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Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or indolo(3,2-*b*)carbazole is associated with oxidative DNA damage

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ABSTRACT **Induction of cytochrome P4501A1 (CYP1A1) in the hepatoma Hepa1c1c7 cell line results in an elevation in the excretion rate of 8-oxoguanine (oxo^{8}Gua), a biomarker of oxidative DNA damage and the major repair product of 8-oxo-2′-deoxyguanosine (oxo^{8}dG) residues in DNA. Treatment of this cell line with 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD), a nonmetabolized environmental contaminant, and indolo(3,2-*b*)carbazole (ICZ), a metabolite of a natural pesticide found in cruciferous vegetables, is shown to both induce CYP1A1 activity and elevate the excretion rate of oxo^{8}Gua; 7,8-benzoflavone (7,8-BF or α-naphthoflavone), an inhibitor of CYP1A1 activity and an antagonist of the aryl hydrocarbon (Ah) receptor, reduced the excretion rate of oxo^{8}Gua. The essential role of Ah-receptor, which mediates the induction of CYP1A1, is shown by the inability of TCDD to induce CYP1A1 and to increase excretion of oxo^{8}Gua in Ah receptor-defective c4 mutant cells. While there was a significant 7.0-fold increase over 2 days in the excretion rate of oxo^{8}Gua into the growth medium of TCDD-treated Hepa1c1c7 cells compared to control, no significant increase was detected in the steady-state level of oxo^{8}dG in the DNA presumably due to efficient DNA repair. Thus, the induction of CYP1A1 appears to lead to a leak of oxygen radicals and consequent oxidative DNA damage that could lead to mutation and cancer.**

Cytochrome P450 (CYP) is a family of enzymes involved in the oxidative metabolism of both synthetic and natural compounds (1, 2). Humans are exposed to many CYP inducers (3–6) from eating plant chemicals such as indolo(3,2-*b*)carbazole (ICZ), from broccoli and other cruciferous vegetables (5), and also from environmental contaminants such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). It is possible that the carcinogenic effects of TCDD (7, 8) and phenobarbital (9) may, in part, reside in the capacity of the induced CYP enzymes to leak oxidants and thus promote cell division (10) and oxidative DNA damage.

The mechanisms of CYP1A1 induction by TCDD (11–13) and its involvement in the metabolism of xenobiotics (4, 14, 15) has been investigated extensively. The CYP catalytic cycle involves binding of the substrate followed by binding and activation of molecular oxygen to a reactive intermediate, which hydroxylates the substrate. Most studies designed to investigate the toxicological consequences of CYP induction have been directed toward understanding pathways by which substrate molecules are bioactivated to reactive and genotoxic compounds. The formation of hydrogen peroxide and water as end products of CYP catalysis has been well described (16–21), but the biological consequences of this oxidant leakage from CYP *in vivo* has received relatively little attention. A postulated mechanism of production of activated O_2 is the autoxidation of the oxycytochrome P450 complex, generating superoxide (16, 17). The superoxide anion can, in turn, spontaneously dismutate, generating hydrogen peroxide as a by-product. It is proposed that if transition metals are present to catalyze the one-electron reduction of hydrogen peroxide, hydroxyl radicals will be produced, leading to the indiscriminant damage to cellular biomolecules (22). The data reported here show that activated O_2 produced during the catalytic cycle of CYP1A1 oxidatively damages DNA and thus could be relevant to carcinogenesis (10).

Evidence indicates an association between CYP activity and oxidant formation. CYP2E1, for example, has been shown in microsomal studies to generate oxidants and lipid peroxidation by-products following treatment of rats with either ingestion of ethanol or exposure to a 95% oxygen atmosphere (16, 17, 19, 23). Generation of superoxide, hydrogen peroxide, and hydroxyl radical has been shown *in vitro* using reconstituted membrane vesicle systems with purified CYP1A2, CYP2B4, or CYP2E1 enzymes (18, 20, 21, 24). *In vivo* studies suggest indirectly the possible involvement of CYP in oxidative damage to DNA as an increase in 8-oxo-2′-deoxyguanosine (oxo^{8}dG) production observed upon treatment of rats with agents (i.e., peroxisome proliferators, phenobarbital) that are known to result in, among other effects, an associated elevation in CYP2B or CYP4 activity (25, 26).

Previous studies have shown associations between TCDD-induced aryl hydrocarbon (Ah) receptor-mediated aryl hydrocarbon hydroxylase (AHH) expression, an indicator of CYP1A1 activity, and toxicity such as thymus involution, teratogenesis, DNA single-strand breaks, hepatic porphyria, and chloracne (4). It is plausible that oxidants may play some role in producing the toxic effects observed following TCDD administration as indicated by increases in lipid peroxidation, DNA single-strand breaks, decreases in membrane fluidity, and decreases in hepatic glutathione and NADPH content (reviewed in ref. 27). However, since many of these indices of oxidative damage required amounts of TCDD much higher than that required to induce AHH activity, the oxidant-producing effects of CYP have been largely neglected.

Thus, the contribution of CYP1A1 activity to oxidative damage to DNA and RNA, unlike that of other endogenous oxidant sources such as mitochondria, peroxisomes, and cytosolic oxidases (27), has not been investigated in detail. Since CYP1A1 is one of the major isozymes of CYP, is present in humans, and is inducible by natural and synthetic compounds, the study of its effects on oxidative DNA and RNA damage could aid us in understanding a possible relationship between CYP induction and carcinogenesis.

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Abbreviations: TCDD, 2,3,7,8-tetrachloro-*p*-dibenzodioxin; ICZ, indolo(3,2-*b*)carbazole; 7,8-BF, 7,8-benzoflavone; Ah, aryl hydrocarbon; CYP1A1, cytochrome P4501A1 monooxygenase; EROD, ethoxyresorufin *O*-deethylase; CYP1A2, cytochrome P4501A2 monooxygenase; CYP2E1, cytochrome P4502E1 monooxygenase; oxo^{8}Gua, 8-oxoguanine; oxo^{8}G, 8-oxoguanosine; oxo^{8}dG, 8-oxo-2′-deoxyguanosine; AHH, aryl hydrocarbon hydroxylase.

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We examine the effects of TCDD or ICZ treatment on "oxidative hit rates" to DNA and RNA as well as steady-state levels of oxidative DNA damage in the Hepa1c1c7 and mutant progeny c4 hepatoma cell lines. These cells are distinguished by the presence and absence of Ah receptor responsiveness, respectively, the latter of which is caused by a defect in the translocation of the Ah receptor complex to the nucleus (28, 29). Our study is aimed at measuring oxidative damage to DNA and RNA produced as a consequence of inducing CYP1A1 in two hepatoma cell lines. These results indicate that in addition to the well documented capacity of CYP to bioactivate compounds to genotoxic species, there may be a further tradeoff associated with this enzymatic activity: leakage of oxidants and damage to DNA.

MATERIALS AND METHODS

Chemicals. TCDD and ICZ were a gift from L. F. Bjeldanes (University of California, Berkeley). Resorufin, ethoxyresorufin (ERF), ethanol, dimethyl sulfoxide, and 7,8-benzoflavone (7,8-BF) were purchased from Sigma. Oxo^{8}dG and 8-oxoguanosine (oxo^{8}G) were synthesized as described (30) and oxo^{8}Guanine (oxo^{8}Gua) (2-amino-6,8-dihydroxypurine) was purchased from Aldrich. Enzymes used in the enzymatic hydrolysis of DNA were obtained from Boehringer Mannheim. Radiolabeled [1′,2′-^{3}H]oxo^{8}dG and [U-^{14}C]oxo^{8}Gua were prepared as described (31, 32).

Cell Culture. Mouse hepatoma cell lines were obtained from O. Hankinson (University of California, Los Angeles). Cells were seeded at a density of 1×10^5 cells in 100-mm culture dishes and were grown to 90% confluency in Dulbecco's modified Eagle's medium (DMEM; Irvine Scientific) supplemented with 10% (vol/vol) dialyzed fetal bovine serum (FBS; Sigma) at 37°C with 100% humidity. The growth media were changed to fresh media containing very low background levels of oxo^{8}Gua, oxo^{8}G, and oxo^{8}dG before incubation with inducer or inhibitor. To prepare media with the lowered background levels of oxo^{8}Gua, oxo^{8}G, and oxo^{8}dG, 10% dialyzed FBS was incubated for 5 min a twice with anti-oxo^{8}dG monoclonal antibody immunoaffinity beads (32–34). The serum was centrifuged to remove the beads prior to addition of DMEM.

Induction. Either TCDD (500 pM) or ICZ (5 μM) was used as an inducer of CYP1A1 (5). 7,8-BF (10, 30, or 75 μM, or a saturating concentration of ~150 μM) was used as an inhibitor. Nontoxic levels of TCDD, ICZ, or the inhibitor were added directly to the fresh growth media of the subconfluent cells. The cells were incubated at 37°C for 48 h unless mentioned otherwise. Cells were either harvested in PBS buffer for measurement of ethoxyresorufin *O*-deethylase (EROD) activity and cell counting or were frozen immediately under nitrogen at −80°C until analysis of oxo^{8}dG in cell DNA. The 48-h growth media were collected and frozen at −80°C until analysis of oxo^{8}Gua, oxo^{8}G, and oxo^{8}dG. Compounds were first dissolved in a minimal amount of dimethyl sulfoxide followed by a 1:20 dilution in ethanol to produce a working stock solution; 150 μl of this working stock solution was added to 10 ml of media. The vehicle for delivering the compounds, 150 μl of 1:20 dilution of dimethyl sulfoxide in ethanol, was used as the control. 7,8-BF was dissolved in 50 μl of acetone.

Measurement of EROD activity. EROD activity was measured as described in intact cells (5, 35).

Determination of Oxidized DNA or RNA. Oxo^{8}Gua, a major product of oxidative damage to guanine residues in DNA, has been shown to be a sensitive marker of oxidative DNA damage *in vivo* (32, 36, 37). Oxo^{8}Gua is released from the DNA by a specific glycosylase during DNA repair (38, 39) and is excreted into the medium (32, 34). Oxo^{8}G and oxo^{8}dG are biomarkers of oxidative damage to RNA and DNA, respectively (31, 40–43). The level of oxo^{8}Gua, oxo^{8}dG, and oxo^{8}G produced by cells in culture was determined as described (32) with a few modifications. A single 100-mm dish of cells (~5×10^6 cells at 100% confluency) was sufficient for one determination of oxo^{8}dG, oxo^{8}G, and oxo^{8}Gua. The growth medium from one 100-mm dish was thawed and centrifuged ($1000 \times g$ for 1 min) to remove cell debris prior to monoclonal antibody-based immunoaffinity column isolation of oxo^{8}Gua, oxo^{8}dG, and oxo^{8}G from the growth media. The purified oxo^{8}Gua, oxo^{8}dG, and oxo^{8}G were separated further by HPLC and measured by electrochemical (HPLC-EC) detection (32, 44). To determine the steady-state level of oxo^{8}dG in cells, DNA was extracted, digested with nuclease P1 and alkaline phosphatase, and analyzed by HPLC-EC detection (32, 44).

Addition of Guanine to Cell Culture. To assess the possibility that some oxo^{8}Gua arises from oxidation of guanine, 350 pmol of guanine was spiked along with TCDD into culture dishes containing 1×10^7 cells. After a 48-h incubation, the growth medium was collected, 3.5 pmol (3400 cpm) per 10^7 cells of [^{14}C]oxo^{8}Gua was spiked as an internal standard, and the level of oxo^{8}Gua was determined as described above.

Microsomal Study. Before isolation of microsomes Hepa1c1c7 cells were incubated for 2 days in culture with either 500 pM TCDD in 1.5% ethanol or 1.5% ethanol alone. CYP1A1 activity was estimated by measuring EROD activity. The assay consisted of 50 μg of microsomes, 5 μM ERF, and 300 μM β-NADPH in phosphate-buffered saline (PBS) buffer (total 1.0 ml, 37°C, pH 7.5). To measure the production of oxo^{8}Gua from guanine as a substrate, 1 mg of microsomes per ml, 100 μM guanine, and 2 mM β-NADPH in PBS buffer (pH 7.5) were incubated at 37°C. At 5 min and at 1, 2, and 5 h, 1-ml aliquots of the reaction mixture were removed and the level of oxo^{8}Gua was measured by HPLC-EC following immunoaffinity purification as described above.

Statistics. Student's two-tailed *t* test or Mann–Whitney's nonparametric test was performed using a Macintosh INSTAT statistical analysis program (Instat, San Diego, CA).

RESULTS

TCDD is one of many chemicals that upon binding to the Ah receptor induces CYP1A1 expression (11–13). Incubation of Hepa1c1c7 cells with 500 pM TCDD for 48 h, a dose that has been shown to be noncytotoxic as measured by cell count (Coulter counter), was performed in order to test a possible association between CYP1A1 induction and oxidative DNA damage. This treatment resulted in a 7-fold increase in the level of oxo^{8}Gua in the growth medium of Hepa1c1c7 cells (Fig. 1*B*), associated with a 30-fold increase in EROD (CYP1A1) activity (Fig. 1*A*). In contrast, no increase in oxo^{8}Gua accumulation in the growth medium nor increase in EROD activity was noted in the mutant cell line that had been similarly pretreated with 500 pM TCDD, indicating the importance of Ah receptor responsiveness in mediating formation of this oxidized nucleobase. 7,8-BF, an inhibitor of CYP, inhibited EROD activity and lowered oxidative DNA damage, lending further support to the notion that CYP induction is associated with oxidative DNA damage (Fig. 1*B*). In contrast to the marked increases in oxo^{8}Gua noted for conditions in which CYP1A1 activity was induced, little change in the levels of oxo^{8}G, a measure of RNA turnover after oxidation, was observed, possibly due to slow RNA turnover (Fig. 1*C*).

To assess the steady-state level of damage to cellular DNA, a similar experiment was performed, in which oxo^{8}dG in DNA was measured in addition to analyzing oxo^{8}Gua and its nucleoside derivatives in growth medium (Fig. 2). Fig. 2 demonstrates, in addition to supporting the results presented in Fig. 1, that the steady-state level of oxo^{8}dG in DNA after a 48-h treatment with TCDD, does not change significantly, possibly due to efficient DNA repair.

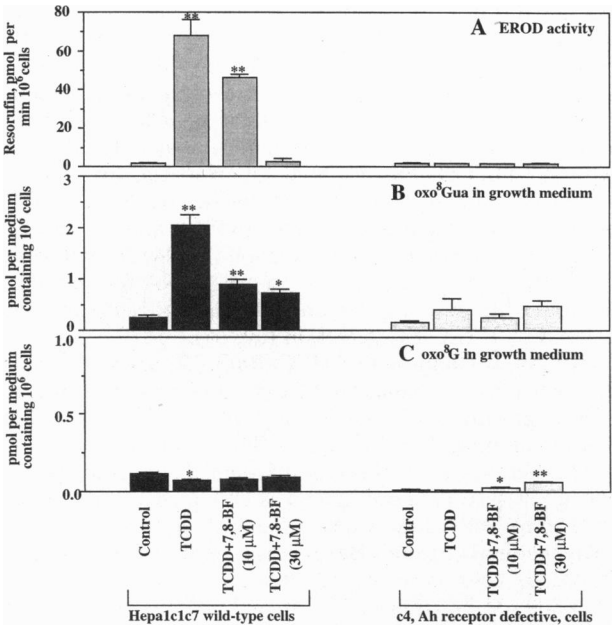

FIG. 1. (*A*) Induction of EROD (CYP1A1) activity (mean of duplicates) by TCDD and its inhibition by two doses of 7,8-BF in Hepa1c1c7 cells. (*B* and *C*) Amount of oxo^{8}Gua and oxo^{8}G, respectively, excreted into growth medium (mean ± SEM; $n = 4$). *, $P < 0.05$; **, $P < 0.001$.

To measure the kinetics of release of oxo^{8}Gua into growth medium, Hepa1c1c7 cells treated with either TCDD or TCDD plus 7,8-BF were collected at 24, 48, or 72 h following addition of these chemicals (Fig. 3). The rate of release of oxo^{8}Gua increased from 0.96 to 1.61 pmol per 10^6 cells per day within 3 days of TCDD treatment. There was no significant change in the level of oxo^{8}G or oxo^{8}dG (data not shown) in the growth medium over this 3-day period.

ICZ, another inducer of CYP1A1 and a natural plant chemical formed in the stomach after ingesting broccoli, cabbage, or other cruciferous vegetables (5), was also examined for its potential to produce oxidative DNA damage. ICZ is not toxic to the cells and is known to bind strongly to the Ah receptor with an affinity close to that of TCDD (5). As with TCDD, binding of ICZ to the Ah receptor results in induction of CYP1A1 expression and activity (5, 45). Fig. 4 shows that 5 μM ICZ is capable of inducing marked increases in both EROD activity and oxo^{8}Gua levels in the growth medium, indicating that production of oxidants following CYP1A1 induction is not unique to TCDD, the effects of which are shown for comparison.

In addition to TCDD and ICZ, several prototype inducers of CYP1A1, such as 3-methylcholanthrene, β-naphthoflavone, or benzo[*a*]pyrene, were also tested but were found to cause significant cytotoxicity to these cells at the doses required to induce CYP1A1. The control contained 1.5% ethanol as a vehicle, which appears to increase oxidative damage slightly (see also Fig. 4) but through a pathway apparently independent of CYP1A1 induction.

To investigate whether the oxo^{8}Gua in the growth medium can be formed in part from oxidation of the cytosolic pool of guanine, 35 pmol per 10^6 cells of guanine base was added to the Hepa1c1c7 cells simultaneously with TCDD. The level of oxo^{8}Gua did not change in the cell cultures incubated with exogenous guanine (0.44 ± 0.19 pmol per 10^6 cells) compared to that of cells treated with TCDD alone (0.52 ± 0.07 pmol per 10^6 cells).

To test whether cytosolic pools of guanine are oxidized by CYP1A1 resulting in the increased level of oxo^{8}Gua observed, microsomes were prepared from TCDD treated or untreated Hepa1c1c7 cells and incubated with guanine. CYP1A1 activity from the isolated microsomes following TCDD treatment produced 30 pmol of resorufin per min per mg of microsomal protein. No detectable CYP1A1 activity was observed with the microsomes isolated from untreated Hepa1c1c7 cells. At each time point from 5 min to 5 h after guanine addition, the level of oxo^{8}Gua detected with microsomes isolated from TCDD-treated cells was approximately the same (and in some instances lower) as that observed with microsomes from untreated cells. This study suggests that none of the oxo^{8}Gua detected in the growth medium of Hepa1c1c7 cells treated with TCDD arises from CYP-catalyzed oxidation of Gua to oxo^{8}Gua.

DISCUSSION

To quantify the leakage of oxidants from CYP we induced CYP1A1 and measured oxidative DNA damage in mouse hepatoma cell lines. 7,8-BF, an inhibitor of this enzyme, was also employed in this study. The hepatoma cell line is also known to have low levels of catalase activity (46), which is likely to make this cell line more susceptible to oxidative damage by hydrogen peroxide as an end product of CYP catalysis when compared to other nontransformed cell lines or cells *in vivo*. TCDD, an environmental contaminant, or ICZ, a natural plant chemical found as a metabolite of cruciferous vegetables, were both shown to increase CYP1A1 activity, as judged by elevations in EROD activity and increase in oxidative damage to DNA as measured by oxo^{8}Gua excretion into growth medium.

The dependence of oxidative damage on the Ah receptor is shown by the lack of oxo^{8}Gua production in a mutant cell line, c4, which lacks a functional Ah receptor. This finding is consistent with the observation that 7,8-BF, an inhibitor of CYP1A1 activity and an antagonist of the Ah receptor, significantly inhibited production of oxidants in a wild-type cell line. Though leakage of oxidants from CYP1A1 is the most plausible explanation of our results, we cannot rule out the possibility that some other gene induced through the Ah receptor causes the oxidative damage.

Increased excretion into the growth medium of oxo^{8}Gua, a major nucleobase lesion implicated in oxidative mutagenesis (47) that is observed whenever CYP1A1 is induced, suggests that oxidants formed as a consequence of this induction can result in oxidative damage to DNA. Excretion of the deoxynucleosides oxo^{8}dG and oxo^{8}G did not increase upon treatment of the Hepa1c1c7 cells with noncytotoxic amounts of TCDD or ICZ. However, unlike oxo^{8}Gua, the major repair product of oxo^{8}dG lesions in DNA (48, 49), oxo^{8}G requires RNA turnover and the oxo^{8}dG may require DNA turnover, thus delaying excretion. In addition to measuring the oxidized residues excreted into the medium, we also investigated the effect of CYP1A1 induction on steady-state levels of oxo^{8}dG. This was not changed significantly, suggesting that oxo^{8}dG repair is adequate under the conditions of our experiment. The results of the 3-day kinetic study shows that the rate of oxo^{8}Gua excretion was increased with incubation time. In contrast to the marked incubation time-dependent increase in oxo^{8}Gua, the steady-state level of oxo^{8}dG did not change significantly at all time points, suggesting that this oxidative lesion is efficiently repaired following induction of CYP1A1 by TCDD, which occurred within 24 h.

A plausible source of oxidants is the oxyform of CYP (17, 19), formed from compounds that can induce CYP but that are not themselves readily oxidizable. This situation may create a condition in which activated oxygen can escape the active site of this enzyme and damage other cellular constituents. Because both ICZ and TCDD are poorly metabolized, they may, in effect, uncouple CYP1A1 by allowing the activated oxygen to leak from the catalytic site.

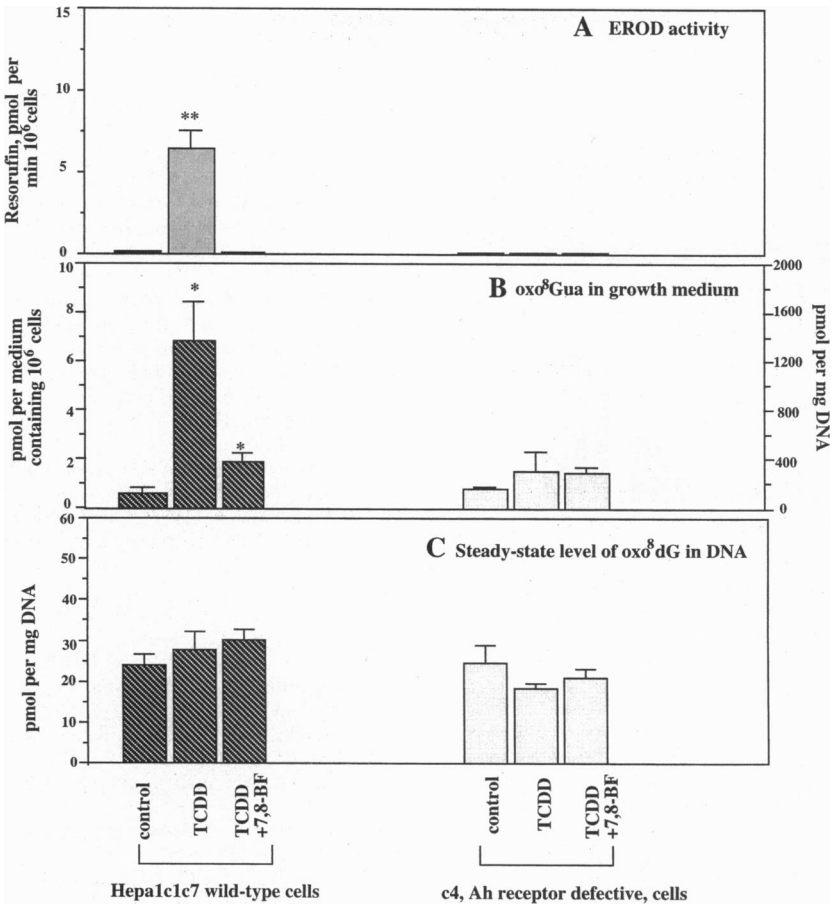

FIG. 2. Growth conditions and treatment of cells were the same as in Fig. 1, except that cells were incubated with a saturating concentration of 7,8-BF (≈150 μM). The 48-h growth media were collected and frozen at −20°C until processed for analysis of oxo^{8}Gua. Cells were harvested, washed, and frozen at −80°C under nitrogen until processed for measurement of oxo^{8}dG. Data in *A* represent mean of duplicates and those in *B* and *C* represent mean ± SEM ($n = 4$). *, $P < 0.05$; **, $P < 0.001$.

Increases in the excretion rate of oxo^{8}Gua following treatment of the Hepa1c1c7 cells with TCDD is consistent with the idea that DNA is being subjected to a high oxidative "hit rate" that could increase mutation. In this context, it is important to recognize the potential influence of this oxidative hit rate on the carcinogenic effects of TCDD and other inducers of CYP. As oxidants are known to contribute to cell proliferation, it is possible that the oxidants formed by CYP induction may be involved in the proliferative, and therefore mutagenic (50), effects of these compounds on the liver.

Consistent with the idea that CYP-derived uncoupled oxidants could play a role in cell proliferation is the finding that the hepatomegaly observed in rats treated with phenobarbital, a classic CYP inducer, was associated with increased hepatocyte proliferation, and it was reduced when a substrate (51) for CYP was coadministered. The observation that liver weight is reduced when CYP is coupled to oxidation of a substrate suggests that leakage of oxidants may play a role in the hepatomegaly observed with many inducers of this enzyme system (52, 53).

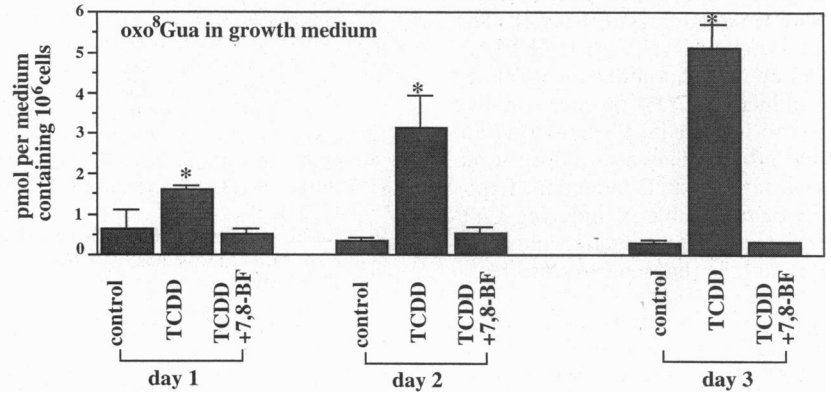

FIG. 3. Cumulative levels of oxo^{8}Gua measured in growth media of Hepa1c1c7 cells after incubation with TCDD or TCDD and 7,8-BF. Growth condition and treatment of cells were the same as in Fig. 1, except that 75 μM 7,8-BF was added with 500 pM TCDD to cells. The growth media or cells were collected at day 1, 2, or 3 after incubation and were frozen at −20°C until processed for analysis. Means ± SEM ($n = 4$) are shown. *, $P < 0.05$.

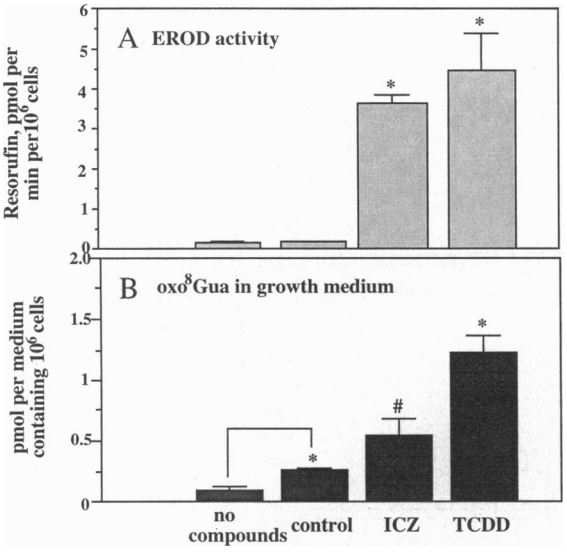

FIG. 4. (*A*) Effect of ethanol alone (1.5%), ICZ, or TCDD on EROD activity. (*B*) Level of oxo^{8}Gua in growth media of Hepa1c1c7 cell lines after CYP1A1 induction by ICZ and TCDD. Cell culture conditions before or after treatment of compounds were the same as in Fig. 1. Ethanol, ICZ (5 μM), or TCDD (500 pM) was added directly to the medium of wild-type cell lines and incubated for 48 h. Cells were harvested in PBS buffer and EROD activity and cell counts were performed immediately (within 1 h of isolation). The 48-h growth medium was collected and frozen at −20°C until the samples were processed for oxo^{8}Gua analysis. Data in *A* are means of duplicates and in *B* are means ± SEM (n = 4). Significance of ICZ and TCDD are relative to control and control (1.5% ethanol plus 0.07% dimethyl sulfoxide) is relative to no compounds. #, $P < 0.06$; *, $P < 0.05$.

The formation of oxo^{8}Gua and its accumulation in the growth medium could also be due, in part, to mechanisms independent of oxidative DNA damage. We investigated the possibility of guanine serving as a substrate for CYP1A1 by employing microsomes isolated from TCDD-treated Hepa1c1c7 cells. Results of our studies indicate that oxo^{8}Gua is unlikely to arise from CYP1A1-catalyzed oxidation of cytosolic guanine. Incubation of guanine solutions that contain residue oxo^{8}Gua show a net consumption of oxo^{8}Gua when microsomes isolated from TCDD-treated Hepa1c1c7 cells are used. This suggests that oxo^{8}Gua, the proposed major repair product excised by a specific glycosylase activity, may be further oxidized by oxidants generated from CYP1A1, possibly leading to an underestimate of oxidative hits.

Our studies emphasize the importance of CYP1A1 induction on nucleic acid oxidation. DNA oxidation may be stimulated upon induction of other CYP450 isozymes as well. The effect of induction of other isozymes of CYP, such as CYP1A2, phenobarbital-inducible CYP2B, ethanol-inducible CYP2E, or peroxisome proliferator-inducible CYP4 on the oxidative damage would be interesting to investigate further in detail. Our experiments suggest that ethanol may also induce some oxidative damage, perhaps through CYP2E induction. Exposure of individuals to compounds capable of inducing CYP isozymes which lead to increased oxidant production and DNA damage may contribute to mutations that lead to cancer.

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