ABSTRACT

The nonsteroidal antiestrogen tamoxifen is widely used in breast cancer treatment and is currently under evaluation as a chemopreventive agent for individuals at high risk of contracting the disease. The effects of tamoxifen administration on the expression of xenobiotic metabolizing enzymes in F344 rat liver have been investigated. Tamoxifen administration for 7 days produced a dose-dependent increase in enzyme expression similar to that reported to be produced by phenobarbital. Increases in CYP1B1, CYP1B2, CYP1A1, and microsomal epoxide hydrolase mRNA and protein levels in males and females were observed by Western and Northern blotting. The expression of CYP1A1, CYP1A2, and γ-glutamyl transpeptidase mRNA was not significantly affected by tamoxifen treatment. Tamoxifen was approximately one-tenth as potent an inducer of combined CYP1B1/2 mRNA compared with phenobarbital when the two drugs were administered at equimolar doses. In addition to the effects observed after short-term tamoxifen exposure, increases in CYP1B1 and CYP1B2 protein levels were noted after 6 and 15 months of 250 ppm tamoxifen in the diet. Taken together, these results suggest that tamoxifen is a weak phenobarbital-like inducer. However, there are significant differences in the induction profiles produced by the two drugs. Most significant of these differences was the relative weak induction of CYP1B1 but striking induction of CYP1B2 by tamoxifen. In addition, females were often more sensitive than males to tamoxifen, especially at low doses. These differences suggest that tamoxifen and phenobarbital do not use identical molecular mechanisms to produce enzyme induction. It is possible that the effects of tamoxifen are a result of phenobarbital-like properties coupled with the effects of tamoxifen-induced hormonal perturbations in the animal. In sum, tamoxifen induces enzyme expression in rats at a dose comparable, on a mg/kg basis, to the dose women receive for disease management, suggesting these results may be significant for human exposure.

INTRODUCTION

The antiestrogen, TAM3 [Z-1-(4-(2-dimethylaminoethoxy)phenyl)1,2-diphenyl-1-butane], is a well-tolerated treatment for breast cancer (1, 2) and requires continuous, long-term administration for optimal therapeutic effectiveness (3, 4). In addition to its current role in breast cancer therapy, the drug is under evaluation as a chemopreventive agent for individuals at high risk of contracting the disease (5). In view of the ongoing chemoprevention trials and the already widespread use of TAM, many hundreds of thousands of women could be taking the drug indefinitely by the year 2000.

Tamoxifen is metabolized similarly in rats and humans to a variety of products, principally the 4-hydroxy-, N-desethyl-, and N-oxide-derivatives (6–8). Hepatic cytochrome P450-dependent monoxygenases and related enzymes are known to be responsible for much of this metabolism. N-desmethyl-TAM is produced by the action of CYP1A1 and possibly CYP2A5 or CYP2C10. The N-oxide metabolite is produced by flavin-dependent monoxygenases (13), and 4-hydroxy-TAM is produced by an as yet unidentified P450 (10).

Although the potent hepatocarcinogenicity of TAM in rodents is well documented (12–15), the mechanism of this carcinogenicity is not known. It has been suggested that this activity may be due to the metabolism of TAM to an activated species (16) that covalently modifies DNA (17–20). Findings which support this hypothesis include binding of TAM metabolites to microsomal protein (16) and DNA (13, 17, 19, 21), TAM-induced micronuclei (18), hepatocyte DNA breakage (20), and unselected DNA synthesis (19). Taken together, these findings suggest that an activated metabolite of TAM is involved in the hepatocarcinogenicity of the drug. Interestingly, more unselected DNA synthesis was produced in hepatocytes harvested from rats pretreated with TAM than in hepatocytes harvested from untreated rats, suggesting that TAM may induce enzymes responsible for its own activation (19).

Recently, a mechanistic hypothesis for DNA adduct formation by TAM was proposed (22) which involves hepatic α-oxidation of the ethyl group. The enhanced genotoxicity of α-hydroxytamoxifen (23) and reduced genotoxicity of [D-ethyl]TAM (24) when compared to the parent compound support this hypothesis. The specific enzymes responsible for the generation of α-hydroxytamoxifen have not been identified; however, the CYP1A1 subfamily has been implicated (25).

Thus, the influence of TAM on hepatic XME expression is of importance, considering the effects that altered enzyme expression could produce. Trans-stilbene oxide, a compound structurally similar to TAM, is a potent inducer of EH and other PB-inducible hepatic enzymes (26, 27). In contrast, initial studies with TAM demonstrated no effect on liver microsomal enzyme activities in female rats treated with 1 or 10 mg/kg for up to 5 days (28, 29). However, recent reports have shown that acute TAM administration induces CYP1B1, CYP1B2, and CYP1A1 mRNA, protein, and/or associated monoxygenase activities in rat liver in a dose-dependent manner (30, 31). Also, moderate induction of hepatic 7-ethoxyresorufin-O-deethylase, 7-ethoxyxoumarin-O-deethylase, and glutathione-S-transferase activities was observed after chronic TAM treatment (32).

The aim of the present investigation was to further characterize the ability of TAM to alter liver XME mRNA and protein expression in female and male rats. The rat was used as a model system because the disposition of TAM is similar in rats and humans (6–8). The range of doses used encompasses two orders of magnitude, from 0.5 to 50 mg/kg/day. Breast cancer patients normally receive 20 mg/day (Ref. 5; approximately 0.3–0.5 mg/kg/day) for disease management (5). The results from this study demonstrate that TAM is a weak PB-like inducer of hepatic enzyme expression in rats and suggest that this induction may be relevant for human exposure.

MATERIALS AND METHODS

Materials. α and γ 125I-labeled radiolabeled nucleotides and Hybond N+ nuclear acid transfer membrane were purchased from Amersham Corporation (Arlington Heights, IL). Protein A-125I conjugate was purchased from ICN corpora-
cDNA probes (25 ng/labeling) were labeled with $\beta$-32P]dATP with the Am-
hardt's = 0.02% Ficoll, 0.02% BSA, and 0.02% polyvinylpyrrolidone), and
amide, 6X SSC, 1 mM EDTA, 1% SDS, 5X Denhardt's, (IX Den-
subjected to UV irradiation in a Stratagene UV-Stratalinker 2400 on automatic
glass homogenizer in 4 volumes of 0.25 M KPO4 (pH 7.5), 0.15 M KC1,
were washed with ice-cold KC1 and homogenized by six passes of a Teflon-
was removed, and the pellet was dissolved in diethyl pyrocarbonate-treated
(49:1) were added. The tube was inverted after each addition. The samples
acetate (pH 4.0), water-saturated phenol, and chlorofornrisoamyl alcohol
in solution D [4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7),
Chomczynski and Sacchi (35). Briefly, crushed frozen liver was homogenized
of Bradford (34) with Bio-Rad reagents and BSA as a protein standard.
were selected for microsome prep-
and the remaining liver was frozen in liquid nitrogen in individual
whirl-pack bags for later RNA preparations. In the chronic treatments, the
entire liver was frozen before a sample was selected for microsome prep-
Preparation of Liver Microsomal Fraction. Microsomes were prepared by the
method of Van Der Hoeven and Coon (33). Briefly, 1.5-g liver samples
were washed with ice-cold KCI and homogenized by six passes of a Teflon-
glass homogenizer in 4 volumes of 0.25 M KPO4 (pH 7.5), 0.15 M KCI,
0.25 mM PMSF, 10 mM EDTA, 0.1 mM DTT at 4°C. All subsequent steps were
for >8 h at 4°C with shaking in blotto [IX TEST (10 mM Tris-HCl(pH 8.0),
protein weight standards. The proteins were electrotransferred to a 0.45 ftm
membranes were exposed to Kodak X-ray film with one intensifying screen for the appropriate
length of time. All numbers are expressed as the average pixel density of each band as detected by the Phosphorimager apparatus. Blots were then stripped
repred with a human $\beta$-actin cDNA to assess loading variability. The B-actin signal was quantified with the Phosphorimager apparatus, and all
samples but one were less than 15% variable; thus, correction for loading was not necessary. The single variable sample (Lane 3 of the 20 mg/kg, females)
was misplaced and was discarded from the analysis. In order to reliably compare male and female samples that were run on separate concurrent blots,
two control samples (3-methylcholanthrene- and PB-treated animals) were loaded on every blot, and their signals were compared between blots. In every
case, the controls were within 10% of each other. The cDNA probes were acquired from the following sources: CYP1B1, Dr. M. Adesnik (New York
University, New York, NY; Ref. 36); CYP1A2, Dr. F. Gonzalez (National
Cancer Institute, Bethesda, MD; Ref. 37); CYP1A1 and EH, Dr. C. Kasper
(University of Wisconsin, Madison, WI; Ref. 38); ALDH, Dr. T. Dunn
(Pacific, CA; Ref. 39); and $\gamma$-glutamyl transpeptidase, Dr. J. Coloma (Uni-
versity of Valencia, Valencia, Spain; Ref. 40). The oligonucleotide probes specific for the variable region of exon 7 of CYP1B1 and CYP1B2 were
synthesized by the UW-Madison Biotechnology Center. The sequences were: CYP1B1, 5'-GGTTGGTACCGGTGTTGACG-3'; and CYP1B2, 5'-
GGATGCGTCGTAGAAGCCA-3'.
Western Blot Procedure. Samples containing 4 μg microsomal protein
were electrophoresed through 7.5% stacking [375 mm Tris-HCI (pH 8.8) and
0.1% SDS] and 10% separating [125 mM Tris-HCI (pH 6.8) and 0.1% SDS]
polyacrylamide gels in 25 mM Tris-HCI, 200 mM glycine, and 0.1% SDS
running buffer. Bio-Rad 14C-labeled rainbow markers were used as protein
molecular weight standards. The proteins were electrotransferred to a 0.45 μm
polyvinylidene difluoride membrane in 25 mM Tris-HCI, 200 mM glycine, and 0.1% SDS
water bath. The supernatant was frozen at —20°C until use. For cDNA probes, blots were prehybridized in 5X SSC-1% SDS for 3 h at 60°C.
Oligonucleotides were end labeled with $\beta$-32P]dATP by use of T4
poly nucleotide kinase. Blots were hybridized at 59°C in 5X SSC-1% SDS with
labeled probe at a concentration of 2.5 × 106 cpm/ml. Blots were washed in 1X SSC-1% SDS at room temperature with shaking three times for 20
min each, followed by washing in 3X SSC-1% SDS in a 60°C shaking water bath for 10 min with three changes of wash buffer. For all Northern blots, the
amount of radioactivity that hybridized to each band was quantitated with a Molecular Dynamics Phosphorimager, and the membranes were then exposed to Kodak X-ray film at ~80°C with one intensifying screen for the appropriate
length of time. All numbers are expressed as the average pixel density of each band as detected by the Phosphorimager apparatus. Blots were then stripped
and reprobed with a human $\beta$-actin cDNA to assess loading variability. The B-actin signal was quantified with the Phosphorimager apparatus, and all
samples but one were less than 15% variable; thus, correction for loading was not necessary. The single variable sample (Lane 3 of the 20 mg/kg, females)
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Western Blot Procedure. Samples containing 4 μg microsomal protein
were electrophoresed through 7.5% stacking [375 mm Tris-HCI (pH 8.8) and
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polyacrylamide gels in 25 mM Tris-HCI, 200 mM glycine, and 0.1% SDS
running buffer. Bio-Rad 14C-labeled rainbow markers were used as protein
molecular weight standards. The proteins were electrotransferred to a 0.45 μm
polyvinylidene difluoride membrane in 25 mM Tris-HCI, 200 mM glycine, and 0.1% SDS
water bath. The supernatant was frozen at —20°C until use. The membranes were incubated with rabbit polyclonal
anti-rat CYP1B1, anti- rat CYP1B1 (generously provided by Dr. Colin Jef-
coate), or anti-rat microsomal EH (generously provided by Dr. Charles Kasper)
for >8 h at 4°C with shaking in blotto [1X TBST (10 mM Tris-HCI (pH 8.0),
150 mM NaCl, and 0.05% Tween 20), 0.03% sodium azide, and 5% nonfat dry
milk). The membranes were rinsed in TBST and incubated in PBS with 0.25
μCi/ml $\beta$-32P]Protein A (specific activity, >80 mCi/mg) and 0.03% sodium azide for 1 h at room temperature. The blots were washed at room temperature in TBST three times for 20 min each. The radioactivity bound to the membrane was quantitated with a Molecular Dynamics Phosphorimager, and then the membranes were exposed to Kodak X-ray film with one intensifying screen at ~80°C for the required amount of time. For Fig. 8, the $\beta$-actin detection method
was not used. Instead, blots were incubated with a goat anti-rabbit-IgG anti-
body conjugated to alkaline phosphatase, followed by detection in nitroblue
tetrazolium/5-bromo-4-chloro-3-indoyl phosphate solution as described by
Sambrook et al. (41).

RESULTS
Effects of TAM Administration on Cytochrome P450 IIB1 and IIB2 mRNA and Protein Expression. Immunoblotting experiments (Fig. 1) demonstrated that the expression of CYP1B1 and CYP1B2
more sensitive at most of the doses tested. However, CYPIIB2 mRNA dropped precipitously in females at the 50 mg/kg dose. This resulted in maximal fold-induction values of 9.3 in females at 35 mg/kg, and 10.6 in males at 50 mg/kg. As shown in Fig. 3, CYPIIB1 mRNA was not increased in either sex at doses of TAM below 35 mg/kg. The overall levels of CYPIIB1 mRNA induction were low in comparison with the limit of quantitation in this assay (Fig. 3, dashed horizontal line). The maximal induction of CYPIIB1 mRNA was greater in males than in females because of a precipitous drop in mRNA levels in females at 50 mg/kg. Overall, there is a rough concordance between the expression of CYPIIB1 and CYPIIB2 protein and their respective mRNAs, as is evident when the results in Figs. 1–3 are compared.

Expression of Cytochrome P450 IIIA Subfamily mRNA and Protein in Response to TAM Administration. As depicted in Fig. 4, males and females exhibited a similar rise in CYPIIA subfamily mRNA, with maximal-fold induction values of 2.1 and 2.3, respectively. However, the absolute levels of induced mRNA were approximately two times higher in males due to 2-fold higher basal expres-

protein was increased after TAM administration. CYPIIB2 protein levels were maximally induced in females up to 3.9-fold at 20 mg/kg, whereas a 6.2-fold increase was achieved at the 50 mg/kg dose in males. Fold induction values cannot be calculated for CYPIIB1 because the protein is not expressed at detectable levels in normal rat liver under our assay conditions. It is interesting to note that for both CYPIIB1 and CYPIIB2, females responded to a greater extent than males at low doses, but males were more inducible at the highest dose tested. This was due to a plateau in the curves for female rats that was absent in the male response. Interestingly, the CYPIIB1 curves were similar in shape but reduced in magnitude compared with their respective CYPIIB2 curves. Fig. 2 depicts CYPIIB2 mRNA levels in response to TAM administration. Clearly, CYPIIB2 mRNA is more inducible in females than in males at low doses, and females remained
TAMOXIFEN INDUCES HEPATIC ENZYME EXPRESSION

Fig. 3. Induction of CYP1B1 mRNA by TAM. Female (●) and male (○) rats were administered TAM citrate, and total RNA was prepared and analyzed by Northern blotting as per “Materials and Methods” and Fig. 2C. An oligonucleotide specific for CYP1B1 was end labeled and used as a probe in this experiment. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test. The estimated limit of quantitation is represented by a dashed horizontal line in the figure. Bars, SD.

Fig. 4. Induction of CYP11A subfamily mRNA in TAM-treated rat liver. Female (●) and male (○) rats were administered TAM, and liver CYP11A subfamily mRNA expression was investigated as per “Materials and Methods” and Fig. 2C. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test. Bars, SD.

Fig. 5. Increase in CYP11A1 protein abundance in response to TAM administration. Female (●) and male (○) rats were administered TAM, and liver microsomes were isolated and analyzed by Western blotting as described in “Materials and Methods.” A rabbit anti-rat CYP11A1 antibody and 125I-protein A detection system were used. The values on the Y-axis are those reported by the PhosphorImager and represent the average pixel density (x 104) of each band. The X-axis is the dose of TAM administered in mg/kg/day. Points, the means of two separate animals. Bars, SD. SDs smaller than the symbol size are not shown. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test.

Fig. 6. Increased expression of EH mRNA in livers of TAM-treated rats. Female (●) and male (○) rats were administered TAM, and liver EH mRNA expression was investigated as per “Materials and Methods” and Fig. 2C. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test.

maximal-fold induction values of 3.3 and 2.8, respectively. Although the fold induction values were similar between the sexes, the basal expression of CYPIIIA1 was 2.5 times higher in males than females, which resulted in a similar disparity in absolute levels of induced protein. Overall, there was a strong concordance between mRNA and protein expression in both sexes, which is evident when Figs. 4 and 5 are compared.

Microsomal Epoxide Hydrolase mRNA and Protein Expression in Response to TAM Administration. Fig. 6 depicts increases in microsomal EH mRNA after TAM administration. Levels of mRNA

Fig. 5. Increase in CYP11A1 protein abundance in response to TAM administration. Female (●) and male (○) rats were administered TAM, and liver microsomes were isolated and analyzed by Western blotting as described in “Materials and Methods.” A rabbit anti-rat CYP11A1 antibody and 125I-protein A detection system were used. The values on the Y-axis are those reported by the PhosphorImager and represent the average pixel density (x 104) of each band. The X-axis is the dose of TAM administered in mg/kg/day. Points, the means of two separate animals. Bars, SD. SDs smaller than the symbol size are not shown. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test.

Fig. 6. Increased expression of EH mRNA in livers of TAM-treated rats. Female (●) and male (○) rats were administered TAM, and liver EH mRNA expression was investigated as per “Materials and Methods” and Fig. 2C. *, the value is significantly different (P < 0.05) from the respective vehicle-treated control group as determined by Dunnett’s t test.

Overall, however, the shapes of the curves are strikingly similar with the exception that the female response dropped off precipitously at the highest dose, whereas male mRNA levels continued to rise. It is of interest that the cDNA probe used in these assays detects the mRNA for all members of the CYPIIIA subfamily. Since the mRNAs for all these members comigrated in our agarose gels, an increase in any one member of the subfamily might be masked by the relatively high background expression of all forms combined. Consequently, the fold increases outlined above may be considered conservative estimates of an increase in any one member of the CYPIIIA subfamily. Immunoblotting experiments (Fig. 5) demonstrated that CYPIIIA1 protein was inducible by TAM in both males and females, with
increased in both males and females (2.2 and 2.9 fold, respectively), but the shapes of the curves varied significantly. Females exhibited a sharp increase at low doses, followed by a plateau phase at the intermediate doses and a precipitous drop at the highest dose, with the resultant response curves resembling an inverted letter “U.” Conversely, males exhibited a relatively linear increase over the entire dose-response. As shown in Fig. 7, both males and females exhibited a modest 45% increase in EH protein expression at the maximally effective dose for this response of 20 mg/kg. At doses higher than 20 mg/kg, females exhibited levels of EH protein expression similar to control values, whereas males exhibited levels similar to the 20 mg/kg dose.

Aldehyde Dehydrogenase mRNA Expression after TAM Administration. Fig. 8 shows the effect of increasing doses of TAM on the phenobarbital-inducible isoform of hepatic ALDH. The most striking feature of this response is the extreme sensitivity of females to low doses of TAM, as well as the fact that females were more responsive than males over the entire dose-response curve. Both sexes exhibited similar-shaped curves, which were reminiscent of the inverted “U” response seen for TAM-induced EH mRNA expression. Maximal-fold induction values were 2.9 for females at 20 mg/kg and 2.0 for males at 35 mg/kg.

Effects of TAM Administration on Cytochrome P450 IA1, IA2, and γ-Glutamyl Transpeptidase mRNA Expression. TAM administration had no significant effect on mRNA expression for CYP1A1, CYP1A2, or γ-glutamyl transpeptidase in males or females as assayed by Northern blot (data not shown).

Cytochrome P450 IIB1 and IIB2 Protein Expression after Chronic Treatment with TAM. Fig. 9 represents an immunoblotting experiment designed to evaluate the expression of CYP1B1 and CYP1B2 protein in female rats after 6 and 15 months of 250 ppm dietary TAM. An elevation of immunoreactive CYP1B1 and CYP1B2 protein was detected after both treatment periods. Interestingly, chronic treatment with TAM appeared to induce the expression of several proteins that are also recognized by the anti-CYP1B1 antibody. The identity of these immunologically related proteins is unknown.

Comparison of the Potency of TAM and PB as Enzyme Inducers. In order to determine the relative potency of TAM and PB as enzyme inducers, equimolar doses of TAM citrate (35 mg/kg) and sodium PB (14.5 mg/kg) were administered to male and female rats, and the induction of CYP1B1/2 mRNA was compared by Northern blot. The results of this experiment are shown in Fig. 10. The cDNA used as a probe in this experiment was the full-length cDNA to CYP2B1, which detects the mRNA for both CYP1B1 and CYP1B2 on Northern blots. Since the mRNAs for the two isoenzymes comigrate, the values in Fig. 10 are an aggregate of mRNA levels for both genes. As shown, PB is approximately ten times more potent than TAM as an inducer of CYP1B1/2 mRNA. This 10-fold difference can be attributed mainly to the strong induction by PB and the minimal induction by TAM of CYP1B1 mRNA at the doses used.

DISCUSSION

Data collected from these studies demonstrated that TAM induced an increase in the expression of CYP1B1, CYP1B2, CYP3A, EH, and ALDH mRNA and/or protein in rat liver. The molecular mechanism(s) by which TAM might affect changes in XME expression is not known. However, it is reasonable to hypothesize that a component of this response is due to a PB-like mechanism. Three general lines of evidence support this hypothesis: (a) PB-like compounds produce a characteristic “pleiotropic” drug-metabolizing enzyme response that
TAMOXIFEN INDUCES HEPATIC ENZYME EXPRESSION

Fig. 10. A comparison of inducer efficacy: TAM versus PB. Female and male rats were administered equimolar doses of TAM citrate (35 mg/kg) or PB sodium (14.5 mg/kg) daily for 7 days, and levels of combined CYP1B1/2 mRNA were evaluated by Northern blot as per "Materials and Methods." In (A), Lanes 1–9 (from left) are female rats, while Lanes 11–18 are male rats. The treatment regimens are indicated above the relevant lanes.

ACKNOWLEDGMENTS

We express our appreciation to Jennifer Vaughan and Robin Crow for their expert assistance with animal care, Gerald Sattler for his expert photography, and Dr. Charles Kasper (CYPVIA and EH cDNAs and EH antibody).

REFERENCES


which result secondarily in altered XME expression. Expression of many of the enzymes investigated in this study can be modulated dramatically by endocrine factors. Well-documented examples of this include growth hormone- and thyroid hormone-induced suppression of CYP1B1 and CYP1B2 expression (51, 53). TAM can attenuate the serum growth hormone profile in male and female rats (54) and reduce growth hormone, prolactin, and luteinizing hormone secretion from pituitary cells in vitro (55). Thus, it is possible that TAM, acting as a mixed agonist/antagonist of the estrogen receptor, produces perturbations in the hormonal milieu of the animal (e.g., growth hormone or thyroid hormone), which facilitates and/or antagonizes XME induction.

Humans are responsive to various classes of enzyme inducers, including polychlorinated biphenyls (56), macrolide antibiotics (57), glucocorticoids (58), and phenobarbital (59, 60); thus, it is possible that TAM administration to breast cancer patients may result in enzyme induction. The potential effects of this induction in humans are difficult to assess because the specific isozymes involved in the metabolism of TAM have not been conclusively identified. However, it is known that the CYPVIA subfamily is involved (9, 10, 25), and that at least one member of this subfamily is inducible in humans (58).

Furthermore, this subfamily has been suggested to be responsible for metabolic activation of TAM to reactive intermediates in rats (25). Overall, this study demonstrates that TAM is an effective inducer of CYP1B1, CYP1B2, CYPVIA, EH, and ALDH expression in rat liver. This induction is accomplished, at least in part, by a PB-like mechanism. Since TAM produced changes in hepatic enzyme expression in rats at doses directly equivalent (on a mg/kg body weight basis) to the therapeutic doses used in humans, enzyme induction may occur in humans and may be relevant for the metabolism and disposition of the drug.

includes induction of CYP1B1, CYPVICA, CYPVIAA, EH, glutathione S-transferase Ya/Yc, and specific glucuronyl transferases (42). TAM induces CYP1B1, CYPVIAA, and EH; (b) the increase in CYP1B1 gene expression is accomplished through increased mRNA levels for both TAM and PB (43); (c) PB-like inducers exhibit a consistent series of effects in the liver besides enzyme induction, including tumor promotion and organ hypertrophy (44–46). TAM is a potent liver tumor promoter in the multitissue model of rat hepatocarcinogenesis (47–48) and results in an increase in the ratio of liver weight to body weight after subchronic treatment (49), which is suggestive of mild liver hypertrophy masked by overall physical atrophy.

Despite the similarities between the responses produced by TAM and PB, there is evidence to suggest that the molecular mechanism of TAM induction may be substantially different from a PB-type response. Most significantly, females appeared to be more responsive than males over most of the dose-response curve for most of the enzymes tested. This is in stark contrast to PB induction, in which males are more responsive (50, 51). In addition, TAM proved to be a more efficient inducer of CYP1B2 than CYP1B1. PB administration produces a 3-to-5-fold greater increase in mRNA and protein for CYP1B1 than CYP1B2 (43, 52). The other only published example of which the authors are aware concerning inducers that selectively elevate CYP1B2 more than CYP1B1 are MIBs (52). However, TAM and MIBs appear to be distinct cases, since MIBs produce noncoordinate mRNA and protein induction (52) not seen with TAM.

Thus, it is possible that induction by TAM is occurring, at least in part, by a second mechanism in addition to the one used by PB. The multiphasic nature of the TAM-induced response curves, the complex pharmacology of TAM, and the significant but slightly deviant PB-like component of the observed responses supports this “dual-mechanism” hypothesis. The most likely candidate for this second mechanism involves perturbations in the hormonal milieu of the animal,
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