Cancer Chemother Pharmacol (1995) 36: 129–135

ORIGINAL ARTICLE

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Comparative in vitro cytotoxic effects of taxol and its major human metabolite 6α-hydroxytaxol

Received: 19 May 1994 / Accepted: 15 October 1994

Abstract Taxol is metabolized by the liver microsomal cytochrome P450 enzyme system into its principal metabolite 6α-hydroxytaxol (6HT). In the present in vitro studies 6HT was compared to taxol with respect to its effects on tubulin depolymerization, mitotic arrest, clonogenic survival and apoptosis in HL-60 cells. 6HT was generated by incubating taxol with human liver microsomes in a NADPH-generating system. HL-60 cells were incubated for 24 h with either taxol or 6HT, washed and placed in drug-free suspension or cultured for colony growth in agarose. For the suspension and colony culture growth of the cells, the IC_{50} concentrations of 6HT were 500 ± 46 and 350 ± 37 n*M*, while those of taxol were 3.2 ± 0.2 and 2.8 ± 0.5 n*M*, respectively. Immediately after a 24-h exposure of HL-60 cells to 50 n*M* taxol, electrophoresis of genomic DNA from HL-60 cells revealed an internucleosomal DNA fragmentation 'ladder'. In addition, 39% of the cells were arrested in mitosis and 16% showed the morphologic features of apoptosis. In contrast, an identical treatment with 6HT resulted in the mitotic arrest of only 2.8% of the cells, with 4.0% displaying apoptosis ($P < 0.01$); internucleosomal DNA fragmentation was not observed. 6HT was also significantly less effective than taxol in inhibiting the temperature-induced depolymerization of microtubules in a cell-free system. However, at equipotent concentrations, the effect of 6HT on tubulin depolymerization, mitotic arrest or apoptosis was similar to that of taxol. In addition, at concentrations of taxol or 6HT at or below their IC_{50}, there was little tubulin depolymerization, mitotic arrest or apoptosis. The results presented here show that the biotransformation of taxol to 6HT substantially detoxifies taxol.

Key words Taxol · 6α-Hydroxytaxol · HL-60 cells

Abbreviations 6HT 6α-hydroxytaxol · *DMSO* dimethyl sulfoxide · *NADPH* nicotinamide adenine dinucleotide phosphate · *HPLC* high-performance liquid chromatography · *FBS* fetal bovine serum · *PBS* phosphate-buffered saline · *EDTA* ethylenediaminetetraacetic acid · *HEPES* *N*-[2-hydroxyethyl]piperazine-*N*′-2-ethanesulfonic acid · *TAE* tris-acetate EDTA · *GTP* guanosine triphosphate · *MES* 2-(N-morpholino) ethanesulfonic acid · *EGTA* ethylene glycol-bis(β-aminoethylether)*N*,*N*,*N*′,*N*′-tetraacetic acid

Introduction

Taxol is a diterpenoid anticancer drug with a broad spectrum of clinical activity against a variety of solid tumors and acute leukemias [18]. Intracellularly, taxol promotes all aspects of tubulin polymerization and inhibits its depolymerization, resulting in stable microtubule bundles [14, 19]. As a result, taxol acts as a mitotic spindle poison, arresting cell cycle progression in the G_2/M phase [12, 17]. Ex vivo sensitivity to taxol-mediated antimicrotubule effects has been correlated with the magnitude of its in vivo antileukemic activity [17]. A more recent report suggests that the exposure to low concentrations of taxol induces the mitotic block and the inhibition of cell proliferation by kinetically stabilizing spindle microtubule dynamics rather than by changing the mass of polymerized microtubules [9]. Taken together, these intracellular effects are considered to be responsible for the inhibition of suspension culture growth and the loss of clonogenic survival of neoplastic cells [1, 9]. Although taxol does not interact with DNA, taxol-induced mitotic arrest is followed by endonucleolytic internucleosomal DNA fragmentation and the morphologic features of apoptosis [1].

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The biologic fate of taxol in humans is not completely understood. Phase I clinical studies of taxol have demonstrated that less than 10% of the administered dose is excreted unchanged in the urine [22], with another 20% appearing in the bile [13]. The remaining 70% is likely to be excreted as metabolites in bile and feces [8, 10]. Taxol is metabolized by the human liver microsomal cytochrome P450 system [3, 11]. The principal human metabolite of taxol has been identified by tandem mass spectroscopy and high-field NMR to be 6α-hydroxytaxol (6HT) [8, 10, 13]. With the use of higher dose taxol regimens administered over 3 h, or in clinical settings where the biliary excretion is impaired, high levels of 6-HT may accumulate. To predict the clinical impact of this, it would be important to ascertain the comparative cytotoxic potency of 6HT and taxol. Therefore, in the present in vitro studies we compared taxol and 6HT with respect to their effects on tubulin polymerization, mitotic arrest, clonogenic survival and apoptosis in human myeloid leukemia HL-60 cells. Our findings indicate that taxol has approximately 100-fold greater activity than 6HT in mediating the inhibition of suspension culture growth and clonogenic survival of HL-60 cells. Correspondingly, taxol is also significantly more potent than 6HT in inhibiting microtubule depolymerization as well as inducing mitotic arrest, internucleosomal DNA fragmentation and apoptosis in HL-60 cells.

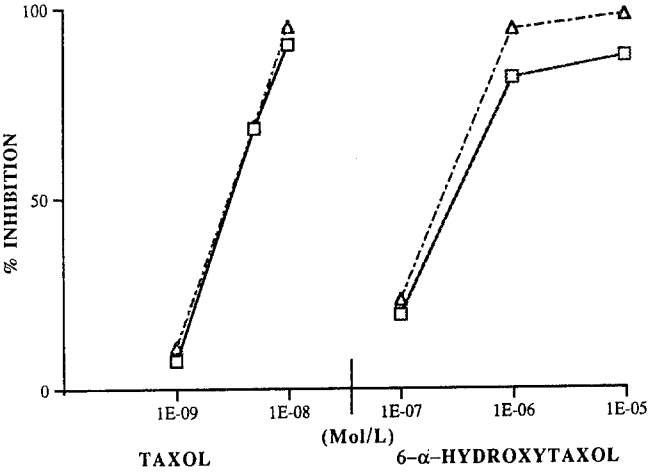


Fig. 1 Logarithmically growing HL-60 cells were exposed to various concentrations of taxol or 6HT for 24 h. Subsequently, cells were washed and cultured in suspension in drug-free medium for 72 h. Following this, the cells were counted and the mean percentage growth inhibition determined relative to control growth of untreated cells. Alternatively, cells were washed after exposure to the drug, then cultured in agarose for 7 days. Subsequently, colony growth was determined and the mean percentage colony growth inhibition determined relative to the control colony growth of untreated cells (△ percentage inhibition of suspension culture growth, □ percentage inhibition of colony culture growth)

Materials and methods

Drugs and other chemicals

Taxol was a kind gift from Dr. Patricia A. Pilia of NaPro Biotherapeutics (Boulder, Calif.). A stock solution (10 m*M*) of taxol was prepared in dimethylsulfoxide (DMSO). Small aliquots of this stock solution were stored at 4 °C. It was diluted in medium to achieve the desired concentrations. In no experiment did the DMSO concentration exceed 0.01%. At this concentration, DMSO by itself does not produce internucleosomal DNA fragmentation in HL-60 cells. RNases A and T1 were purchased from Sigma Chemical Co. (St. Louis, Mo.). Proteinase K and ultrapure DNA grade agarose were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and Bio-Rad Laboratories (Hercules, Calif.), respectively. All other chemicals were of analytical grade.

Preparation of 6α-hydroxytaxol

6HT was generated by human liver microsomes using an incubation system consisting of 1 mg/ml of microsomal protein, an NADPH-generating system and taxol (final concentration, 30 μ*M*) in HEPES buffer. The incubation was performed for 1 h at 37 °C. 6HT was isolated by extraction with ethyl acetate and purified by reversed-phase HPLC as previously described [11]. About 250 μg of 6HT was isolated in this manner and the structure was confirmed by tandem mass spectrometry and high-field nuclear magnetic resonance spectrometry [10]. There were no detectable impurities by HPLC. The extraction step with ethyl acetate precluded contamination of 6HT with inorganic impurities.

Cells

HL-60 cells were freshly grown as suspension culture in RPMI-1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (HyClone, Logan, Utah) and antibiotics at 37 °C in humidified air containing 5% CO_2 [6]. The viability of the cells was assessed by their ability to exclude 0.5% trypan blue. Cell density in the culture was determined using a Coulter Counter (Model ZM; Hialeah, Fl.). Only exponentially growing, viable cells were used for the studies. Cells were seeded at a final density of 0.3×10^6 cells/ml in all experiments, and treated with the designated dose and exposure to the drug. After drug treatment, when necessary, cells were washed twice with PBS and reincubated in drug-free medium.

Effects of taxol and 6HT on morphology, suspension culture and colony growth of leukemic cells

The suspension culture and colony growth of HL-60 cells were determined by a minor modification of a previously described method [1]. Briefly, following 24 h incubations with different concentrations of taxol or 6HT, cytospin preparations of the cells were stained with Wright stain and their morphology was determined by light microscopy [1]. Five different fields were randomly selected for counting of at least 500 cells. The percentage of apoptotic cells was calculated for each experiment. Cells designated as apoptotic were those that displayed the characteristic morphologic features of apoptosis including cell volume shrinkage, chromatin condensation and the presence of membrane-bound apoptotic bodies [21]. The assessment of the percentage of apoptotic cells was confirmed by an additional independent observer who was blinded to the results of the first observer. In parallel studies, following incubation with taxol or 6HT, as mentioned above, cells were washed and placed in suspension culture at a concentration of 2×10^5 cells/ml. Aliquots of cells were withdrawn daily and cell counts determined. In alternate experiments, following taxol exposure, cells were washed and plated at a cell density of 5×10^3 cells/ml in 0.3% agarose in the presence of 20% FBS and 10% 5637 conditioned medium in 18-mm 12-well tissue culture plates. The plates were

incubated at 37 ° in an atmosphere containing 5% CO_2, and colonies of 50 or more cells were counted at the end of 10 days [1, 2].

Genomic DNA extraction

Following incubations with the designated concentrations and schedules of taxol or 6HT, 1×10^6 cells were pelleted in a 1.5-ml Eppendorf tube by centrifugation at 1000 rpm for 5 min, and washed twice with PBS, pH 7.3. Cells were gently resuspended in 50 µl of the lysis buffer (200 m*M* NaCl; 10 m*M* Tris-HCl, pH 8.0; 40 m*M* EDTA, pH 8.0; 0.5% SDS; 200 ng/µl of RNase A, 10 U/µl RNase T1), and incubated at 37 °C for 1 h. To the cell lysate was added 200 µl of the digestion buffer (200 m*M* NaCl; 10 m*M* Tris-HCl, pH 8.0; 0.5% SDS; 125 ng/µl proteinase K). The contents were mixed by inverting several times, and then incubated at 50 °C for 3 h. An equal volume of a mixture of phenol (pH 8.0) and chloroform (1:1 v/v) was added, followed by gentle mixing for 10 min and storage at room temperature for 2 min. The two phases were separated by centrifugation at 3000 rpm for 10 min. The viscous aqueous phase (not the interphase) was transferred to a fresh tube with a wide-bore pipette tip. The phenol/chloroform extraction was repeated. The aqueous phase was extracted with an equal volume of chloroform. To enhance the precipitation of fragmented DNA (if any), 1.0 *M* $MgCl_2$ was added to the aqueous phase to a final concentration of 10 m*M*. The total DNA was precipitated by the addition of two volumes of absolute ethanol with several inversions. DNA was pelleted by centrifugation at 3000 rpm for 15 min, washed twice with 70% ethanol, and air-dried. The pellet was dissolved in 25 µl of 10 m*M* Tris-HCl (pH 8.0) and 1.0 m*M* EDTA (pH 8.0). The concentration and purity of DNA was spectrophotometrically determined.

Agarose gel electrophoresis

Completely melted agarose (1.8% w/v) in TAE (40 m*M* Tris, 40 m*M* sodium acetate, 1.0 m*M* EDTA, pH 8.3) was poured onto the tray to form a 1.5-mm thick gel. The agarose gel was solidified and dried for 1 h at room temperature and was transferred to a Mini-Sub cell (Bio-Rad) containing 1 × TAE, pH 8.3. The volume of the running buffer was adjusted to 1.0 mm depth from the surface to the submerged gel, and pre-run at 2 V/cm for 1 h. Wells were cleaned by flushing the running buffer in and out with a pipette tip. Samples were prepared by mixing DNA (< 1.0 µg) with 0.2 volume of loading dye mixture (0.25% bromophenol blue, 0.25% xylene cyanol, 40% sucrose) and heating at 65 °C for 3 min. They were then rapidly chilled in ice-water, loaded onto the gel and subjected to electrophoresis at 2 V/cm for 3.5 h under ice-cold conditions. The gel was stained with ethidium bromide (0.5 µg/ml) for 15 min with slow rocking, and destained in water until the background fluorescence disappeared. DNA-intercalated ethidium fluorescence was observed in a UV (302 nm) transilluminator, and photographed on Polaroid film no. 665 (P/N) using an orange filter. Subsequently, the negative was developed for the DNA profile.

Flow-cytometric cell cycle analyses

Flow-cytometric analysis of the cellular DNA content by propidium iodide staining was performed in 6HT- or taxol-treated HL-60 cells according to a previously described technique [16]. Cells (1×10^6) were treated with the various concentrations of taxol or 6HT for 24 h. Following this, the cells were withdrawn, stained with propidium iodide and analyzed for DNA content using a Coulter Elite flow cytometer. The percentage of cells in each of the G_1, S, G_2/M phases was calculated using Multicycle Software (Phoenix Flow Systems, San Diego, Calif.).

Table 1 Effects of taxol and 6HT on the suspension and colony culture growth of HL-60 cells. Values are the means ± SEM of three experiments (IC_{50}, 50% inhibitory concentration, IC_{90}, 90% inhibitory concentration, *CCG*, 7-day colony culture growth following 24 h exposure to taxol or 6HT, *SCG*, 3-day suspension culture growth after 24 h exposure to taxol or 6HT

		IC_{50} (n*M*)	IC_{90} (n*M*)
Taxol	CCG	3.2 ± 0.2	9.6 ± 0.6
	SCG	2.8 ± 0.5	8.8 ± 1.0
6HT	CCG	500 ± 46	4515 ± 121
	SCG	350 ± 37	2433 ± 79

Analyses of microtubular structure by antitubulin immunofluorescent labeling

These studies on untreated and taxol- or 6HT-treated HL-60 cells were performed with a monoclonal rat antitubulin antibody (YL1/2, Accurate Chemical, Westbury, N.Y.) and a rhodamine-conjugated goat anti-rat IgG (Jackson Immuno-Research, West Grove, Pa.) using a previously published immunofluorescent labeling technique [4].

Tubulin preparation and depolymerization assay

Calf brain tubulin was isolated using temperature-induced polymerization cycles in the presence of GTP [20]. The effect of taxol or 6HT on the depolymerization of microtubules was measured by following the absorbance at 350 nm using a Cary spectrophotometer (Varian Associates, Palo Alto, Calif.) connected to a circulating water bath [7]. An aliquot of tubulin in PEM buffer was treated with activated charcoal to remove traces of GTP, and this solution was used as the reference [15]. Typically, test samples contained 1 milligram of tubulin per milliliter of 1 m*M* GTP in PEM buffer. The polymerization was started by warming the cuvette holder to 37 °C. Once equilibrium had been achieved, depolymerization was induced by cooling to 0 °C. Taxol or 6HT, diluted in DMSO (final concentration 0.33% v/v) was added to the reaction mixture. This concentration of DMSO did not alter the polymerization–depolymerization of tubulin. The extent of depolymerization in the presence of taxol or 6HT was compared after 16 min of cooling, the time at which complete depolymerization was observed in the control sample.

Statistical analysis

The significance of the differences between values obtained in the population of HL-60 cells subjected to different treatments was determined by paired *t*-tests.

Results

The effect of exposures to various concentrations of taxol or 6HT ranging from 2 to 1000 n*M* for 24 h on suspension culture growth (SCG) and colony culture growth (CCG) was examined in HL-60 cells. Figure 1 demonstrates that for both SCG and CCG inhibition, the dose-response curves for 6HT were shifted at least 2 log units of concentration to the right of those for taxol. The IC_{50} and IC_{90} concentrations (50% and 90% inhibitory concentrations, respectively) of taxol for SCG and CCG were between 2 and 10 n*M*, while the IC_{50} and IC_{90} concentrations of 6HT were significantly higher, ranging from 0.35 to 5.0 µ*M* (Table 1).

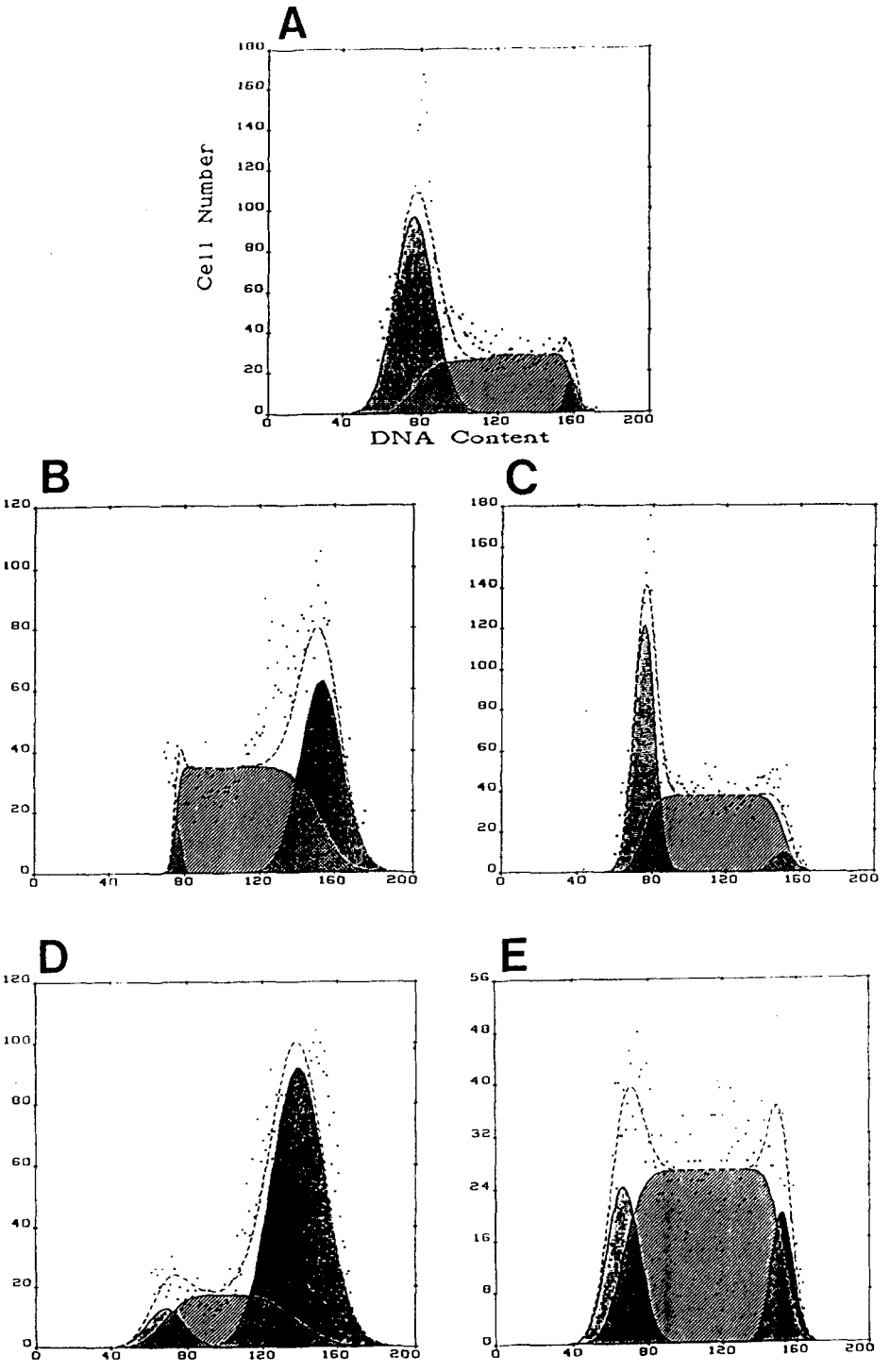


Fig. 2A–E Flowcytometric analysis of the DNA content of HL-60 cells following treatment with 50 or 500 n*M* taxol or 6HT for 24 h. **A** Untreated cells; **B** and **D**, cells treated with 50 and 500 n*M* taxol, respectively; **C** and **E**, cells treated with 50 and 500 n*M* 6HT, respectively

Therefore 6HT was substantially less cytotoxic than taxol toward HL-60 cells.

The SCG and CCG inhibitory effects of taxol and 6HT were found by flow-cytometric DNA analyses to be correlated with their ability to arrest HL-60 cells in the G_2/M phase of the cell cycle (mean of three experiments; Fig. 2). These studies demonstrated that immediately after exposure to 50 n*M* taxol for 24 h, 68.0% of HL-60 cells had accumulated in the G_2/M phase of the cell cycle (Fig. 2, panel D). In contrast, exposure to 500 n*M* 6HT caused the mitotic arrest of only 16.0% of the cells (Fig. 2, panel E). Less than 3% of untreated control HL-60 cells were in the G_2/M phase of the cell cycle. Exposure to ≈10 n*M* of either taxol or 6HT had no significant effect on the percentage of cells in G_2/M phase of the cell cycle (data not shown). These results indicate that a significant mitotic arrest did not occur immediately after exposure to ≈10 n*M* taxol or <500 n*M* 6HT, concentrations which produced approximately 50% inhibition of SCG (evaluated 72 h after the drug exposure) and the loss of clonogenic survival (evaluated 7 days after drug exposure).

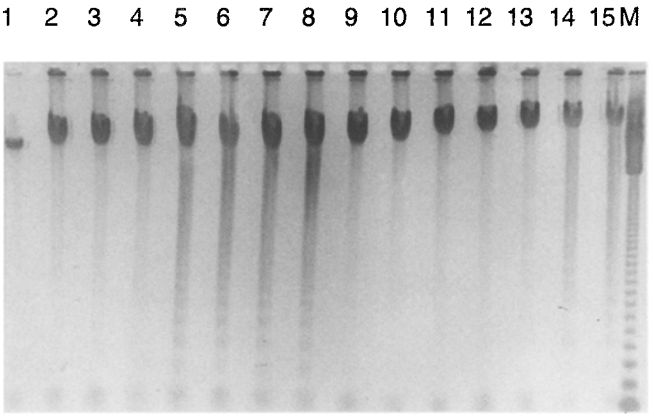


Fig. 3 Taxol- or 6HT-induced internucleosomal DNA fragmentation in HL-60 cells. Each lane of the gel was loaded with 600 ng genomic DNA purified from cells immediately following exposure to varyious concentrations of taxol (*lanes 2 to 8*) or 6HT (*lanes 9 to 15*). *Lane 1* Untreated cells, *lanes 2 and 9* 2 n*M*, *lanes 3 and 10* 5 n*M*, *lanes 4 and 11* 10 n*M*, *lanes 5 and 12* 50 n*M*, *lanes 6 and 13* 100 n*M*, *lanes 7 and 14* 500 n*M*, *lanes 8 and 15* 1000 n*M* (*M* 123 bp DNA ladder)

Internucleosomal DNA fragmentation, the biochemical hallmark of apoptosis, was examined by an improved method of agarose gel electrophoresis of genomic DNA from HL-60 cells following treatment for 24 h with taxol or 6HT at concentrations ranging from 2 to 1000 n*M*. The method was sufficiently sensitive to detect the 'ladder' pattern of internucleosomal DNA fragmentation in 400–800 ng of the electrophoresed genomic DNA sample. The method also allowed the accurate comparison of the amount of fragmented DNA in each lane of the same gel, since an identical amount of genomic DNA purified from the cells subjected to different treatments was loaded into the well of each lane. Figure 3 demonstrates that internucleosomal DNA fragmentation was observed in the HL-60 cells following the exposure to 50 and 500 n*M* taxol or 6HT, respectively. However, higher concentrations of either of the two agents did not substantially increase

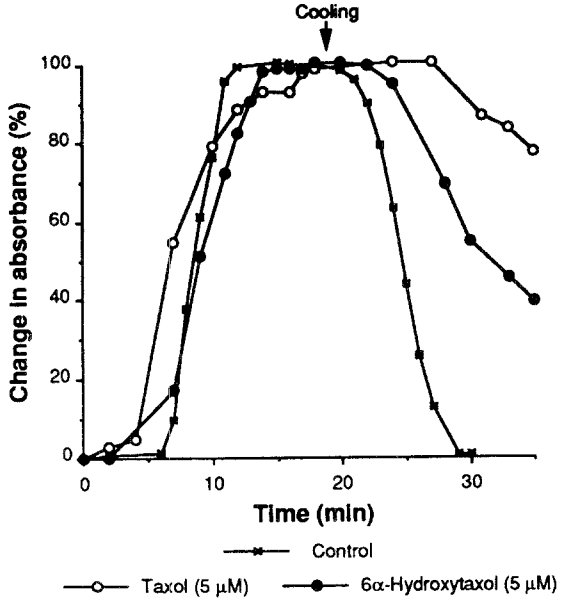
Table 2 Effect of taxol or 6HT on the percentage of HL-60 cells demonstrating morphologic features of apoptosis. Values are the means of two experiments performed in duplicate. HL-60 cells were treated for 24 h with concentrations of taxol or 6HT ranging 2 to 100 n*M*. Cells demonstrating light microscopic morphologic features of apoptosis were counted and expressed as a percentage of total the number of cells examined (see text).

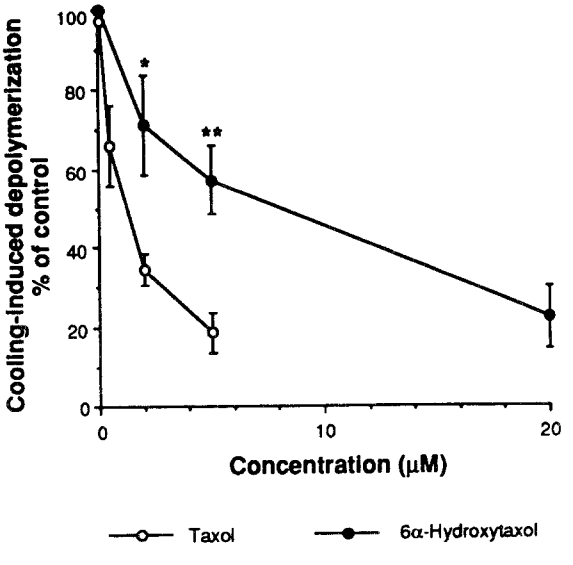
Concentration of taxol or 6HT (n*M*)	Apoptotic cells (%)	
	Taxol	6HT
0	3.3±1.1	3.2±1.0
2	4.3±1.5	3.0±1.2
5	5.0±1.0	3.5±0.5
10	7.3±1.6	3.8±1.1
50	16.0±2.5	4.0±2.0*
100	16.3±2.0	7.3±2.1*
500	21.0±1.5	11.6±1.5*
1000	23.3±1.5	12.8±1.2*

* $P < 0.01$ versus taxol-treated cells

internucleosomal DNA fragmentation. Immediately after a 24 h treatment with 50 or 500 n*M* of taxol, a mean of 16.0% and 21.0% of cells displayed the morphologic criteria of apoptosis, while approximately 10% of cells were non-viable as determined by the trypan blue exclusion method (data not shown). In contrast, identical treatment with 6HT resulted in apoptosis in 4.0% and 11.6% of cells, respectively ($P < 0.01$; Table 2). Taken together, these results also show that immediately after the exposure to IC_{50} concen-

Fig. 4 *Left panel* Temperature-induced polymerization and depolymerization of calf brain tubulin. At 0 min, polymerization was started by warming to 37 °C. Depolymerization was started at 19 min by cooling to 0 °C. The extent of depolymerization in the presence of taxol or 6HT was compared after 16 min of cooling. *Right panel* Dose-response curves for the effect of taxol and 6HT on the cooling-induced depolymerization. Each point is the mean of three determinations ± SEM. * $P < 0.05$, ** $P < 0.02$, versus the values obtained following treatment with the same concentrations of taxol





trations of taxol or 6HT, apoptosis occurred in only a minority of the HL-60 cells.

The effect of taxol and 6HT on the depolymerization of microtubules was examined using a turbidimetric method. Both taxol and 6HT were found to inhibit the temperature-induced depolymerization of microtubules in a dose-dependent manner (Fig. 4). The IC_{50} values calculated for the inhibition of depolymerization were 1.2 μM for taxol and 8.0 μM for 6HT. As compared to taxol, 6HT was significantly less potent at both the 2 μM ($P<0.05$) and the 5 μM ($P<0.02$) levels. These data indicate that inhibition of temperature-induced depolymerization of microtubules occurred at concentrations of taxol which were significantly lower than those of 6HT. Antitubulin antibody labeling and immunofluorescent microscopy [2] of HL-60 cells exposed to 50 nM taxol or 6HT also revealed microtubular bundling in taxol-treated but not in 6HT-treated cells (data not shown).

Discussion

The biotransformation of taxol to its principal metabolite 6HT is mediated by the liver microsomal cytochrome P450 enzyme system [3, 11]. This study clearly demonstrated that the IC_{50} concentrations of 6HT for SCG and CCG were approximately 100-fold greater than those for taxol. A comparison of taxol and 6HT with respect to their intracellular antimicrotubule effects, their ability to induce mitotic arrest or apoptosis and their inhibition of tubulin depolymerization also demonstrated that 6HT was significantly but variably less potent than taxol in mediating these biologic effects.

In a previous study, 6HT purified from the bile of a patient receiving taxol caused approximately 30-fold less growth inhibition of human leukemia MOLT-4 and U-937 cells than taxol [8]. However, in that study the cytotoxic effects were compared utilizing a schedule which involved a 72-h exposure to 6HT or taxol. In contrast, in the present study using HL-60 cells, following 24-h exposures to taxol or 6HT, we evaluated both the growth inhibition (after 3 days) and the loss of clonogenic survival (after 7 days). This difference in the design of the two studies may account for the observed disparity between the cytotoxic potential of 6HT relative to taxol. In human leukemia cells the cytotoxic effect of taxol has been correlated with the sensitivity to taxol-induced intracellular microtubule bundle formation [15, 17]. However, in the present study, the IC_{50} concentrations of taxol or 6HT for the SCG and CCG appeared to be lower than those that caused intracellular microtubular bundling (data not shown), accumulation of the cells in the G_2/M phase of the cell cycle or apoptosis in HL-60 cells. It should be noted that in the present studies these biologic effects were evaluated immediately after a 24-h exposure to taxol or 6HT, while growth inhibition for the determination of IC_{50} concentrations was evaluated 3 days (suspension culture) or 7 days (colony culture) after exposure to taxol.

A recent study has indicated that at low concentrations (approximately 10 nM), which are growth inhibitory, taxol causes inhibition of the mitotic index without enhancing microtubule polymerization [9]. This is achieved by suppressing dynamic instability at the ends of microtubules. Dynamic instability of microtubules, which takes the form of transitions between phases of growing and phases of shortening at microtubule ends, is a characteristic of mitotic spindle microtubules [9]. Other studies have also suggested a possible correlation between taxol-induced microtubule flexibility and cytotoxicity [5]. Antimicrotubule effects by any of these mechanisms may be responsible for taxol-induced mitotic arrest, which appears to be a prerequisite for taxol-induced apoptosis [17, 22]. However, there is evidence to suggest that mitotic block induced with low concentrations of taxol is not sustained, and a large percentage of cells escape and become abnormal multinucleate cells. These cells may die without displaying the characteristic biochemical and morphologic features of apoptosis [22].

IC_{50} concentrations of taxol were significantly lower than those of 6HT for the inhibition of cold-induced depolymerization of microtubules in a cell-free system, as determined by the in vitro turbidimetric assay. The IC_{50} concentrations of 6HT were in the micromolar range and appeared to be approximately 1000-fold higher than the growth-inhibitory concentrations of taxol. However, in HeLa cells exposed to 10 nM taxol, the drug has been shown to be concentrated 480-fold to an intracellular level of 4.8 μM [9]. Therefore, the IC_{50} concentrations of taxol for the inhibition of cold-induced depolymerization of microtubules in the cell-free system is clearly in the range of the taxol concentrations that can be concentrated intracellularly.

In conclusion, for each of the intracellular biologic effects studied, the IC_{50} concentrations of 6HT were significantly higher than those of taxol. Therefore, it appears that metabolism by cytochrome P450 enzymes detoxifies taxol to a less active metabolite. Since, in patients receiving a 3-h infusion of taxol the plasma AUC of 6HT is only about 10% of the AUC of taxol, these results also indicate that plasma and intracellular levels of 6HT that would contribute toward the cytotoxicity of taxol are unlikely to be achieved clinically.

Acknowledgments This work was supported by NIH grant CA63382-01 (K.B.) and by the Hollings Cancer Center, Charleston, S.C. K.B. is the recipient of a Leukemia Scholar Award of the Leukemia Society of America. The technical support of U. Kristina Walle is greatly appreciated.

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