Genoprotection by UDP-Glucuronosyltransferases in Peroxidase-dependent, Reactive Oxygen Species-mediated Micronucleus Initiation by the Carcinogens 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanolone and Benzo[a]pyrene

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ABSTRACT

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation and elimination of putative tobacco carcinogens such as benzo[a]pyrene (B[a]P) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanolone (NNK), which may reduce competing bioactivation and toxicity. B[a]P-initiated cytotoxicity and micronucleus formation, believed to reflect carcinogenic initiation, are enhanced in UGT-deficient rat fibroblasts, and UGTs may provide some genoprotection against NNK. Using skin fibroblasts from wild-type UGT-normal (+/+), congenic heterozygous (+/j) and homozygous (j/j) UGT-deficient rats, this study evaluated NNK in relation to B[a]P with respect to the mechanism of genotoxicity, evidenced by micronucleus formation, and genoprotection by UGTs. Molecular mechanisms were determined by changes in B[a]P- and NNK-initiated micronucleus formation when cells were incubated with the antioxidative enzyme superoxide dismutase (1680 U/ml), inhibitors of cytochrome P450 (1 mM 1-aminobenzotriazole and benzofajpyrene1; 20, 3,7,8-tetrachlorobenzofajpyrene-2,3,7,8-tetrachlorodibenzo-p-dioxin; 0.625 ng/ml (0.0367 mM) interleukin 1α; 12-O-tetradecanoylphorbol-13-acetate). In +/+ fibroblasts, NNK and B[a]P initiated concentration-dependent, respective maximum 2.7-fold and 1.7-fold increases over DMSO controls in micronucleus formation (P < 0.05), with 10 μM NNK being 2.4-fold more genotoxic than B[a]P (P < 0.05). In both +/+ and j/j UGT-deficient cells, micronuclei initiated by NNK and B[a]P each were over 2-fold higher than that in +/+ UGT normal cells (P < 0.05). Both NNK- and B[a]P-initiated micronuclei were decreased by superoxide dismutase and cytochrome P450 inhibitors, while only that initiated by B[a]P was enhanced, up to 2.4-fold, by inducers, of which only interleukin 1α was effective in all UGT phenotypes (P < 0.05). These results provide the first evidence that: (a) UGTs may be genoprotective for NNK, with even heterozygous UGT deficiencies being toxicologically critical; and (b) peroxidase-catalyzed bioactivation, reactive oxygen species, and molecular target oxidation may contribute differentially to the genotoxicity of both NNK and B[a]P.

INTRODUCTION

The elimination of endogenous chemicals and xenobiotics, including the tobacco-specific carcinogen B[a]P (4), is catalyzed by the superfamily of membrane-bound isoforms known collectively as UGTs (5–7). UGTs catalyze the conjugation of xenobiotics with the hydrophilic molecule UDP-glucuronic acid, allowing for excretion in urine or feces (Fig. 1). Recent evidence indicates that human deficiencies in UGTs may lead to increased susceptibility to xenobiotic bioactivation and toxicity (8, 9), including carcinogenesis (10) and teratogenesis (11).

UGTs exist as two families (UGT1 and 2) located on separate chromosomes (12, 13) that are regulated by distinctly different mechanisms. The different UGT1 isoforms are produced from a single gene complex consisting of multiple isoform-specific exons located at the 5′ variable/specific region, which are alternatively spliced with exons at the 3′ constant region (14, 15). In contrast, UGT2 isoforms are produced from the transcription of separate and complete genes. Approximately 2–5% of the human population have hereditary deficiencies in at least the bilirubin UGT isozyme (UGT1*1; Gilbert’s and Crigler-Najjar types I and II syndromes; Ref. 16), which results from various mutations (15, 17–20). These mutations lead to the loss of bilirubin UGT protein or activity (21), which is phenotypically expressed as jaundice due to abnormally elevated bilirubin blood concentrations (6, 15, 19, 20).

UGT-deficient Gunn and RHA rats have decreased acetaminophen glucuronidation, resulting in enhanced bioactivation, hepatotoxicity, and nephrotoxicity (8, 22, 23), and humans with Gilbert’s syndrome have decreased acetaminophen glucuronidation and increased acetaminophen bioactivation (24). Similarly, in vitro and in vivo studies using UGT-deficient Gunn and RHA rats showed decreased glucuronidation of B[a]P metabolites, with enhanced B[a]P bioactivation and covalent binding to protein and DNA (25), and enhanced embroytotoxicity (11). Additional studies showed that lymphocytes obtained from UGT-deficient rats accurately reflected the decreased glucuronidation and enhanced bioactivation and covalent binding of B[a]P in hepatic microsomes from the same animals (26). Lymphocytes from normal human volunteers demonstrated a 200-fold variation in UGT activity for B[a]P metabolites, and decreased glucuronidation correlated with enhanced cytotoxicity, suggesting that human UGT deficiencies may be an important determinant of toxicological susceptibility to B[a]P and related xenobiotics (9). A recent in vitro study demonstrated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was a potent inducer of CYP1A1/2ÜO nM 2,3,7,8-tetrachlorodibenzo-p-dioxin; 0.625 ng/ml (0.0367 mM) interleukin 1α; 12-O-tetradecanoylphorbol-13-acetate; 1680 U/ml cytochrome P450; 1 mM 1-aminobenzotriazole, inhibitors of cytochrome P450 (1 mM 1-aminobenzotriazole and benzofajpyrene1; 20, 3,7,8-tetrachlorobenzofajpyrene-2,3,7,8-tetrachlorodibenzo-p-dioxin; 0.625 ng/ml (0.0367 mM) interleukin 1α; 12-O-tetradecanoylphorbol-13-acetate). In +/+ fibroblasts, NNK and B[a]P initiated concentration-dependent, respective maximum 2.7-fold and 1.7-fold increases over DMSO controls in micronucleus formation (P < 0.05), with 10 μM NNK being 2.4-fold more genotoxic than B[a]P (P < 0.05). In both +/+ and j/j UGT-deficient cells, micronuclei initiated by NNK and B[a]P each were over 2-fold higher than that in +/+ UGT normal cells (P < 0.05). Both NNK- and B[a]P-initiated micronuclei were decreased by superoxide dismutase and cytochrome P450 inhibitors, while only that initiated by B[a]P was enhanced, up to 2.4-fold, by inducers, of which only interleukin 1α was effective in all UGT phenotypes (P < 0.05). These results provide the first evidence that: (a) UGTs may be genoprotective for NNK, with even heterozygous UGT deficiencies being toxicologically critical; and (b) peroxidase-catalyzed bioactivation, reactive oxygen species, and molecular target oxidation may contribute differentially to the genotoxicity of both NNK and B[a]P.

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3 The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanolone; +/+, homozygous UGT normal Gunn rats; +/-, heterozygous UGT-deficient Gunn rats; j/j, homozygous UGT-deficient Gunn rats; ABT, 1-aminobenzotriazole; B[a]P, benzo[a]pyrene; CYP1A1, cytochrome P450A1; DAPI, 4′,6-diamidino-2-phenylindole; ETYA, 5,8,11,14-eicosatetraynoic acid; IL-1α, interleukin 1α; LPO, lipoxgenase 5; P450, cytochrome P450; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; PHS, prostaglandin H synthase; SOD, superoxide dismutase; TPA, 12-O-tetradecanoylphorbol-13-acetate; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; UGT, UDP-glucuronosyltransferase; ROS, reactive oxygen species; pen/strep, penicillin/streptomycin.


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Fig. 1. Metabolism of NNK to its major glucuronide metabolites. NNK is nonenzymatically converted to NNAL and also is α-hydroxylated by various P450s to α-hydroxymethyl NNK, the precursor of the putative reactive intermediate thought to pyridyloxobutylate DNA. NNK also is hydroxylated by P450s at the methylene carbon, forming a reactive intermediate that can methylate DNA. Finally, NNK initiates DNA oxidation, possibly via the formation of ROS, but the bioactivating enzymes have not been characterized. OGluc, glucuronide conjugate; HPB, 4-hydroxy-N-(3-pyridyl)-1-butanone.

methylations (36, 37), arylation (38, 39), and strand breaks (Refs. 40 and 41; Fig. 1). UGTs not only help eliminate NNK through the glucuronidation of the carbonyl-reduced metabolite NNAL, but also directly eliminate or detoxify the α-methyl-hydroxylated NNK metabolite that is a proximate DNA-arylating agent (Ref. 42; Fig. 1). In this study, we investigated NNK-initiated micronuclear formation in skin fibroblasts cultured from UGT normal (+/+) and heterozygous (+/j) and homozygous (j/j) UGT-deficient Gunn rats to determine the potential genoprotective role of UGTs in NNK carcino genesis. We further evaluated the role of bioactivation and ROS in NNK-initiated micronuclear formation, in comparison to B[a]P, using inducers and inhibitors of potential bioactivating enzymes (CYP1A1 and peroxidases) and the antioxidative enzyme SOD.

These results constitute the first evidence that UGTs may be critical in protecting against NNK genotoxicity, with even heterozygous UGT deficiencies being important. The mechanism of NNK genotoxicity, like that for B(a)P, appears to involve peroxidase- as well as P450-dependent bioactivation, although the relative contributions may differ, with ROS ultimately contributing to micronuclear formation.

MATERIALS AND METHODS

Animals. Male HsdBlu/Gunn rats, 180–200 g (Harlan Sprague-Dawley Inc., Indianapolis, IN) were housed in separate plastic cages. All animals were kept in a temperature-controlled room with a 12-h light-dark cycle automatically maintained. Food (Laboratory Rodent Chow 500, PMI Feeds, Inc., St. Louis, MO) and tap water were provided ad libitum. Animals were acclimated for a minimum of 1 week. All animal studies were approved by the University Animal Care Committee in accordance with the standards of the Canadian Council on Animal Care.

Chemicals. The CYP1A1 inducer TCDD (1 dpm = 1 x 10⁻¹⁴ mol) was a gift from Dr. Allan B. Okey (University of Toronto, Toronto, Ontario, Canada). The P450 inhibitor ABT was a gift from Dr. Jack P. Uetrecht (University of Toronto). ETYA was a gift from Hoffmann LaRoche Ltd. (Etobicoke, Ontario, Canada). B[a]P, formaldehyde, TPA (phorbol 12-myristate 13-acetate), IL-1α, SOD, and DAPI were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of an analytical grade.

DMEM, fetal bovine serum, lyophilized pen/strep, HBSS (without calcium chloride, magnesium chloride, and magnesium sulfate) and 0.25% trypsin were purchased from GIBCO-BRL (Toronto, Ontario, Canada).

Cell Culture Studies. The various methods used in these studies have been described in length elsewhere (10).

Cell Culture Method. In brief, rats were sacrificed by CO₂ asphyxiation, bathed in 70% ethanol, and two 2 x 2-cm pieces of skin were removed from the dorsal surface and placed in HBSS with 2% pen/strep. Skin was cultured immediately.

All succeeding steps were conducted in a laminar flow hood. The skin was minced into 1-mm³ pieces, stored in 20 ml HBSS (2% pen/strep), and transferred to sterile 100-mm polystyrene tissue culture dishes and arranged to fit under square 18-mm coverslips. Medium (DMEM 500 ml with 75 ml fetal bovine serum and 5 ml pen/strep) was added at the margin of the coverslip, allowing it to move across by capillary action, and then another 5 ml medium
allowed to undergo one complete mitotic cycle, which previously was shown 5 ml fresh medium were added, and cells were This concentration of SOD was shown in embryo culture to be embryoprotective against phenytoin-initiated DNA oxidation and embryotoxicity (43). preincubated with the cells for 24 h before addition of either B[a]P or NNK. concentrations of each chemical used were discussed by Kim et al.* The vehicle for ETYA, NNK, and B[a]P was DMSO. and PBS was the vehicle for ABT.

Micronucleus Formation. For studies using potential inhibitors of bioactivation, skin fibroblasts from either homozygous UGT normal (+/+) or heterozygous (+/j) or homozygous (j/j) UGT-deficient Gunn rats. Cells were incubated with the DMSO vehicle or NNK (10, 100, or 500 µM) for 5 h, washed, and cultured for the rest of one mitotic cycle (26 h). *, †, ‡, and ‡‡, means of four fibroblast cultures. * difference from respective NNK-treated +/+ UGT normal groups (P < 0.05). All NNK concentrations were significantly different from controls, fl±, ß, and γ, means of four fibroblast cultures. * difference from the respective treatment in +/+ UGT normal cells (P < 0.05). NNK-initiated micronucleus formation in +/+ and j/j cells was similar at 10 and 100 µM NNK, whereas at 500 µM, j/j cells had a lower incidence than +/+ cells (P < 0.05), likely due to enhanced NNK cytotoxicity in j/j cells. In untreated cells, the incidence of micronucleus formation was not different among the UGT phenotypes.

Comparative Genotoxic Potencies of NNK and B[a]P. When the potencies of 10 µM B[a]P and NNK were compared in the +/+ and j/j UGT normal cells, increased micronucleus formation initiated by NNK was 2.4-fold higher than that by B[a]P (P = 0.002; Fig. 3). Although NNK also appeared to be more potent than B[a]P in +/+ and j/j UGT-deficient cells, these differences were not statistically significant.

Modulators of B[a]P-initiated Micronucleus Formation. As previously reported (10, 27, 28), 5 10 µM B[a]P initiated substantial micronucleus formation compared to DMSO controls (P < 0.05), the magnitude of which was genotype dependent (Figs. 3 and 4). B[a]P-initiated micronucleus formation was increased respectively by 3.1- and 3.6-fold in cells cultured from both +/+ heterozygous (P = 0.06) and j/j homozygous UGT-deficient (P = 0.0003) Gunn rats over +/+ UGT normal congenic controls (Fig. 3). There was no difference in micronucleus formation between B[a]P-treated +/+ and j/j UGT-deficient cells.

Preincubation with the antioxidative enzyme SOD decreased B[a]P-initiated micronucleus formation by 78% (P = 0.027) and 41%
UDP-GLUCURONOSYLTRANSFERASE GENOPROTECTION

Fig. 4. Effect of UGT deficiency, CYPIA1/2, and/or peroxidase induction and antioxidative cytoprotection on B[a]P-initiated micronucleus formation. Skin fibroblasts were cultured from either homozygous UGT normal (+/+) or heterozygous (+/j) or homozygous (j/j) UGT-deficient Gunn rats. Cells were preincubated with either the dual CYPIA1/2/peroxidase inducer TCDD (10 nM), peroxidase inducers IL-1α (0.625 ng/ml, 0.0367 μM) or TPA (1 μM), or antioxidative enzyme SOD (1680 IU/ml, 500 μg/ml) for 24 h prior to B[a]P (10 μM) for 5 h. Results are the means of four fibroblast cultures after subtracting the respective mean values for the DMSO controls for each UGT phenotype (+/+ , 12.25 ±2.87; +/j, 9.5 ±4.43; j/j, 11.25 ±2.75, mean ±SD). *, difference from B[a]P-treated cells (P < 0.05). Bars, SD.

Modulators of NNK-initiated Micronucleus Formation. NNK (10 μM)-treated cells cultured from +/+ UGT normal, +/j, and j/j UGT-deficient Gunn rats showed respective 2.7- (P = 0.0001), 5.5- (P = 0.008), and 4.6-fold (P = 0.0001) increases in micronucleus formation compared to DMSO vehicle controls (Figs. 3 and 5).

Preincubation with SOD caused respective 65%, 56%, and 49% decreases in NNK-initiated micronucleus formation in cells cultured from +/+ UGT normal (P = 0.001) and +/j (P = 0.08) and j/j (P = 0.02) UGT-deficient Gunn rats (Fig. 5).

When cells were preincubated with ABT, a known P450 inhibitor, NNK-initiated micronucleus formation was respectively decreased by 81% and 62% in +/+ UGT normal (P = 0.001) and j/j UGT-deficient (P = 0.0095) cells (Fig. 5). The dual PHS and LPO inhibitor, ETYA, reduced NNK-initiated micronucleus formation respectively by 44% and 40% in +/+ UGT normal (P = 0.006) and j/j UGT-deficient (P = 0.04) cells. ABT and ETYA were not tested in +/j cells.

Surprisingly, TCDD reduced NNK-initiated micronucleus formation in +/+ UGT normal cells compared to cells treated with NNK alone (P = 0.008), whereas TCDD had no effect in +/j or j/j UGT-deficient cells (Fig. 5). Both IL-1α and TPA failed to increase micronucleus formation in either +/+ UGT normal, +/j, or j/j UGT-deficient cells.

DISCUSSION

UGTs catalyze the elimination of most xenobiotics, including both B[a]P (4) and NNK (2, 42, 44, 45), which can reduce competing bioactivation and thus decrease or eliminate toxicity (Fig. 1). Similar
UDP-GLUCURONOSYLTRANSFERASE GENOPROTECTION

Fig. 5. Effect of UGT deficiency. CYP1A1/2.

and/or peroxidase induction or inhibition and antioxidative cytoprotection on NNK-initiated micronucleus formation. Skin fibroblasts were cultured from either homozygous UGT normal (+/+), or heterozygous (+/j) or homozygous (j/j) UGT-deficient Gunn rats. Cells were preincubated with either the dual CYP1A1/2/peroxidase inducer TCDD (10 nm), peroxidase inducers IL-1α (0.625 ng/ml, 0.0367 nm), or TPA (1 μM), dual P450/peroxidase inhibitor ABT (1 μM), selective peroxidase inhibitor ETYA (40 μM), or antioxidative enzyme SOD (1680 IU/ml, 500 μg/ml) for 24 h prior to NNK (10 μM) for 5 h. Results are the means of four fibroblast cultures after subtracting the respective mean values for the DMSO controls for each UGT phenotype (+/+, 12.25 ± 2.87; +/-, 9.5 ± 4.43; j/j, 11.25 ± 2.75, mean ± SD). *, difference from NNK-treated cells (P < 0.05); ND, treatment groups not determined. Bars, SD.

to a previous report on B[a]P by Vienneau et al. (10), skin fibroblasts cultured from +/j and j/j UGT-deficient rats showed increased NNK- and B[a]P-initiated micronucleus formation with increasing UGT deficiencies, which in the present study was reduced by the antioxidative enzyme SOD. Results from these and previously reported studies of the toxicological relevance of UGT deficiencies in animals (8, 22, 23, 25, 26) and humans (9, 24) have demonstrated a reduction in glucuronidation, with enhanced bioactivation and toxicity, for heterozygous UGT deficiencies that in several cases, including the present study, were equivalent to the toxicological predisposition of the homozygous UGT-deficient group. These observations suggest that hereditary heterozygous UGT deficiencies, which are common in the human population, may be an important clinical determinant of toxicological susceptibility. With homozygous UGT deficiencies, studies in pregnant UGT-deficient Gunn rats suggests that in utero exposure to xenobiotics like B[a]P may be embryo lethal (11).

The tobacco-specific carcinogen NNK initiated micronucleus formation in skin fibroblasts cultured from Gunn rats (Figs. 2–4). Micronucleus formation initiated by NNK at 10, 100, and 500 μM demonstrated a concentration-response relationship in cells cultured from +/- Gunn rats, and a similar pattern was seen in both the +/j and j/j UGT-deficient cells (Fig. 2). Interestingly, micronucleus formation in j/j UGT-deficient cells incubated with 500 μM NNK was lower than that of +/- UGT-deficient cells, which may have resulted from increased NNK-mediated cytotoxicity in j/j UGT-deficient cells. This is supported by the fact that 100 μM B[a]P is cytotoxic to cultured rat skin fibroblasts (10), and since the potency of 10 μM NNK in the +/- cells in initiating micronucleus formation is over 2-fold higher than that of B[a]P (Fig. 3), both 100 and 500 μM NNK would be expected to be cytotoxic. Furthermore, previous studies found that an NNK concentration of 200 μg/ml (190 μL) in transformed C3H/10T1/2CL8 mouse embryo fibroblasts was severely cytotoxic (33).

Similar to previous reports (27, 28), results from this study in cells cultured from +/-j and j/j UGT-deficient Gunn rats showed that preincubation with the respective CYP1A1 and peroxidase inducers TCDD and/or IL-1α and TPA significantly enhanced B[a]P-initiated micronucleus formation (Fig. 4). Although P450s (46), UGTs (47, 48), and PHS (49) all are known to be induced by TCDD, we would expect preincubation with either TCDD, IL-1α, or TPA to induce...
bioactivation pathways (P450, PHS) of B[a]P, while leaving UGT-catalyzed elimination at deficient or basal levels in +/+j and j/j UGT-deficient cultured cells. We hypothesized that this differential induction of the two competing pathways would cause an imbalance favoring bioactivation and a substantial enhancement in B[a]P-initiated micronucleus formation in +/+j and j/j UGT-deficient cells to congregate normal controls. However, preincubation with TCDD, IL-1α, or TPA in +/+j and j/j UGT-deficient cells, prior to B[a]P, caused a similar fold enhancement in micronucleus formation to that seen in ++ UGT normal controls (Fig. 4). This lack of enhanced differential susceptibility to B[a]P-initiated micronucleus formation may be explained by the broad spectrum of gene induction known to occur from TCDD, IL-1α, or TPA exposure, and such broad induction may significantly complicate interpretation of the results (46, 50). However, since induction of both CYP1A1 and peroxidases are major determinants in B[a]P bioactivation and micronucleus formation, as seen by significant enhancement upon CYP1A1 and peroxidase induction (27, 28) (Fig. 5), a more likely explanation for the lack of enhancement may be explained by increased cytotoxicity of +/+j and j/j UGT-deficient cells to enhanced B[a]P bioactivation. A similar effect likely would explain the decrease in NNK-initiated micronucleus formation in j/j UGT-deficient versus +/+j UGT-deficient cells at the highest NNK concentration (Fig. 2).

In this study, preincubation with SOD protected against B[a]P-initiated micronucleus formation, thus suggesting a role for ROS-mediated DNA damage as a molecular mechanism of initiation (Fig. 4). These results are supported by a previous report that ROS-mediated DNA oxidation is a likely molecular mechanism in B[a]P-initiated micronucleus formation (28). Interestingly, although SOD was protective against micronucleus formation initiated by B[a]P, the level of micronuclei in j/j UGT-deficient cells was not reduced down to DMSO controls, indicating significantly increased B[a]P bioactivation and ROS production, above the protection afforded by exogenous SOD (Fig. 4). Conversely, the lack of total SOD protection may indicate that severe UGT deficiencies may enhance micronucleus formation by allowing for greater peroxidase- and/or CYP1A1-catalyzed B[a]P bioactivation and formation of the electrophilic reactive intermediate B[a]P-7,8-diol-9,10-epoxide, which can covalently damage DNA. However, the above evidence and the study by Kim and Wells (28) suggest that DNA oxidation initiated by B[a]P is the predominant mechanism in B[a]P-initiated micronucleus formation in ++ UGT normal and +/+j UGT-deficient cultured rat skin fibroblasts, and at least a major contributor in j/j UGT-deficient cells.

Evidence to date has linked various P450s (CYP1A1, CYP1A2, CYP2A6, CYP2B1, CYP2B7, CYP2D6, and CYP2E1) in the bioactivation of NNK and its major metabolite NNAL to reactive intermediates that can oxidize, methylate, and pyridyloxobutylate DNA (29–33, 35). A P450-catalyzed bioactivation pathway is supported by the reduction in NNK-initiated micronucleus formation by ABT, a known P450 inhibitor (Refs. 51 and 52; Fig. 5).

Interestingly, when cells were preincubated with TCDD, there was a significant reduction in NNK-initiated micronucleus formation in ++ UGT normal cells, with no effect in +/+j or j/j UGT-deficient cells (Fig. 5). TCDD preincubation may have induced UGT isozymes responsible for NNK elimination, thereby avoiding NNK bioactivation. This is supported by the fact that NNK-initiated micronucleus formation in +/+j and j/j UGT-deficient cells, which respectively are less and non-UGT-inducible compared to ++ UGT normal cells, was not reduced by TCDD preincubation (Fig. 5). Alternative mechanisms, such as TCDD induction of CYP1A1, PHS (49), or other enzymes that might produce less toxic NNK metabolites, are possible, although such effects also would be expected in UGT-deficient cells. Since many xenobiotics such as B[a]P (53–55) and the teratogen phenytoin (56) are bioactivated by both P450s and peroxidases, we investigated the role of peroxidase-catalyzed NNK bioactivation. ETYA, a dual inhibitor of both PHS and LPOs, significantly reduced NNK-initiated micronucleus formation, suggesting peroxidase-mediated NNK bioactivation. However, this bioactivation pathway remains questionable, since the peroxidase inducers IL-1α or TPA failed to enhance micronucleus formation (Fig. 5). In fact, others have found that NNK-initiated DNA oxidation was not reduced by various nonsteroidal anti-inflammatory drugs (35), which are known to inhibit peroxidases such as PHS.

Similar to B[a]P, exogenous SOD significantly reduced NNK-initiated micronucleus formation, indicating the involvement of NNK-mediated ROS production (Fig. 5). SOD or catalase also has been shown to inhibit NNK-initiated DNA strand breaks in MRC-5 fetal human lung fibroblasts (40), which suggests that, in our study, DNA damage may be a direct mechanism in NNK-initiated micronucleus formation. However, unlike with B[a]P, SOD in all three UGT phenotypes was only marginally protective against micronucleus formation initiated by NNK. As discussed above, the inability of exogenous SOD to totally protect against micronucleus formation may be due to excessive ROS production initiated by NNK, which may result in the saturation of exogenous SOD. Conversely, unlike with B[a]P, micronucleus formation initiated by NNK in +/+ UGT normal, +/+j, and j/j UGT-deficient cells may involve not only ROS-mediated DNA damage, but also damage in the form of both methylation and pyridyloxobutylatation.

These results demonstrate that UGT-catalyzed NNK elimination in cultured rat skin fibroblasts may be an important cytoprotective determinant in NNK-initiated micronucleus formation, and suggest that the involvement of ROS-mediated damage to essential macromolecules such as DNA may constitute a potential molecular mechanism. These results also show that similar to NNK carcinogenicity, NNK potency in initiating micronucleus formation at least in cells cultured from +/+ UGT normal rats is greater than that of B[a]P, and that UGTs also may be critical determinants in peroxidase-dependent, B[a]P-initiated ROS production and micronucleus formation. Additional research on the toxicological relevance of even heterozygous human hereditary UGT deficiencies is warranted.

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