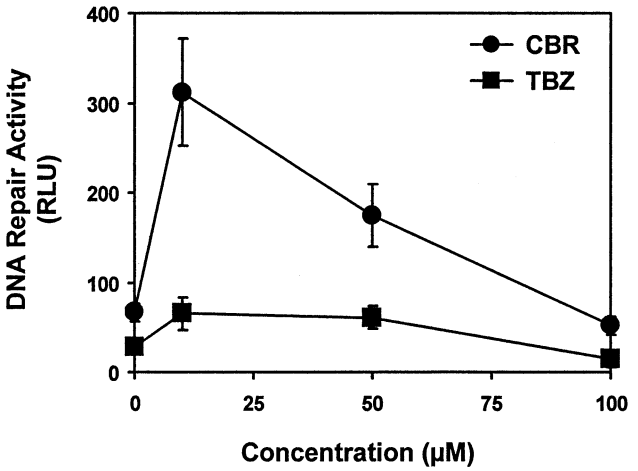


Fig. 5. Effect of carbaryl (CBR) and thiabendazole (TBZ) on DNA repair in HepG2 cells. Cells were treated with DMSO (0.5%), carbaryl or thiabendazole at lower doses (10, 50, and 100 μM) for 72 hr with a change of chemical-containing medium every 24 hr. DNA damage was monitored by unscheduled DNA synthesis (UDS) 3D assay. The data are expressed as means ± SD from three determinations. RLU, relative light unit.

Second, we performed similar experiments on the HepG2 cell line. Fig. 5 demonstrates that carbaryl and thiabendazole also induced DNA repair in these cells. However, the comparison of Figs. 4 and 5 shows two important differences. First, carbaryl appears as a more potent genotoxic compound (4.6-fold increase compared to control) than thiabendazole (2.4-fold). These results are in agreement with those obtained for oxidative stress. The second difference is that the dose responsible for genotoxicity (10 μM) is much lower in HepG2 than the effective dose (250 μM) in the transfected lymphoblastoid cell line h1A1v2. This could suggest a tissue-specific different sensitivity or at least a difference between these two cell lines.

4. Discussion

Recently, it has been demonstrated that two pesticides, namely carbaryl and thiabendazole, induce CYP1A1 without being capable of displacing dioxin from its binding sites to the Ah receptor, although this induction was inhibited by molecules known to antagonize ligand binding to the AhR (α-naphthoflavone) and XRE (8-methoxypsoralen). We therefore investigated the transcriptional regulation of *CYP1A1* as well as other genes induced by these molecules in various HepG2 cell lines stably transfected with the CAT reporter gene, cloned under the control of promoters (*CYP1A1*, *GSTYa*, *HMTIIA*, *Fos*, *XHF*, *HSP70*, *GADD153*, *GADD45*, *GRP78*) and responsive elements (*XRE*, *NF-κBRE*, *CRE*, *p53RE*, *RARE*) of relevant stress genes. These genes are considered to be important elements in stress responses in mammalian cells [27].

Using such stably transfected cell lines, we first confirm that the two pesticides significantly induced CYP1A1 expression at the transcriptional level, as characterized by the steady-state concentration of CYP1A1 mRNAs and the induction of *CYP1A1* promoter-directed CAT gene expression (Figs. 1 and 2). The apparent discrepancy between the concentrations provoking CYP1A1 induction in the *Hep*G2.241c.1 (10 μg/mL or less, Fig. 1) and Xenometrix cell lines (25–50 μg/mL, Fig. 2) could be explained by the fact that two complementary but different techniques in terms of sensitivity were used. Moreover, the construct used in HepG2.241c.1 contains a sequence upstream of CYP1A, from −1140 to +59, while in the Xenometrix cell line, the promoter region goes from −1646 to +57. It is therefore possible that in the −1646 to −1140 region, some sequence(s) have a down-regulatory effect. The insertion site of the two promoter:reporter constructs within the genome of the host cell differs, and the "context sequence" might also modify the overall expression of the construct. Finally, for ribonuclease protection assay (RPA) analysis, cells were treated for 16 hr, while for CAT Elisa detection, treatment lasted only 4 hr, and the concentration ratio of xenobiotics per cell, XRE copy numbers, or protein content might thus be different in the two transfected cell lines.

On the other hand, we also highlight that carbaryl and thiabendazole induce CYP1A1 via the Ah transduction signaling pathway, since we show that both compounds were able to activate the CAT reporter gene under the control of the *XRE* response element. However, if this response was statistically significant for *XRE*, this was not the case for the *CYP1A1* and *GSTYa* constructs, since both compounds exerted only a very weak effect. These results could be explained by the fact that the *XRE* construct used in our assay was a synthetic tandem array of two consensus *XRE* motifs which proved to be more sensitive than either the *CYP1A1* or *GSTYa* constructs, both of which contain physiological XREs. It is therefore not surprising to see XRE induction in the absence of either CYP1A1 or GSTYa elevations.

The ability of the two pesticides to induce CYP1A1 expression through the classical Ah cascade of events without apparently being capable of binding to the AhR in competitive assays is difficult to reconcile. Several hypotheses have been or can be formulated in an attempt to explain these contradictory data. The first notion holds that metabolites of these xenobiotics might be the AhR ligand(s) and thereby the vector of the observed induction. The possibility of carbaryl's being transformed into a metabolite which in turn could bind to the AhR has been considered. However, naphthalene and naphthol, the major metabolites of this pesticide, were not able to displace [^{3}H]TCDD from its binding site [34] to the AhR, but the AhR-binding capability of another metabolite cannot be excluded. Another hypothesis could be that the compounds mentioned above may in fact be very weak ligands of the AhR, but the demonstration of their ability to competitively bind to the receptor has been difficult, due to the extremely high AhR-binding affinity of TCDD. This has been observed for carbaryl by using a modification of the classical AhR ligand binding assay (reduction of the [^{3}H]TCDD concentration and increase in the competitor concentrations) and hepatic cytosols from guinea pigs to allow direct demonstration of competitive AhR binding by lower-affinity ligands [35]. However, these results have to be confirmed with human cytosols because of large interspecies differences in terms of affinity of the ligand for the receptor.

The last hypothesis would be that these compounds that induce CYP1A1 without binding to the AhR may act through another signaling pathway. Indeed, XRE induction is generally thought to indicate interactions between the compound and the Ah receptor. This is, however, not necessarily the case, since the activation of the *XRE* construct merely indicates that an interaction involving the XRE itself has occurred. Our results indicate that like 3-MC, carbaryl and thiabendazole were not able to induce CAT activity through the RARE, suggesting that they do not activate CYP1A1 via this pathway [32,33]. It has recently been demonstrated that omeprazole, a component of the same family (benzimidazole) as thiabendazole, induces CYP1A1 by indirect activation of the AhR complex via intracellular signal transduction systems, a process which is distinct from induction mediated by AhR ligands [36]. It is therefore possible that thiabendazole acts through this new pathway. Carbaryl, which belongs to another chemical family, could also use this signaling cascade, but this remains to be investigated.

Furthermore, we observed that, in addition to genes containing an *XRE* sequence, the two pesticides also activate, at the transcriptional level, genes directed by five other stress promoters or response elements which respond to chemical oxidants (*c-fos* and *NF-κBRE*), protein pertubations (*HSP70* and *GRP78*), and DNA damage (*GADD153*). Therefore, both molecules appear to generate oxidative stress, although the data indicate that this effect is more pronounced with carbaryl than thiabendazole.

Finally, since carbaryl and to a lesser extent thiabendazole seem to elicit oxidative stress, the question of their genotoxicity must be addressed. Indeed, several studies have revealed that carbaryl has genotoxic effects in animals [37], but these data are not extrapolable to human because of interspecies variations in term of bioactivation. The use of two human lymphoblastoid cell lines (either transfected or not with constitutively expressed *CYP1A1* human cDNA) allowed us to demonstrate that carbaryl and thiabendazole (at 100 and 250 μM, respectively) induce a much stronger DNA repair in *CYP1A1*-transfected cells than in parental ones. Carbaryl and to a lesser extent thiabendazole were also able to induce DNA repair in HepG2 cells. These results indicate a relationship between CYP1A1 induction, oxidative stress, and genotoxicity, which suggests that reactive metabolite(s) would be produced by CYP1A1, which in turn may be at the origin of the observed effects. An important question to be addressed is the nature of the metabolite(s) responsible for cellular and molecular pertur-

bations. At this time, we have no exact idea of the metabolite(s) likely causing the damage, but for carbaryl, numerous bioproducts such as epoxy derivatives [37] could be good candidates for such an oxidative action.

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