Biochemical Pharmacology, Vol. 52, pp. 1561–1568, 1996.
Copyright © 1996 Elsevier Science Inc.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00560-6

Regulation of Gene Expression of Various Phase I and Phase II Drug-Metabolizing Enzymes by Tamoxifen in Rat Liver

Edward T. Hellriegel, George A. Matwyshyn,† Peiwen Fei,* Konstantin H. Dragnev,‡ Raymond W. Nims,‡ Ronald A. Lubet,‡ and Ah-Ng Tony Kong*†§*

*Division of Clinical Pharmacology, Department of Medicine, Thomas Jefferson University, Philadelphia, PA; †Center for Pharmaceutical Biotechnology, Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, IL; and ‡Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD, U.S.A.

ABSTRACT. The objective of the present investigation was to evaluate the effect of tamoxifen (TAM) on the gene expression of different phase I and phase II drug-metabolizing enzymes. Groups of male and female F344/NCr rats were administered either corn oil or TAM (2.8 to 45 mg/kg body wt × 14 days) dissolved in corn oil by gavage. An additional group of rats received a diet supplemented with phenobarbital (PB, 500 ppm). Northern blot analyses of total liver RNA were conducted using [^{32}P]-labeled cDNA or oligonucleotide probes coding for different sulfotransferase (ST), UDP-glucuronosyltransferase (UGT), glutathione *S*-transferase (GST), epoxide hydrolase (EPH) or cytochrome P450 (CYP) mRNA transcripts. In male rats, TAM increased the levels of ST_{el}, ST_a and ST_{pl} mRNAs, whereas PB increased only the ST_{el} mRNA. In female rats, there was no expression of ST_{el} and ST_{HA} mRNA in either control or TAM-treated animals. TAM and PB increased $UGT_{Br/p}$ mRNAs in all rats, whereas UGT_{ml} mRNA was elevated only in PB-treated animals. EPH mRNA was elevated markedly in all rats treated with TAM and PB, whereas $GST_{ya/yc}$ mRNA was highly increased by PB, but only marginally increased by TAM. Finally, TAM increased CYP3A1 mRNA, and slightly increased CYP2B1 mRNA, whereas PB highly elevated mRNAs for both of these *CYP* genes. In conclusion, treatments of rats with TAM increased the mRNA levels of many phase I and phase II drug-metabolizing enzymes, and this pleiotypic response to TAM seems to be different from other prototype inducers such as PB or dioxin (TCDD). BIOCHEM PHARMACOL 52;10:1561–1568, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. tamoxifen, chemoprevention; UDP-glucuronosyltransferase; sulfotransferase; glutathione *S*-transferase; epoxide hydrolase; cytochrome P450; rat liver; mRNA/gene regulation

The anti-estrogen drug TAM[‖] is presently the most widely used therapeutic agent for the treatment of breast cancer in post-menopausal women [1, 2]. Recently, TAM has shown a great deal of promise for use as a chemopreventive agent [3–6], and large-scale clinical trials are currently underway to evaluate its efficacy as a prophylactic for breast cancer [7, 8].

TAM is presumed to exert its antitumor and chemoprotective effects by competitively antagonizing the binding of estradiol to estrogen receptors, which ultimately leads to the inhibition of gene transcription and protein synthesis [9]. However, recent evidence suggests that the effects of TAM may additionally involve mechanisms that are unrelated to its abilities to interact with the estrogen receptor [10, 11]. Presently, there are a number of questions remaining with respect to the ability of TAM to influence various phase I and phase II drug-metabolizing enzymes. First, it is possible that some portion of the pharmacological effects of TAM may be mediated, in part, via the induction of phase I and phase II drug-metabolizing enzymes. Previous reports have shown that induction of phase II detoxifying enzymes by certain xenobiotics, i.e. phenolic antioxidants, confers protection against chemically induced carcinogenesis [12–14]. In addition, it has been shown that induction of both phase I and phase II drug-metabolizing enzymes by com-

§ Corresponding author: Dr. Ah-Ng Tony Kong, Center for Pharmaceutical Biotechnology (M/C 870), Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois, 900 South Ashland Ave., Chicago, IL 60607-7173. Tel (312) 413-9646; FAX (312) 413-9303.

‖ *Abbreviations:* TAM, tamoxifen; PB, phenobarbital; ST, sulfotransferase; UGT, UDP-glucuronosyltransferase; GST, glutathione *S*-transferase; EPH, epoxide hydrolase; CYP, cytochrome P450; and SSC, 0.15 M sodium chloride + 0.015 M sodium citrate.

Received 3 October 1995; accepted 6 June 1996.

pounds such as indole-3-carbinol sufficiently alters endogenous hormone metabolism to inhibit "spontaneous" mammary carcinogenesis in mice [15] or tumor "promotion and progression" in an *N*-methyl-*N*-nitrosourea (NMU) induced rat mammary model that does not require carcinogen activation [16]. Second, TAM has proven to be a potent liver carcinogen in both rats and hamsters [17]. This effect is presumably attributable, at least in part, to the fact that TAM can readily form DNA adducts in the livers of treated rodents [18, 19]. However, prior reports of both the promoting effects of TAM [20] and its ability to induce CYP2B implied that some portion of its carcinogenic activity may be related to its abilities to induce phase I drug-metabolizing enzymes. Finally, the common use of TAM in humans opens the possibility of certain drug–drug interactions that might either enhance or diminish its biologic activity. In view of these facts, the primary objectives of the present investigation were to examine the effects of TAM on the regulation of gene expression of various phase I and phase II drug-metabolizing enzymes.

In the present report, we focused specifically on the mRNA expression of different STs,¶ UGTs, GST and microsomal EPH isoenzymes, as well as certain major inducible forms of CYPs. ST, UGT, GST, and EPH belong to large multigene families of metabolizing enzymes that are distributed in many organs throughout the body including the liver, lung, and kidney. These enzymes catalyze the metabolism of a number of xenobiotics and endogenous compounds. The STs, for example, are responsible for sulfoconjugating compounds such as biogenic amines (i.e. norepinephrine), steroid hormones (i.e. β-estradiol, dehydroepiandrosterone), bile acids (i.e. hyodeoxycholic acid), drugs (i.e. acetaminophen, propranolol) and carcinogens (i.e. *N*-hydroxy-2-acetylaminofluorene) [21]. UGTs conjugate compounds such as bilirubin, bile acids (i.e. hyodeoxycholic acid), drugs (i.e. acetaminophen, morphine) and carcinogens (i.e. *N*-hydroxy-2-acetylaminofluorene) [22]. The GSTs play an important role in the conjugation of numerous electrophilic drugs (i.e. melphalan, ethacrynic acid) and carcinogens (i.e. 4-nitropyridine-*N*-oxide, benzo[*a*]pyrene, aflatoxin B) [23], whereas EPH catalyzes the conversion of arene and alkene oxides to vicinal dihydrodiols [24]. Conjugation by these phase II enzymes increases the polarity and water solubility of the parent compounds, which facilitates biliary and/or urinary excretion and detoxification. Finally, we examined the effect of TAM on certain of the major inducible families of cytochrome P450 genes (*CYP2B* and *CYP3A*) that are involved with the metabolism of various endogenous ligands as well as activation and detoxification of various carcinogens.

¶ ST(s) is used to denote sulfotransferase protein(s) and italic *ST* with subscript, e.g. ST_{pl}, indicates the gene that encodes the protein. The names of individual enzymes/genes are abbreviated ST_a (alcohol/hydroxysteroid ST), ST_{pl} (phenol ST), ST_{HA} (*N*-hydroxyarylamine ST), ST_{el} (estrogen ST), $UGT_{Br/p}$ (family 1–bilirubin/phenol UGT), and UGT_{mi} (family 2–bile acid/hydroxysteroid UGT).

MATERIALS AND METHODS

Chemicals

TAM and the free acid of PB were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade and were purchased from the Fisher Scientific Co. (Pittsburgh, PA).

Animals and Treatments

Male and female F344/NCr rats were obtained at 6 weeks of age from the Animal Production Area, Frederick Cancer Research and Development Center (FCRDC, Frederick, MD). The animals were maintained on hardwood chips at 68–72°F and ~50% humidity in an American Association for Laboratory Animal Care-certified laboratory. The experimental protocols were reviewed and approved by the FCRDC Animal Care and Use Committee, in accordance with the Declaration of Helsinki and the NIH Guide for the Care and Use of Laboratory Animals. After a 2-week acclimation period, rats of the same sex were randomized by body weight into control and TAM-treated groups (N = 3 rats/group). Rats in the control group were maintained on a standard diet of Purina Lab Chow (No. 5010) and administered corn oil (0.4 mL/day) via oral gavage as a vehicle control. TAM-treated groups received TAM dissolved in corn oil at doses of 2.8 (low dose), 11 (medium dose), or 45 mg/kg body weight (high dose). An additional group of rats receiving the control diet supplemented with PB (500 ppm) served as the positive control for the experiment. This dose of PB would yield a daily dose of about 40 mg/kg body weight. Animals in all treatment groups were allowed free access to food and water throughout the experiment. After 14 days of treatment, individual body weights were recorded, and the rats were killed between 9:00 and 10:30 a.m. by CO_2 asphyxiation. Immediately thereafter, the livers were removed, trimmed free of extraneous tissue, and stored at −70° pending analysis of mRNA.

Isolation of Total RNA and Northern Blot Analysis

Total cellular RNA was isolated from a pooled sample of about 0.7 g (equal amount of liver tissue from each animal per treatment group) by the guanidine-thiocyanate method as described by Chomczynski and Sacchi [25] using the RNAgents Total RNA Isolation System (Promega, Madison, WI). For studies on the phase II genes (*STs*, *UGTs*, *GST* and *EPH*) 15 μg of total RNA was electrophoretically separated on 1% agarose gels containing 5% formaldehyde and transferred onto Hybond™-N nylon membranes (Amersham, Arlington Heights, IL). The blot was prehybridized in 48% formamide, 5x SSC, 20 mM Tris–HCl (pH 7.6), 0.1% SDS and 1x Denhardt's solution at 42° for 2 hr. After prehybridization, the blot was incubated overnight at 42° in fresh prehybridization solution containing 100 μg/mL sheared salmon sperm DNA and eight different ^{32}P-labeled cDNA probes coding for various ST, UGT and EPH mRNA transcripts. The specific cDNA probes utilized

were: rST_{el} [26], rST_{HA} [27], rST_{a} [28], mST_{pl} [29], $mUGT_{Br/p}$ [30], $mUGT_{ml}$ [31] and $pEPH_{302}$ [32]. A separate blot was obtained for each cDNA probe. After hybridization, the blots were initially washed for 15 min at room temperature in 2x SSC and 0.1% SDS, and then for an additional 15 min at 65° in 0.2x SSC and 0.1% SDS. Finally, the washed blots were autoradiographed at −70°. For normalization of the amount of RNA in each lane, blots were stripped of radioactivity and reprobed with a human β-*actin* cDNA probe (ATCC, Rockville, MD). The methodologies employed to probe for *CYP2B1*, *CYP3A1*, *EPH* and $GST_{ya/yc}$ in the slot-blot analysis have been described previously [33].

Preparation of cDNA Probes

The rST_{el} [26], rST_{HA} [27] and rST_{a} [28] cDNAs were prepared from total RNA that was reverse-transcribed to cDNA and subsequently amplified using standard polymerase chain reaction techniques as described previously [34]. The $rGST_{ya/yc}$ [35] and $pEPH_{302}$ [32] cDNAs were obtained from Dr. Cecil B. Pickett (Schering-Plough Research Institute, Kenilworth, NJ). The $mUGT_{ml}$ [31] cDNA was provided by Dr. Ida S. Owens (NIH, Bethesda, MD). The mST_{pl} [29] and $mUGT_{Br/p}$ [30] were described previously. All cDNA probes were labeled with [α-^{32}P]dCTP (New England Nuclear, Boston, MA) using the Multiprime DNA labeling system (Amersham). The specific oligonucleotide probes for *CYP2B1* and *CYP3A1* have been described previously [33].

Densitometry

The density of the bands of the northern blots was visualized by autoradiography and quantitated with a phosphorImager (AMBIS, Inc., San Diego, CA).

RESULTS

Effect of Tamoxifen on Levels of Sulfotransferase mRNA in Rat Liver

The effect of TAM on hepatic sulfotransferase mRNA is shown in Fig. 1. Hybridization of the northern blot to an rST_{el} cDNA probe (Fig. 1A) revealed a prominent RNA transcript of about 1.4 kb in male control rats (lane 1). As shown in Table 1, this transcript was 0.7, 1.6, and 1.9 times that of control in male rats treated with low, medium, and high doses of TAM, respectively. An increase in ST_{el} mRNA (2.4-fold) was also observed in male rats treated with PB. In contrast to male rats, female rats lacked both basal and drug-induced expression of ST_{el} mRNA.

Figure 1B shows an autoradiogram from a northern blot hybridized to an rST_{HA} cDNA probe. A prominent transcript of approximately 1.4 kb was observed in male control rats (lane 1), which was relatively unchanged in male rats treated with low, medium, or high doses of TAM or with PB (Table 1). In contrast to male rats, female rats lacked both basal and drug-induced expression of ST_{HA} mRNA.

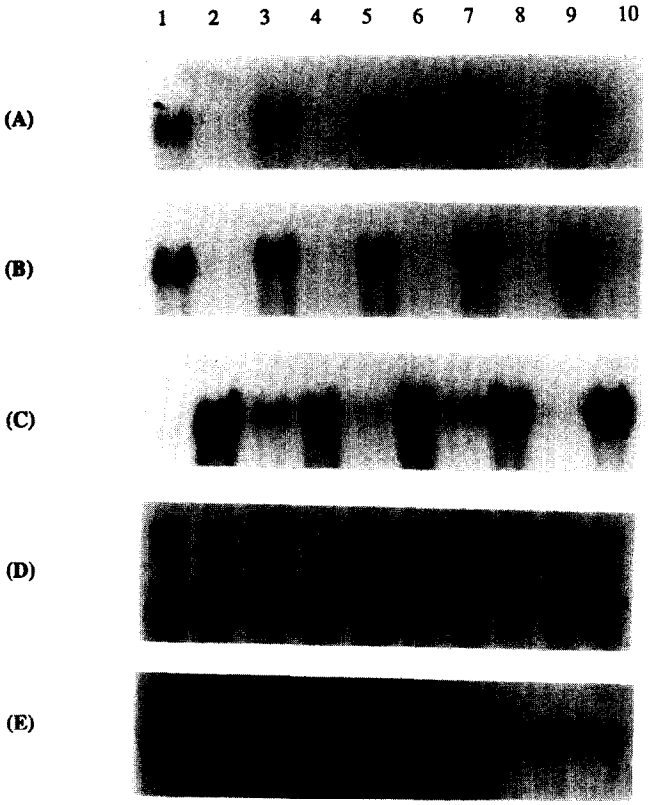

FIG. 1. Northern blot analysis of the effect of TAM on sulfotransferase mRNA levels in rat liver. Total hepatic RNA was isolated from a tissue sample pooled from groups of three rats administered either corn oil, TAM (2.8 to 45 mg/kg body wt) dissolved in corn oil, or a diet supplemented with PB (500 ppm) for 14 days. The blots were hybridized to (A) rST_{el}, (B) rST_{HA} (C) rST_{a}, (D) mST_{pl} and (E) human β-*actin*. Lane 1, male control; lane 2, female control; lane 3, male TAM (11 mg/kg); lane 4, female TAM (2.8 mg/kg); lane 5, male TAM (2.8 mg/kg); lane 6, female TAM (11 mg/kg); lane 7, male TAM (45 mg/kg); lane 8, female TAM (45 mg/kg); lane 9, male PB (500 ppm); and lane 10, female PB (500 ppm).

Hybridization of the northern blot to an rST_{a} cDNA probe (Fig. 1C) showed a single transcript of about 1.2 kb in both male and female control rats (lanes 1 and 2). However, ST_{a} mRNA expression tended to be less abundant in the male control animals than in the female. The low basal level of expression of ST_{a} mRNA in male control rats increased following treatment with TAM. As shown in Table 1, mRNA levels for this particular ST isoform were 3.7, 7.6, and 3.0 times that of control in male rats administered low, medium, and high doses of TAM, respectively. While TAM dramatically increased the level of ST_{a} mRNA in male rats, PB appeared to have an opposite effect, slightly decreasing mRNA levels to 0.7 times that of control (Table 1). In contrast to male rats, female rats that displayed high basal levels of ST_{a} showed little response to any of the drug treatments. Figure 1D shows hybridization of the same blot to an mST_{pl} cDNA probe. Two dominant transcripts of 1.5 and 3.5 kb were observed in both male and female control

TABLE 1. Effect of TAM on hepatic mRNA levels of different phase II detoxifying enzymes in male and female F344/NCr rats

cDNA probe	mRNA (fold change vs control)							
	Male				Female			
	TAM (mg/kg)			PB (ppm)	TAM (mg/kg)			PB (ppm)
	2.8	11	45	500	2.8	11	45	500
rST_{el}	0.7	1.6	1.9	2.4				
rST_{HA}	0.9	0.9	0.8	0.7				
rST_{a}	3.7	7.6	3.0	0.7	1.3	1.1	0.9	0.8
mST_{pl}	0.9*	1.8	3.8	1.4	1.0	0.9	1.9	1.7
	1.1†	1.0	1.3	1.2	0.9	0.8	1.3	1.0
$mUGT_{Br/p}$	2.0	2.9	1.8	1.9	2.1	1.9	1.7	2.2
$mUGT_{ml}$	1.4	1.7	2.0	1.6	1.3	1.1	1.5	1.4
$pEPH_{302}$	1.9	4.8	11.2	16.0	2.8	4.2	6.1	7.1

Total hepatic RNA was isolated from a tissue sample pooled from groups of three rats administered either corn oil, TAM (2.8 to 45 mg/kg body wt) dissolved in corn oil, or a diet supplemented with PB (500 ppm) for 14 days. The blots were hybridized to cDNA probes coding for various ST, UGT, and EPh mRNA transcripts. Values (β-actin normalized) were obtained by densitometry of the autoradiograms from northern blots.

* 3.5 kb mST_{pl} mRNA transcript.

† 1.5 kb mST_{pl} mRNA transcript.

rats (lanes 1 and 2). Whereas the smaller 1.5 kb mRNA transcript showed no substantial response to any of the drug treatments, the larger 3.5 kb transcript was increased with high doses of TAM (Table 1).

To normalize the amount of RNA loaded onto each lane, all blots used to probe the different cDNAs were hybridized to a human β-*actin* cDNA probe. A typical autoradiogram is pictured in Fig. 1E, and shows even loading for all lanes. Ethidium bromide staining of the 18S and 28S ribosomal RNA also indicated that all lanes were loaded evenly (data not shown).

Effect of Tamoxifen on Levels of UGT mRNA in Rat Liver

The effect of TAM on hepatic UGT mRNA is shown in Fig. 2. Hybridization of the northern blot to a family 1 UGT $mUGT_{Br/P}$ cDNA probe (Fig. 2A) showed a prominent RNA transcript of about 2.5 kb in male and female control animals (lanes 1 and 2). Densitometric analysis revealed that the expression of this mRNA transcript was consistently increased in both sexes of animals following

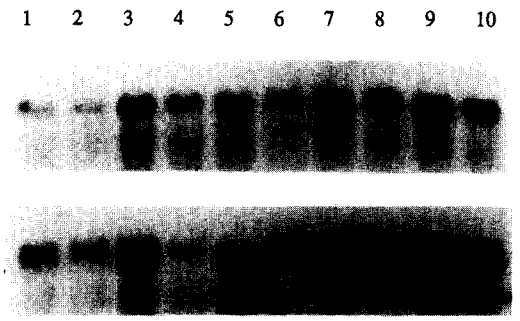

FIG. 2. Northern blot analysis of the effect of TAM on UGT mRNA levels in rat liver. The blots were identical to those described in the legend of Fig. 1 and were hybridized to (A) $mUGT_{Br/p}$, and (B) $mUGT_{ml}$.

treatment with TAM. As shown in Table 1, $UGT_{Br/p}$ mRNA levels were 2.0, 2.9, and 1.8 times that of control in male rats, and 2.1, 1.9, and 1.7 times that of control in female rats treated with low, medium, and high doses of TAM, respectively. Similar to TAM, PB was also found to be a potent inducer of $UGT_{Br/p}$ expression, increasing mRNA levels 1.9 and 2.2 times that of control in male and female rats, respectively, as been shown by other investigators [36].

Figure 2B shows the northern blot hybridized to a family 2 UGT $mUGT_{ml}$ cDNA probe. A single transcript of approximately 2.5 kb was evident in both groups of control animals (lanes 1 and 2). This mRNA transcript was 1.4, 1.7, and 2.0 times that of control in male rats, and 1.3, 1.1, and 1.5 times that of control in female rats treated with low, medium, and high doses of TAM, respectively (Table 1). In addition, treatment of both male and female rats with PB increased UGT_{ml} mRNA levels 1.6- and 1.4-fold when compared with their respective control groups (Table 1).

Effect of Tamoxifen on Levels of EPH and GST mRNA in Rat Liver

The effect of TAM on hepatic microsomal EPH mRNA probed by $pEPH_{302}$ cDNA is shown in Fig. 3. A prominent mRNA transcript of about 2.0 kb was observed in both male and female control animals (lanes 1 and 2), and the level of expression of this mRNA transcript was increased markedly in both sexes of animals following treatment with TAM. Densitometric analysis shows that EPH mRNA levels increased 1.9, 4.8, and 11.2 times that of control in male rats, and 2.8, 4.2, and 6.1 times that of control in female rats treated with low, medium, and high doses of TAM, respectively (Table 1). Similar to TAM, PB was also found to elevate EPH mRNA to levels of 16.0 and 7.1 times that of control in male and female rats, respectively, consistent with that reported in the literature [32]. The elevation of

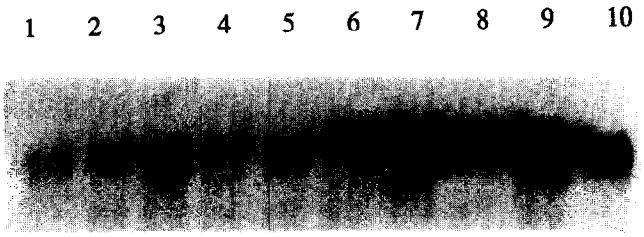

FIG. 3. Northern blot analysis of the effect of TAM on EPH mRNA levels in rat liver. The blot was identical to that of Fig. 1 and was hybridized to $pEPH_{302}$.

EPH mRNA level by TAM and PB was further confirmed in Fig. 4 using slot blot. Also in Fig. 4, slot-blot analysis of hepatic RNA showed relatively strong elevation of the $GST_{ya/yc}$ family by PB and a somewhat weaker increase by the highest dose of TAM.

*Effect of Tamoxifen on the Induction of Various CYP Genes***

The induction of *CYP2B1* and *CYP3A1* by the highest dose of TAM (45 mg/kg body wt) or PB is presented in Fig. 4. As can readily be seen, TAM had minimal effects on the mRNA level of CYP2B1, while PB highly increased this specific isozyme of CYP. We have confirmed these results (data not shown) employing both enzymatic methods, dealkylation of benzyloxyresorufin, and immunochemical methods employing slot-blot analysis. These studies showed that although TAM did increase the levels of CYP2B1, the maximal levels obtained were never greater than 8% of the levels obtained with PB. Interestingly, we found that CYP3A1 was highly induced by both PB and the highest dose of TAM. We have similarly confirmed this finding employing immunochemical analysis (data not shown). In contrast, enzymatic methods examining dealkylation of methoxyresorufin (*CYP1A2*) or ethoxyresorufin (*CYP1A1*) showed minimal induction by any of the doses of TAM or PB (data not shown).

DISCUSSION

Chemopreventive agents, as proposed by Wattenberg [37] can potentially act by one of the following pathways: (1) compounds that prevent the formation of carcinogens from precursor substances; (2) compounds that act subsequent to exposure to carcinogenic agents [these have been called "suppressive agents" since they act by suppressing the expression of neoplasia in cells previously exposed to doses of a carcinogenic agent that will cause cancer, and examples of which include the retinoids, protease inhibitors, and non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin]; and (3) compounds that inhibit carcinogenesis by preventing carcinogenic agents from reaching or reacting with critical target sites, i.e. DNA in tissues (these have been termed "blocking agents"). This third group of agents "block" the action of carcinogens either via the induction of carcinogen-detoxifying enzymes in tissues such as the liver, leading to detoxification and subsequent excretion of the carcinogen in the urine or bile, before they have a chance to react with DNA molecules, or via blocking the metabolic activation process by enzymes such as the cytochrome P450s [38]. Hence, in theory, chemical carcinogenesis can be prevented, in most cases, either by blocking the metabolic activation process or by induction of enzymes that preferentially detoxify carcinogens. In fact, PB, which was examined in the present study, has proven to be a highly effective chemopreventive for aflatoxin-induced liver carcinogenesis in both rat and hamster [39] apparently by inducing a specific glutathione transferase gene that preferentially conjugates aflatoxin 8,9-epoxide [40].

As alluded to at the beginning of the paper, there are a variety of reasons for more detailed studies examining the widest range of potential biological effects of TAM. First, TAM has proven to be a highly effective chemopreventive and chemotherapeutic agent in the breast in a variety of species [1, 2]. Although it has been proposed that most of the efficacy of TAM is due to its antiestrogenic/estrogenic properties, drugs that highly induce various phase I and phase II drug-metabolizing enzymes may alter levels of endogenous substrates including estrogens [15, 19]. Second, TAM is being employed extensively in both therapeutic trials and in chemoprevention trials in humans; thus, any further information relative to potential drug effects or drug–drug interactions is of great clinical relevance. Finally, TAM has proven to be a potent liver carcinogen in rats and

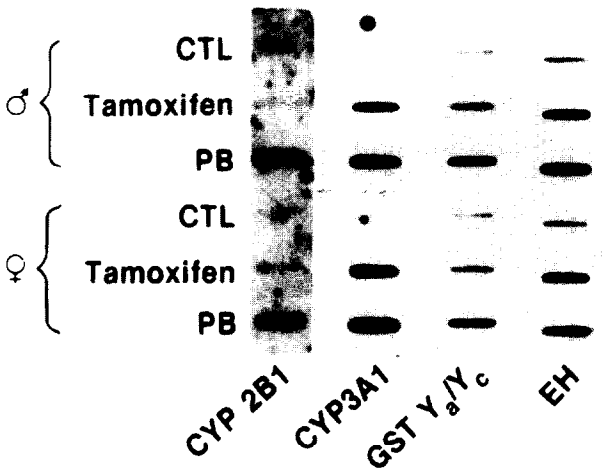

FIG. 4. Slot-blot analysis of the effect of a high dose of TAM (45 mg/kg body wt), or a diet supplemented with PB (500 ppm) for 14 days on the mRNA levels of EPH, GST, CYP2B1 and CYP3A1 in rat liver. The blots were hybridized to $rGST_{ya/yc}$ or $pEPH_{302}$ cDNAs or to oligonucleotide probes for *CYP2B1* or *CYP3A1*.

** Prior experiments with the various phase II genes implied that the higher dose of TAM did appear to give results that were quantitatively similar to those of PB.

hamsters [17]. [The doses of TAM employed in the present studies (2.8 to 45 mg/kg body wt/day) were the same as those used in carcinogenesis assays with TAM and were similar to concentrations of TAM employed in therapy studies in rats.].

One of the most interesting aspects of the present studies is the striking sex-dependent differences in the expression of certain of the rat STs. Thus, ST_{el} and ST_{HA}, both of which are expressed at readily observable levels in male rats, are virtually unexpressed in either control or drug-treated female rats. In contrast ST_a mRNA is expressed at low level in male rats, is highly elevated by TAM, but not by PB, and is not affected by either drug in female rats (although female rats have much higher basal level transcript).

We employed PB as a control in these experiments for three reasons. First, PB induces a variety of phase I and phase II drug-metabolizing enzymes [41] and, therefore, can serve as a positive control for induction of the various *UGT* and *CYP* genes examined. Second, previous reports had contended that TAM could induce metabolism of certain substrates associated with CYP2B (pentoxyresorufin *O*-dealkylase), implying that TAM may be a PB-type inducer [19]. Third, we have shown previously that most strong PB-type inducers [42, 43] are effective liver tumor promoters in initiated rats, and such a mechanism could help to explain the carcinogenic properties of TAM in addition to its known abilities to form DNA adducts. The data with the STs, UGTs and EPH show a similar response of TAM and PB with respect to induction with the exception of ST_a. This ST mRNA transcript was highly elevated by TAM while being suppressed by PB treatment. The results with the *CYP* genes reinforce the clear dichotomy between these two inducers. Thus, CYP2B1 is highly induced by PB but is only very weakly induced by even the highest dose of TAM. The finding that TAM is an inducer of CYP3A and at best a weak inducer of CYP2B1 is in agreement with the recent results of Nuwaysir *et al.* [44]. This limited induction of CYP2B is in contrast to strong induction by classic promoters, e.g. PB-type inducers [42, 43]. These results indirectly argue that the "PB-type" effect is unlikely to be a primary contributor to the carcinogenic/promoting potential of TAM [42, 43]. Perhaps the most intriguing result is the finding that TAM appears to increase the levels of CYP3A1 as determined by RNA or immunochemical analysis (data not shown). This finding supports observations by White and coworkers [19] of induction of CYP3A1 by TAM. This finding is of particular interest since it is this form of P450 that has been implicated in the major metabolic routes for TAM and is apparently involved in its activation to reactive metabolites in rats and humans [45]. In view of certain of the known drug–drug interactions associated with CYP3A1, this is potentially of some interest in women exposed long term to TAM. In summary, TAM appears to induce a wide variety of phase I and phase II drug-metabolizing enzymes although this pleiotypic response appears to be different from that observed with other prototype inducers such as PB, dioxin (TCDD), dexamethasone, or peroxisome proliferators.

Although we have examined the ability of TAM to alter the mRNA levels of phase I and phase II drug-metabolizing enzymes, it alters levels of other genes. Thus, TAM had been shown to stimulate the transcription of many estrogen-regulated genes in the uterus, including ovalbumin [46], insulin-like growth factor-1 (IGF-1) [47], alkaline phosphatase [48], complement C3 [49], progesterone receptor [50], and collagenase [51].*** Webb *et al.* [51] recently reported that TAM activates transcription of human collagenase promoter containing a consensus AP-1 site via the estrogen receptor and the AP-1 pathway. Examination of the xenobiotic detoxifying genes show that $gpST_e$ [52], and $rGST_{ya}$ [53] contain AP-1 and AP-1-like sites, respectively, at the 5′-flanking region. Future studies will elucidate whether TAM regulates phase I and phase II gene expression through transcription activation of the AP-1 complexes and/or other transcription factors, or through some other pathway such as mRNA stabilization.

We thank T. Nguyen and Dr. C. B. Pickett (Schering-Plough Research Institute, Kenilworth, NJ), and Dr. T. H. Rushmore (Merck Research Laboratories, West Point, PA) for providing $rGST_{ya}$, *and* $pEPH_{302}$ *cDNAs, and Dr. I. S. Owens (NIH, Bethesda, MD) for providing* $mUGT_{ml}$ *cDNA. This work was supported, in part, by Grants GM 49172 and ES 06887 from the National Institutes of Health, and a PhRMA Foundation Research Grant (Washington, DC). A.T.K. was the recipient of a 1994 American Association of Pharmaceutical Scientists Young Investigator Award.*

References

1. Harris JR, Lippman ME, Veronesi U and Willett W, Breast cancer. *N Engl J Med* **327:** 473–480, 1992.
2. Jordan VC, The development of tamoxifen for breast cancer therapy. *Breast Cancer Res Treat* **11:** 197–209, 1988.
3. Sharma S, Stutzman JD, Kellof GJ and Steele VE, Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer Res* **54:** 5848–5855, 1994.
4. Bhimani RS, Troll W, Grunberger D and Frenkel K, Inhibition of oxidative stress in HeLa cells by chemopreventive agents. *Cancer Res* **53:** 4528–4533, 1993.
5. Lippman SM, Benner SE and Hong WK, Chemoprevention. Strategies for the control of cancer. *Cancer* **72:** 984–990, 1993.
6. Gottardis MM and Jordan VC, Antitumor actions of keoxifene and tamoxifen in the *N*-nitrosomethylurea-induced rat mammary carcinoma model. *Cancer Res* **47:** 4020–4024, 1987.
7. Fisher B and Redmond C, New perspective on cancer of the contralateral breast: A marker for assessing tamoxifen as a preventive agent. *J Natl Cancer Inst* **83:** 1278–1280, 1991.
8. Raloff J, Tamoxifen quandary. *Sci News* **141:** 266–269, 1992.
9. Heel RC, Brogden RN, Speight TM and Avery GS, Tamoxifen: A review of its pharmacological properties and therapeu-

*** Some of these effects are presumably due to the fact that the estrogen receptor has at least two sites that may serve in activation of various genes. Although TAM inhibits activity of one of the sites, it actually is an agonist for the other sites.

tic use in the treatment of breast cancer. *Drugs* **16:** 1–24, 1978.
10. Sunderland MC and Osborne CK, Tamoxifen in premenopausal patients with metastatic breast cancer: A review. *J Clin Oncol* **9:** 1283–1297, 1991.
11. Buckley M and Goa KL, Tamoxifen: A reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* **37:** 451–490, 1989.
12. Wattenberg LW, Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. *J Natl Cancer Inst* **48:** 1425–1430, 1972.
13. Prochaska HJ, De Long MJ and Talalay P, On the mechanisms of induction of cancer-protective enzymes: A unifying proposal. *Proc Natl Acad Sci USA* **82:** 8232–8236, 1985.
14. Zhang Y, Kensler TW, Cho C, Posner GH and Talalay P, Anticarcinogenic activities of sulforaphane and structurally related synthetic norbomyl isothiocyanates. *Proc Natl Acad Sci USA* **91:** 3147–3150, 1994.
15. Bradlow HL, Michnovicz JJ, Telang NT and Osborne MP, Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* **12:** 1571–1574, 1991.
16. Grubbs CJ, Steele VE, Casebolt T, Juliana MM, Eto I, Whitaker LM, Dragnev KH, Kelloff GJ and Lubet RL, Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. *Anticancer Res* **15:** 709–716, 1995.
17. Williams GM, Iatropoulos MJ, Djordjevic MV and Kaltenberg OP, The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis* **14:** 315–317, 1993.
18. Han X and Liehr JG, Induction of covalent DNA adducts in rodents by tamoxifen. *Cancer Res* **52:** 1360–1363, 1992.
19. White IN, Davies A, Smith LL, Dawson S and De Matteis F, Induction of CYP2B1 and 3A1, and associated monooxygenase activities by tamoxifen and certain analogues in the livers of female rats and mice. *Biochem Pharmacol* **45:** 21–30, 1993.
20. Dragan YP, Xu Y-D and Pitot HC, Tumor promotion as a target for estrogen/antiestrogen effects in rat hepatocyte. *Prev Med* **20:** 15–26, 1991.
21. Mulder GJ and Jakoby WB, Sulfation: In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 107–161. Taylor & Francis, London, 1990.
22. Mulder GJ, Coughtrie MWH and Burchell B, Glucuronidation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 51–106. Taylor & Francis, London, 1990.
23. Ketterer B and Mulder GJ, Glutathione conjugation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 307–364. Taylor & Francis, London, 1990.
24. Guenthner TM, Epoxide hydrolases. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 365–404. Taylor & Francis, London, 1990.
25. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
26. Demyan WF, Song CS, Kim DS, Her S, Gallwitz W, Rao TR, Slomczynska M, Chatterjee B and Roy AK, Estrogen sulfotransferase of the rat liver: Complementary DNA cloning and age- and sex-specific regulation of messenger RNA. *Mol Endocrinol* **6:** 589–597, 1992.
27. Nagata K, Ozawa S, Miyata M, Shimada M, Gong D-W, Yamazoe Y and Kato R, Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyzes activation of *N*-hydroxy-2-acetylaminofluorene. *J Biol Chem* **268:** 24720–24725, 1993.
28. Ogura K, Kajita J, Narihata H, Watabe T, Ozawa S, Nagata K, Yamazoe Y and Kato R, cDNA cloning of the hydroxysteroid sulfotransferase STa sharing a strong homology in amino acid sequence with the senescence marker protein SMP-2 in rat livers. *Biochem Biophys Res Commun* **166:** 1494–1500, 1990.
29. Kong A-NT, Ma M, Tao D and Yang L, Molecular cloning of cDNA encoding the phenol/aryl form of sulfotransferase (mST_{pl}) from mouse liver. *Biochim Biophys Acta* **1171:** 315–318, 1993.
30. Kong A-NT, Ma M, Tao D and Yang L, Molecular cloning of two cDNAs encoding the mouse bilirubin/phenol family of UDP-glucuronosyltransferases ($mUGT_{bi/p}$). *Pharm Res* **10:** 461–465, 1993.
31. Kimura T and Owens IS, Mouse UDP glucuronosyltransferase: cDNA and complete amino acid sequence and regulation. *Eur J Biochem* **168:** 515–521, 1987.
32. Ding VD-H, Cameron R and Pickett CB, Regulation of microsomal, xenobiotic epoxide hydrolase messenger RNA in persistent hepatocyte nodules and hepatomas induced by chemical carcinogens. *Cancer Res* **50:** 256–260, 1990.
33. Nims RW, Sinclair PR, Sinclair JF, Dragnev KH, Jones CR, Mellini DW, Thomas PE and Lubet RA, Dose–response relationships for the induction of P450 by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBPO) in rat and cultured rat hepatocytes. *Xenobiotica* **23:** 1411–1426, 1993.
34. Kong A-NT, Yang L, Ma M and Tao D, Molecular cloning of the alcohol/hydroxysteroid form (hST_{al}) of sulfotransferase from human liver. *Biochem Biophys Res Commun* **187:** 448–454, 1992.
35. Pickett CB, Telakowski-Hopkins CA, Ding GJ-F, Argenbright L and Lu AYH, Rat liver glutathione S-transferases: Complete nucleotide sequence of a glutathione S-transferase mRNA and the regulation of the Ya, Yb, and Yc mRNAs by 3-methylcholanthrene and phenobarbital. *J Biol Chem* **259:** 5182–5188, 1984.
36. Burchell B, Nebert DW, Nelson DR, Bock KW, Iyanagi T, Jansen PLM, Lancet D, Mulder GJ, Roy Chowdhury J, Siest G, Tephly TR and Mackenzie PI, The UDP glucuronosyltransferase gene superfamily: Suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* **10:** 487–494, 1991.
37. Wattenberg LW, Prevention-therapy-basic science and the resolution of the cancer problem: Presidential address. *Cancer Res* **53:** 5890–5896, 1993.
38. Yang CS, Smith TJ and Hong JY, Cytochrome P450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: Opportunities and limitations. *Cancer Res* **54:** 1982s–1986s, 1994.
39. Swenson DH, Lin J-K, Miller EC and Miller JA, Aflatoxin B_1-2,3-oxide as a probable intermediate in the covalent binding of aflatoxin B_1 and B_2 to rat liver DNA and ribosomal RNA *in vivo*. *Cancer Res* **37:** 172–181, 1977.
40. McLellan LI, Judah DJ, Neal GE and Hayes JD, Regulation of aflatoxin B_1-metabolizing aldehyde reductase and glutathione S-transferase by chemoprotectors. *Biochem J* **300:** 117–124, 1994.
41. Lubet RA, Dragnev KH, Chauhan DP, Nims RW, Diwan BA, Ward JM, Jones CR, Rice JM and Miller MS, A pleiotropic response to phenobarbital-type enzyme inducers in the F344/NCr rat. Effects of chemicals of varied structure. *Biochem Pharmacol* **43:** 1067–1078, 1992.
42. Rice JR, Diwan BA, Hu H, Ward JM, Nims RW and Lubet RA, Enhancement of hepatocarcinogenesis and induction of specific cytochrome P450 dependent monooxygenase activities by the barbiturates allobarbital, aprobarbital, pentobarbital, secobarbital and 5-phenyl and 5-ethyl barbituric acids. *Carcinogenesis* **15:** 395–402, 1994.
43. Lubet RA, Nims RW, Ward JM, Rice JM and Diwan BA, Induction of cytochrome P_{450b} and its relationship to liver tumor promotion. *J Am Coll Toxicol* **8:** 259–268, 1989.

44. Nuwaysir EF, Dragan YP, Jefcoate CR, Jordan VC and Pitot HC, Effects of tamoxifen administration on the expression of xenobiotic metabolizing enzymes in rat liver. *Cancer Res* **55:** 1780–1786, 1995.
45. Mani C, Pearce R, Parkinson A and Kupfer D, Involvement of cytochrome P4503A in catalysis of tamoxifen activation and covalent binding to rat and human liver microsomes. *Carcinogenesis* **15:** 2715–2720, 1994.
46. Berry M, Metzger D and Chambon P, Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J* **9:** 2811–2818, 1990.
47. Huynh HT and Pollak M, Insulin-like growth factor I gene expression in the uterus is stimulated by tamoxifen and inhibited by the pure antiestrogen ICI 182780. *Cancer Res* **53:** 5585–5588, 1993.
48. Albert JL, Sundstrom SA and Lyttle CR, Estrogen regulation of placental alkaline phosphatase gene expression in a human endometrial adenocarcinoma cell line. *Cancer Res* **50:** 3306–3310, 1990.
49. Sundstrom SA, Komm BS, Xu Q, Boundy V and Lyttle CR, The stimulation of uterine complement component C3 gene expression by antiestrogens. *Endocrinology* **126:** 1449–1456, 1990.
50. Jamil A, Croxtall JD and White JO, The effect of anti-oestrogens on cell growth and progesterone receptor concentration in human endometrial cancer cells (Ishikawa). *J Mol Endocrinol* **6:** 215–221, 1991.
51. Webb P, Lopez GN, Uht RM and Kushner PJ, Tamoxifen activation of the estrogen/AP-1 pathway: Potential origin for the cell-specific estrogen-like effects of antiestrogens. *Mol Endocrinol* **9:** 443–456, 1995.
52. Komatsu K, Oeda T and Strott CA, Cloning and sequence analysis of the 5′-flanking region of the estrogen sulfotransferase gene: Steroid response elements and cell-specific nuclear DNA-binding proteins. *Biochem Biophys Res Commun* **194:** 1297–1304, 1993.
53. Rushmore TH and Pickett CB, Transcription regulation of the rat glutathione *S*-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. *J Biol Chem* **265:** 14648–14653, 1990.