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Tamoxifen cytotoxicity in hepatoblastoma cells stably transfected with human CYP3A4

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Abstract

Tamoxifen can exert its effects through the competitive inhibition of estrogen receptors or other mechanisms. HepG2 cells lacking estrogen receptors and engineered to overexpress CYP3A4, the most important CYP to metabolize the drug, appear to be a good model to study the effects of tamoxifen metabolites. Tamoxifen altered cell cycle of transduced HepG2 cells, decreased G0/G1 cell numbers, diminished proliferation index and induced cell death mostly in cells overexpressing CYP3A4 but was without significant effect on cytotoxicity or proliferation of cells engineered to overexpress CYP2E1 or on empty vector transfected cells. Tamoxifen did not change MDR1 levels irrespectively on CYP450s expression, but inhibited by approximately 50% *p*-gp functions in all cell types. Drug treatment significantly increased dehydroepiandrosterone sulfotransferase activity and sulfotransferase inhibition significantly decreased tamoxifen cytotoxicity. Our results support the view that metabolic activation of tamoxifen in liver cells may proceed via CYP450-mediated metabolism and subsequent sulfotransferase-mediated activation and point to the role of CYP3A4 and dehydroepiandrosterone sulfotransferase in adverse tamoxifen effects.

Keywords: Tamoxifen; HepG2 cells; CYP3A; *p*-glycoprotein; Dehydroepiandrosterone; Sulfotransferase

1. Introduction

Anti-estrogens used mostly as anti-breast cancer drugs, can be classified into two major groups: triphenylethylene-like analogs of tamoxifen (Tam) with mixed estrogenic/anti-estrogenic properties or pure anti-estrogens [1]. Tamoxifen was approved in 1985 as an adjuvant in chemotherapy of postmenopausal women with node-positive breast cancer and is currently most widely prescribed for breast cancer therapy. Tam can exert its effects through competitive inhibition of estrogen receptors (ER) or non-receptor mechanisms. Detection of human uterine carcinomas or rat liver carcinogenesis related to Tam use stimulated research to elucidate mechanisms of drug action [2,3]. It is known, that the drug undergoes metabolic conversion to Tam-*N*-oxide by the hepatic flavin-containing monooxygenase and into *N*-desmethyl- and 4-OH-Tam by cytochrome P450 [2,4]. Although Tam metabolism is complex and yet not understood, hydroxylted derivatives were mostly considered as detoxication products while other compounds like arene oxides, their precursors or intermediates were supposed to be involved in drug-induced liver carcinogenesis due to their ability to covalently bind DNA and protein [4,5]. Recent studies indicate however, that hydroxylated Tam metabolites, particularly the α-OH-Tam, may be transformed by sulfotransferases to sulfuric acid ester metabolites and form DNA adducts [6–8]. Thus, the α-OH-Tam, a major allylic alcohol metabolite of Tam, may be responsible for the adverse carcinogenic drug effects.

HepG2 cells lack functional estrogen receptors and may serve as a model to study Tam effects which are not related to ER-inhibition. HepG2 cells, however also lack CYP450 enzymes that can metabolically activate Tam, thus cells overexpressing CYP450s are more natural model to study non-anti-estrogen effects of Tam metabolites.

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Abbreviations: DHEA, dehydroepiandrosterone; MDR1, multidrug resistance protein; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PAPS, 3′-phospho-adenosine 5′-phosphosulfate; PCP, pentachlorophenol; *p*-gp, *p*-glycoprotein; PI, propidium iodide; Rh, rhodamine 123; SULT, sulfotransferase; Tam, tamoxifen.

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2. Materials and methods

2.1. Cells and reagents

Human hepatoblastoma cells, subline-HepG2, transfected with an empty vector (C34 cells); HepG2 cells stably transfected with human CYP3A4 (3A4 cells) or cells overexpressing human CYP2E1 (2E1 cells) were used in this study. All cells originate from Department of Biochemistry, Mt. Sinai School of Medicine in New York and were kindly provided by Drs. D. Feierman and A. Cederbaum. Cells were grown in minimal essential medium (MEM) supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 unit/mL) and streptomycin (100 μg/mL). To maintain CYP expression media were additionally supplemented with G418-sulfate (200 μg/mL), the antibiotic used for selection processes. The cell cultures were incubated at 37° in a humidified atmosphere of 5% CO_2. *N*-Dealkylation of fentanyl and GC/MS detection of the reaction product [9] evidenced that 3A4 cells metabolized 1.5 pmol of fentanyl/min/mg cell protein. 2E1 cells hydroxylated *p*-nitrophenol (PNP) at the rate of 320 pmol/min/mg microsomal protein. Ketoconazole (2 μM) added to the culture media blocked CYP3A4-mediated reaction by more than 95% while 4-methylpyrazole (2 mM) blocked CYP2E1 by 77%. Neither control nor Tam-treated C34 cells catalyzed *N*-dealkylation of fentanyl or hydroxylation of *p*-nitrophenol indicating that CYP3A4 or CYP2E1 were not expressed by these cells to any significant extent.

2.2. Tam treatment

Cells were grown in Falcon flasks (Falcon) in MEM. Forty-eight hours prior to the drug treatment the cells were trypsinized, split and divided for the particular assays; 24 hr before the drug treatment, the attached cells were switched to an antibiotic-free media. Tam was dissolved in ethanol and added to the culture media to produce a range of drug concentrations (0.1–20 μM) and the cells were incubated with the drug for additional 48 hr. Ethanol concentrations added with Tam to the culture media were less than 0.3% and were without effect on cell growth, viability and any other parameter tested. Tam effects were tested also in separate experiments in the presence of anti-MDR1 Ab (10 μg/mL) to block *p*-glycoprotein (*p*-gp) function, 2 μM ketoconazole to block CYP3A4-mediated metabolism of Tam (and *p*-gp function) or 50 μM pentachlorophenol (PCP) to block sulfotransferase activity.

2.3. Determination of cell morphology, viability test and proliferation assay

Tam cytotoxicity was determined by flow cytometric quantification of the cellular DNA, using propidium iodide (PI) staining in permeabilized cells [10] and by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test [11]. Briefly, cellular DNA degradation and cell cycle analysis were performed on cells stained for 30 min with PI (50 μg/mL) in Tris buffer (100 mM; pH 7.5), containing potassium cyanide (0.1%), NP-40 (0.01%), RNase (40 μg/mL; Type III-A, 4 kU/mL) and NaN_3 (0.1%). The analysis was performed on an aligned Coulter Epics Profile flow cytometer (Coulter) equipped with an argon laser operating at 488 nm. PI fluorescence was measured in 10,000 cells with appropriate bandpass filters. DNA histograms were further analyzed by DNA analysis software (MultiCycle, Phoenix Flow Systems Inc.). Cells were quantified by their relative distribution in the damaged-hypodiploid (“early” G0/G1 zone of the DNA fluorescence histograms), diploid (G0/G1 zone-pre-DNA synthesis/resting), S-phase (DNA synthesis), and G2/M (post-DNA-synthesis/mitosis) phases of the cell cycle.

MTT test was performed using 5 mM MTT solution in cell culture medium without phenol red. After 4 hr of incubation at 37°, the medium was removed, the crystallized dye was solubilized with 1 mL of isopropanol acidified with HCl (0.05 M) and the optical density of the solution was determined at 570 and 650 nm. The net absorbance ($OD_{570} - OD_{650}$) of untreated cells was taken as the 100% viability value.

2.4. Externalization of phosphatidylserine and Tam toxicity

Phosphatidylserine residues appear on outer surface of plasma membrane in early stages of apoptosis and can bind annexin V [12]. For the analyses, the control and Tam-treated cells were harvested, washed twice with phosphate-buffered saline (PBS) and incubated for 15 min with labeling solution (Clontech Laboratories), containing 2% fluorescein isothiocyanate (FITC)-conjugated annexin V in 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl and 5 mM $CaCl_2$. After that, the cells were washed twice with the same HEPES buffer without labeling reagent and a second fluorescent dye–PI was added to final concentration 5 μg/mL. Green and red fluorescences were simultaneously analyzed using Coulter flow cytometer calibrated on Fl1 (annexin V–FITC) channel and Fl3 (PI channel) using cells stained with annexin V–FITC or PI only. To quantitate early apoptotic cells, annexin V–FITC stained cells that did not fix PI were gated, their green fluorescences were digitized and shown as histograms of fluorescence distribution.

2.5. Immunodetection of extracellular and cytoplasmic epitopes of MDR1 protein

Control and Tam-treated C34, 2E1 and 3A4 cells were scraped, counted, resuspended in 200 μL PBS followed by 20-min incubation (room temperature) with 0.25 μg anti-human MDR1 phycoerythrin-conjugated monoclonal

antibody (Pharmingen) recognizing extracellular domain of the plasma membrane associated *p*-gp. After incubation, the cells were washed twice with PBS containing 0.2% bovine serum albumin and 0.02% NaN_3. MDR1 expression was assessed in 5000 cells per sample on a Coulter Epics Profile flow cytometer using a 585 nm filter. Nonspecific binding was determined using an appropriate isotype control antibody. Cytoplasmic epitope of MDR1 protein was detected in Western blot using a mouse monoclonal antibody anti-*p*-gp, clone C219 (Dako) and a secondary peroxidase-coupled antibody.

2.6. Determination of MDR1 function and mitochondrial dynamics

Rhodamine 123 (Rh) is actively pumped outside the cells expressing multidrug resistance protein (MDR1), therefore it is a valuable probe to functional assay of the MDR1 transporter [13]. To assess MDR1 function, control and Tam-treated cells were incubated with 1 μM Rh for 30 min at 20° with agitation in the presence or absence of mouse mAb against *p*-gp (MDR1; 10 μg/mL) proteins. Subsequently, the cells were trypsinized, washed twice with Tyrode–HEPES–Mg^{2+} free buffer (pH 7.4), resuspended in Rh-free buffer at 37° and time scanned in Coulter Epics Profile flow cytometer every 15 min during 1 hr. The green fluorescence emission from Rh was measured on Fl1 using 525 nm bandpass filter. The registered Rh spectral emissions from 5000 cells were shown as fluorescence distribution histograms, mean fluorescence intensities were calculated and compared.

2.7. Sulfotransferase (SULT2A1) assay

SULT2A1 enzymatic activity was measured in cytosolic fractions of control and Tam-treated cells using a precipitation assay that removes [^{35}S]-3′-phosphoadenosine 5′-phosphosulfate (PAPS). Briefly, cells were scraped, suspended in 20 mM HEPES buffer (pH 7.0) containing 250 mM sucrose, 30 μg/mL aptotonin and 1 mM PMSF and briefly sonicated in ice. The crude homogenates were centrifuged at 100,000 *g* for 2 hr at 4° and the supernatant was dialyzed overnight against 10 mM HEPES buffer, pH 7.0 containing 250 mM sucrose. The dialyzed cytosolic fractions (50 μg of protein) were diluted to 2000 ng/mL in pre-chilled 5 mM potassium phosphate buffer pH 6.5, containing 1.5 mg/mL BSA and 10 mM dithiothreitol. To start the reaction a 100 μL aliquot of the diluted extract was mixed with 5 mL of 160 μM dehydroepiandrosterone (DHEA; final concentration 5 μM), 5 μL of 10 mM magnesium chloride, 50 μL of 25 mM potassium phosphate buffer pH 6.5, containing 25 mM dithiothreitol and 1.28 μM [^{35}S]-PAPS. The mixture was then incubated at 37° for 20 min. The reaction was stopped by addition of a mixture (1:1) of 0.1 M barium acetate and 0.1 M barium hydroxide. Precipitation of free [^{35}S]-PAPS was performed by addition of 50 μL 0.1 M zinc sulfate, followed by short centrifugation in a microfuge at maximum revolution. Then, 50 μL of 0.1 M barium hydroxide and 50 μL of 0.1 M zinc sulfate were added and samples were again centrifuged. Three hundred microliters of supernatant was removed, mixed with 4.5 mL of scintillation cocktail and the cpm determined. Control samples included assays without exogenous substrate, heat-inactivated homogenate or commercial cytosolic extracts (PanVera). The enzymatic activity was calculated by assuming that the soluble cpm were due to sulfate-conjugated DHEA and expressed as pmol/min/mg cytosolic protein.

2.8. SULT inhibition assay

Sulfotransferase (SULT) inhibition assay was performed using 50 μM PCP co-administered with Tam. Such concentration of PCP was not toxic to the cells as assessed in flow cytometry after cell DNA staining with PI.

2.9. Data analysis

Statistical analysis was performed with a statistics package—Statistica 6.0 software (Statsoft) using one-way ANOVA followed by Bonferroni post-tests for comparisons of selected pairs of data. Linear regression and correlation between assayed parameters were calculated according to Pearson test. P values less than 0.05 were considered significant.

3. Results

Figure 1 shows Tam cytotoxicity measured with MTT test. C34 cells and cells overexpressing CYP2E1 or CYP3A4 were treated with different Tam concentrations for 48 hr. Important dose-dependent drug cytotoxicity was found only in cells overexpressing CYP3A4 but not in cells transfected with CYP2E1 or empty vector. Treatment with 20 μM Tam resulted in 60% toxicity in 3A4 cells. Linear regression coefficient was 4-fold lower ($P < 0.01$) in 3A4 cells than in C34 cells.

Table 1, Figs. 2 and 3 show Tam cytotoxicity and the effect of the drug on cell proliferation determined in flow cytometry after DNA staining with PI. Tam increased damaged cell numbers, decreased G0/G1 cells and diminished proliferation index in cells overexpressing CYP3A4 cells, but the drug had only little effect on cell cycle in C34 and 2E1 cells.

DNA fluorescence histograms representative of control (A) and Tam-treated (20 μM; B) 3A4 cells are shown in Fig. 3. Mean fluorescence of diploid peak in control cells was 9.1 and covered 68% of cells, while 32% of cells proliferated. In Tam-treated 3A4 cells (B) an additional fluorescence peak is seen at 4.9 channel and encompass majority (58%) of cells. In 3A4 cells Tam treatment shifted DNA fluorescence to the left side, significantly increased

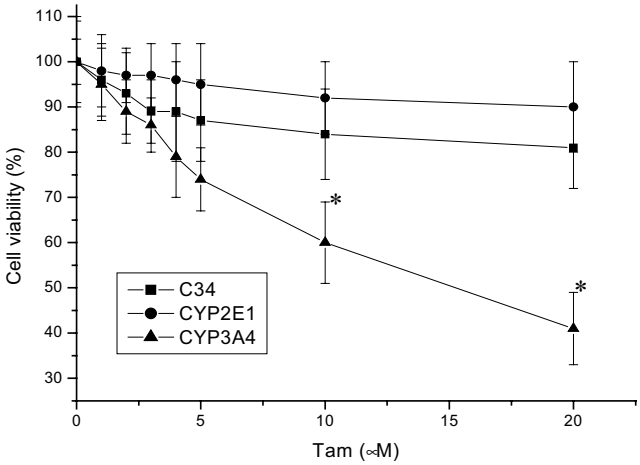

Fig. 1. Tam cytotoxicity in C34 cells and cells overexpressing CYP3A4 or CYP2E1. Cells were treated with Tam for 48 hr and cell viability was assessed with MTT test. Each point represents mean of five assays ± SD. $^{*}P < 0.05$ vs. C34 cells grown in medium containing corresponding Tam concentrations.

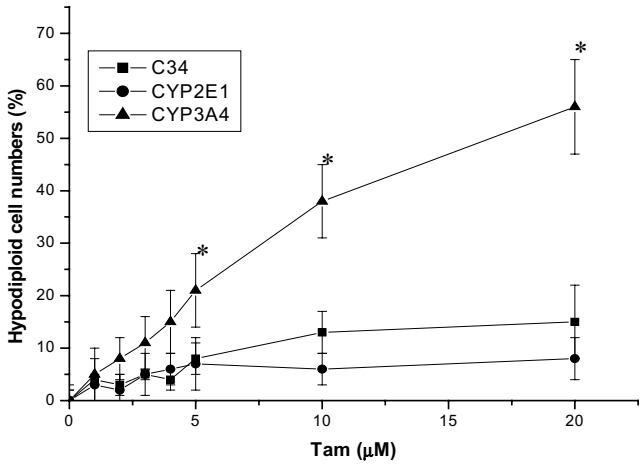

Fig. 2. Tam cytotoxicity measured as hypodiploid cell numbers (%). Cells were treated with Tam for 48 hr, stained with PI and red fluorescence was analyzed in flow cytometry. Each point represents mean of five assays ± SD. $^{*}P < 0.05$ vs. C34 cells grown in medium containing corresponding Tam concentrations.

hypodiploid cell numbers and decreased G0/G1, S and G2/M cell numbers. Also diploid cell numbers and proliferating fraction were significantly reduced. These changes were absent in C34 and 2E1 cells.

Tam induced cytotoxicity in 3A4 cells but not in C34 or 2E1 cells. Phosphatidylserine externalization quantification (Figs. 4 and 5) shows that early apoptotic cell numbers increase with growing Tam concentrations reaching almost 50% of apoptotic cells after 20 μM Tam. Double fluorescence cytograms in Tam-treated 3A4 cells may suggest that important fraction of cells dies via apoptotic pathway (Fig. 5).

MDR1 protein expression (extracellular and intracellular epitopes) was the same in all cell types as determined by

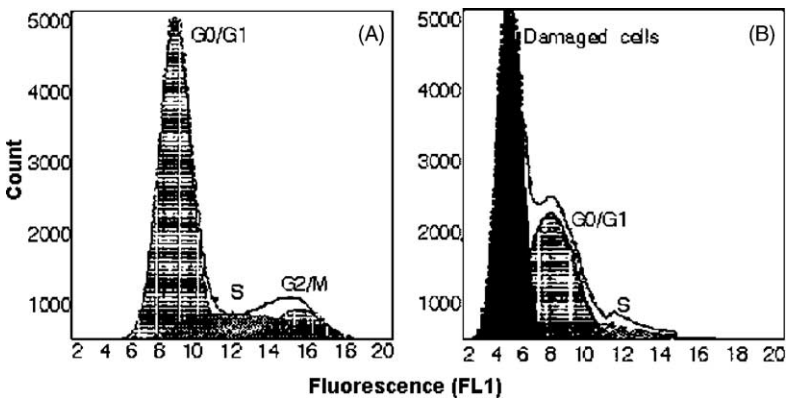

Fig. 3. Typical DNA fluorescence histograms from cells overexpressing CYP3A4 untreated (A) or treated with 20 μM Tam for 48 hr (B). Tam treatment significantly increased hypodiploid cell numbers (B).

Table 1
The effect of 20 μM Tam of cell cycle in empty vector transfected HepG2 cells (C34 cells) and HepG2 cells transduced with CYP2E1 or CYP3A4

	C34 cells		CYP2E1 cells		CYP3A4 cells	
	Control	Tam	Control	Tam	Control	Tam
"Early" G0/G1	7.1 ± 3.4	14.7 ± 7.1	6.2 ± 2.4	8.7 ± 6.2	7.4 ± 3.1	56.6 ± 9.3[*,#]
G0/G1	63.2 ± 8.7	56.5 ± 6.2	66.8 ± 8.1	59.9 ± 8.2	61.2 ± 7.7	29.7 ± 7.2[*,#]
S + G2/M	29.7 ± 5.7	28.8 ± 4.2	27.0 ± 6.1	31.4 ± 7.1	31.3 ± 8.3	13.7 ± 6.3[*,#]

Values represent mean hypodiploid cell numbers (cytotoxicity), diploid cell numbers and S + G2/M fractions (proliferation index). Results are mean of five experiments ± SD.

[*] $P < 0.01$ vs. corresponding control.

[#] $P < 0.01$ vs. C34 cells.

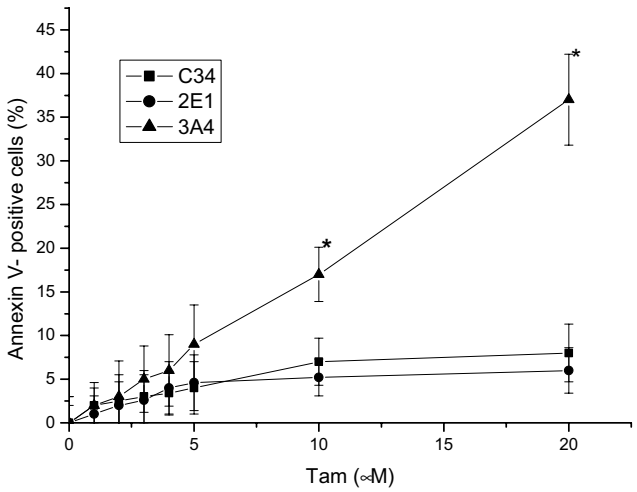

Fig. 4. Tam-induced cytotoxicity in C34, 2E1 and 3A4 cells. Green fluorescence from FITC–annexin V + cells which did not bind PI were quantified in flow cytometry. Each point represents mean of five assays ± SD. $^{*}P < 0.05$ vs. C34 cells grown in medium containing corresponding Tam concentrations.

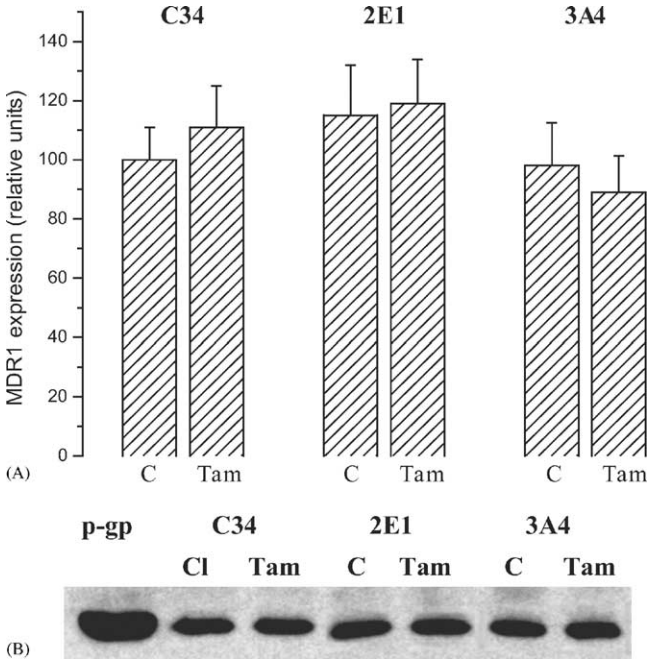

Fig. 6. Mean MDR1 expression in control C34, 2E1 and 3A4 cells treated with 10 μM Tam for 48 hr. (A) Data from flow cytometry quantification of extracellular epitope of MDR1 protein with fluorescent monoclonal antibody. Each bar represents mean relative MDR1 expression of six assays ± SD. (B) Representative Western blot analysis of MDR1 protein in control and Tam-treated cells.

immunofluorescence and flow cytometry detection (Fig. 6A) and Western blot (Fig. 6B).

Rhodamine washout reflecting *p*-gp function was lower by about 40% in cells transfected with CYP3A4 comparing to C34 cells (Fig. 7), but cells overexpressing CYP2E1 had the same *p*-gp function as C343 cells. One micromolar Tam blocked rhodamine washout in all cell types by 47, 55 and 56% in C34, 2E1 and 3A4 cells, respectively.

Tam significantly increased specific cytosolic DHEA sulfotransferase activity in 3A4 cells (about 80% increase), but not in C34 or 2E1 cells (Fig. 8).

Pretreatment of 3A4 cells with CYP3A4 inhibitor-ketoconazole and/or with sulfotransferase inhibitor PCP decreased Tam cytotoxicity by 76 and 63%, respectively (Fig. 9). Pretreatment of 3A4 cells with both inhibitors reduced drug cytotoxicity by 83%.

4. Discussion

Tam has relatively low toxicity and is less harmful than most chemotherapeutics. On the other hand, increased occurrence of endometrial cancer has been reported in breast cancer patients and even in healthy women taking Tam [2]. Tam undergoes metabolic activation and drug metabolism supplies not only effective antitumor compounds but also numerous species that bind DNA. Animal studies show that Tam metabolism produce potent hepatocarcinogens

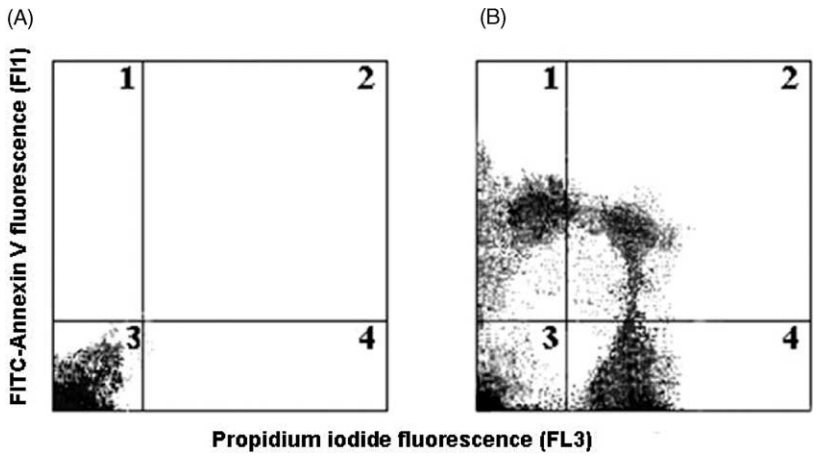

Fig. 5. Typical bivariate fluorescence distribution cytogram of FITC–annexin V (Fl1) and PI (Fl3)-stained 3A4 cells (A: control cells; B: 20 μM Tam; 48 hr). Squares 1, 2, 3 and 4 include Annexin+/PI− cells, Annexin+/PI+ cells, Annexin−/PI− cells and Annexin−/PI+ cells, respectively.

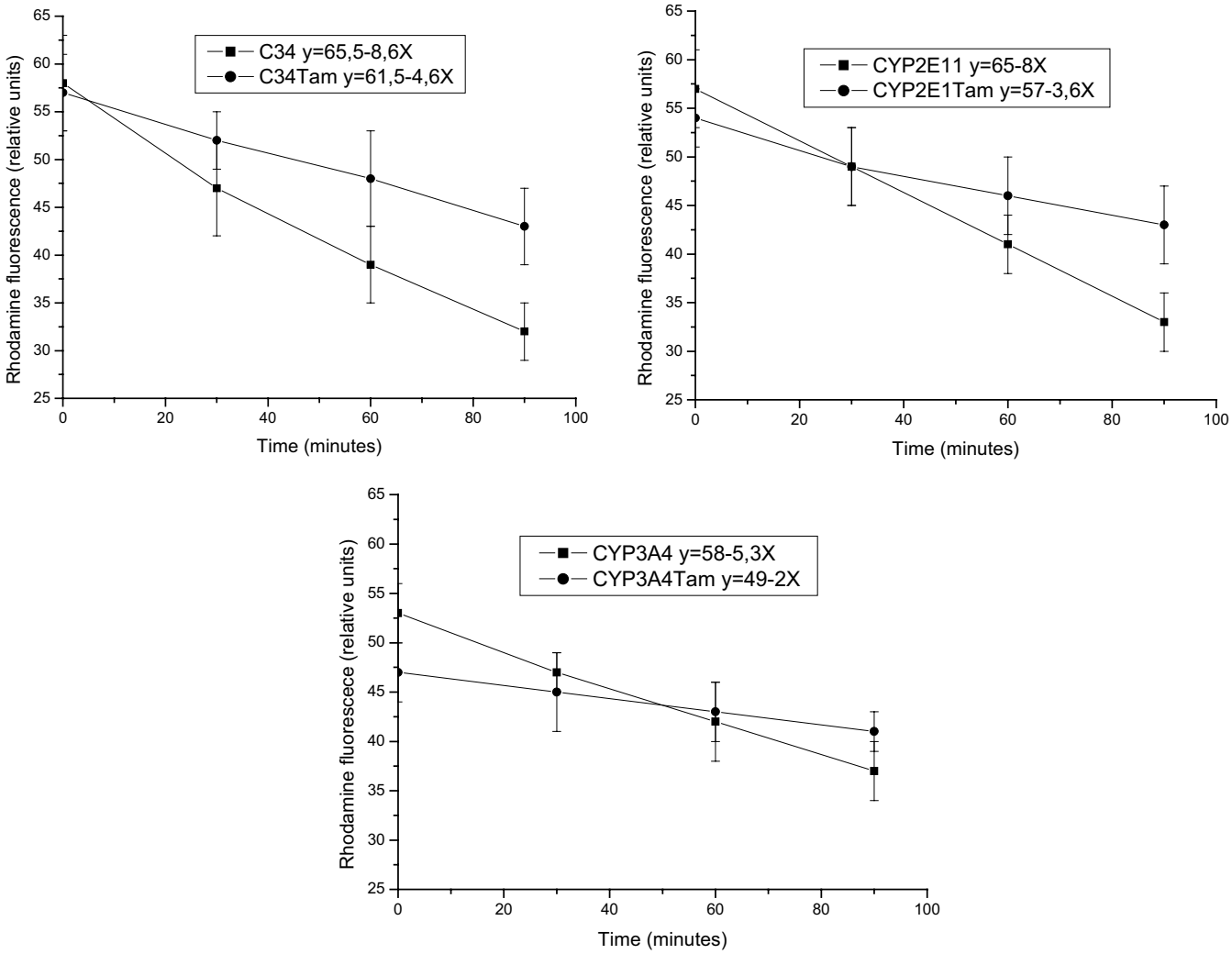

Fig. 7. Rhodamine 123 washout in C34, 2E1 and 3A4 cells treated with 10 μM Tam for 48 hr. Each point represents mean of five assays ± SD. One micromolar Tam blocked rhodamine washout in all cell types by 47, 55 and 56% in C34, 2E1 and 3A4 cells, respectively.

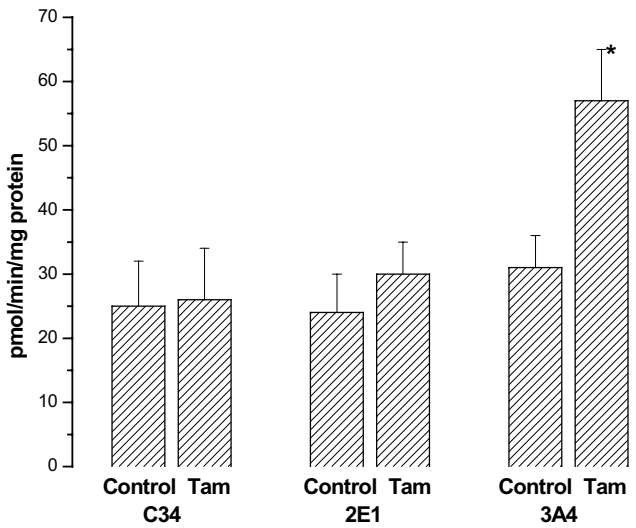

Fig. 8. Sulfotransferase activity in control and Tam-treated (20 μM) C34, 2E1 and 3A4 cells. Enzymatic activity was determined in cytosolic fractions using [^{35}S]-PAPS and DHEA. Each bar is mean of six assays ± SD. $^{*}P < 0.05$ vs. control 3A4 cells.

[3,14,15]. In humans, the major pathway in Tam biotransformation proceeds via *N*-demethylation catalyzed mostly by CYP3A enzymes [16,17]. Another important drug metabolite, 4-OH-Tam, is produced in humans by CYP2D6, CYP2C9, CYP2E1 and CYP3A4 [18,19]. Both metabolites may be further sulfated by sulfotransferases (SULTs), but according to the current knowledge, only *O*-sulfonation of α-hydroxy Tam generates DNA-reacting carbocation [6,20,21]. Consequently, α-hydroxytamoxifen is most probably involved in adverse Tam effects while 4-hydroxytamoxifen is considered as a detoxication product.

To test Tam cytotoxicity, we have used hepatoblastoma cells (HepG2 subline) engineered to overexpress CYP3A4 necessary for oxidative drug activation. HepG2 cells expressing CYP2E1 with minor *in vivo* role in Tam oxidation and cells without any CYP450s activity were used as controls. Tam treatment altered cell cycle and induced cell death mostly via apoptotic pathway, but in the used range of Tam concentrations, a dose-dependent cytotoxicity was

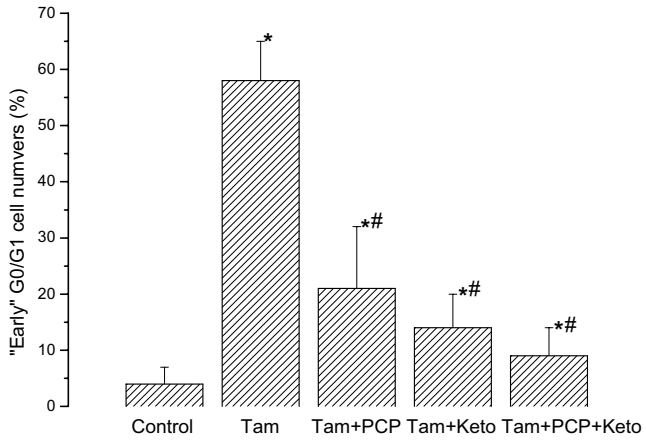

Fig. 9. Tamoxifen cytotoxicity in 3A4 cells pretreated with ketoconazole (Keto; 2 μM, 3A4 inhibitor) and/or PCP (50 μM, sulfotransferase inhibitor). Cells were treated for 48 hr with 20 μM tamoxifen stained with PI and tested using flow cytometry. Each bar represents mean of 10 assays ± SD of "early" G0/G1 cell numbers. Tam treatment resulted in almost 60% toxicity while pretreatment of 3A4 cells with CYP3A4 inhibitor-ketoconazole (Keto) and/or with sulfotransferase inhibitor–PCP decreased Tam cytotoxicity to 24 and 35%, respectively. Pretreatment of 3A4 cells with both inhibitors significantly reduced but not completely abolished Tam toxicity. $^{*}P < 0.05$ vs. control cells; $^{\#}P < 0.05$ vs. Tam-treated cells.

found only in cells overexpressing CYP3A4. Apart from cytotoxic action, Tam decreased G0/G1 cell numbers and diminished proliferation index but was without evident effect on cells transfected with an empty vector or on cells expressing CYP2E1.

Tam exerts different effects in HepG2 cells. The drug has been shown to be toxic [22], to induce apoptosis in native cells through elevation of intracellular Ca^{2+} [23], but also to be without effect on the growth of cultured cells [24,25]. HepG2 cells however, are not of clonal origin therefore experimental data from different laboratories may vary depending on particular cell features. Four days exposure of our cells to Tam resulted in cytotoxicity even in cells lacking CYP3A4 (results not shown), however, CYP3A4 clearly promoted cell death and increased proapoptotic Tam action.

Tam treatment did not affect the *p*-gp protein levels, irrespectively of CYP450 expression, but decreased rhodamine washout in all cell types indicating functional inhibition of *p*-gp mediated drug transport. Earlier studies have shown that Tam, 4-OH-Tam and *N*-desmethyl-Tam can bind *p*-gp protein [26] and this binding may probably affect *p*-gp function. Our results point to Tam-induced modulation of *p*-gp mediated drug resistance that occur intracellulary, but most probably not at the level of *mdr* gene.

Significantly lower Tam toxicity after SULT inhibition suggests that apart from CYP3A4, these enzymes may participate in further metabolic activation of the drug. On the other hand, the *in vivo* situation is more complex because there is great individual variability in SULT isoenzyme in humans [27,28] and laboratory animals [29]. Since Tam metabolites may be sulfated by both hydroxysteroid sulfotransferase and phenol or dopamine-sulfating sulfotransferases, it is hard to predict individual patient susceptibility to the drug. Among major human liver sulfotransferases, SULT1A1 was suggested to play the most important role in the sulfation of HO-Tam isomers [6,30]. Our data point to increased cytosolic dehydroepiadrosterone sulfotransferase activity. Increased SULT2A subfamily, responsible for metabolic activation of α-hydroxytamoxifen was also reported after chronic treatment of rats with Tam [31]. Therefore, our results support the view that Tam cytotoxicity may be related to sulfonation of the Tam metabolites.

Experimental data point to mutatagenic action of α-hydroxytamoxifen, while other drug metabolites seem to have significantly less harmful properties [32]. SULT-dependent genotoxicity of α-hydroxytamoxifen was clearly detected in recombinant cells [33,34] and short treatment of rats with α-hydroxytamoxifen resulted in DNA damage [35]. In a human study, Tam did not prolong survival of patients with inoperable hepatocellular carcinoma and increasing drug doses had apparent negative effect [36]. Since both CYP3A4 and SULT2A are easily inductible enzymes, also by Tam itself, it is possible that such induction may make some patients prone to adverse Tam effects.

Acknowledgments

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