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TAXOL, A POTENT PROMOTER OF MICROTUBULE ASSEMBLY, INHIBITS SECRETION OF PLASMA PROTEINS IN CULTURED RAT HEPATOCYTES

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SUMMARY: Effects of taxol, a potent promoter of microtubule polymerization, on protein secretion were studied in primary cultured rat hepatocytes. Treatment of cells with 5×10^{-5} M taxol caused 70% inhibition of secretion as compared with that of the control cells. However, analyses of newly synthesized albumin and α_1-protease inhibitor demonstrated that the intracellular processing of these proteins was not affected by the drug.

INTRODUCTION

The intracellular pathway of secretory proteins after their synthesis on the membrane-bound polysomes has now been well established as typically shown for albumin in hepatocytes (1-3). It is also known that most secretory proteins are modified during their intracellular transport by glycosylation, selective proteolysis, sulfation and/or phosphorylation (4). However, there is still a paucity of direct evidence on how the secretory proteins are moved from one cellular compartment to another, and on how and where the post-translational modifications occur. The use of drugs, which inhibit the secretory process at distinct sites in the cell, may prove valuable for more detailed studies of specific steps in secretion and may lead eventually to an understanding of the molecular basis of the mechanism involved in intracellular transport. Since Lacy et al.(5) proposed that microtubules were involved in cell secretion, a substantial number of reports have corroborated the inhibition of secretion by colchicine and other agents which depolymerize microtubules (6-8).

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Abbreviations: MEM, minimum essential medium; PBS, phosphate buffered saline; IgM, immunoglobulin M; SDS, sodium dodecyl sulfate; DMSO, dimethyl sulfoxide; endoglycosidase H, endo-β-N-glucosaminidase H.

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Taxol, an alkaloid isolated from Taxus brevifolia which has antitumor and antimitotic activities (9), has recently been reported to affect microtubules by a somewhat different mechanism. In contrast to compounds such as colchicine and vinblastin, taxol acts as a promoter of microtubule assembly and renders microtubules resistant to depolymerization by cold (4°C), Ca^{2+} and other depolymerizing agents in vitro (10,11) and in cultured cells (12,13).

We report here that taxol inhibits protein secretion in cultured rat hepatocytes but does not affect the intracellular processing of albumin (conversion of proalbumin) and α_1-protease inhibitor (glycosylation).

MATERIALS AND METHODS

Materials. Taxol was obtained from the National Products Branch, Division of Cancer Treatment, National Cancer Institute. The drug was dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C. L-[^{35}S]Methionine (1005.2 Ci/mmol), L-[4,5-^{3}H]leucine (53.4 Ci/mmol), Omnifluor, Protosol and Enhance were purchased from New England Nuclear (Boston, MA). Insulin and dexamethasone were from Sigma Chemicals (St. Louis, MO), and endo-β-N-acetylglucosaminidase H was from Seikagaku Kogyo (Tokyo, Japan). Newborn calf serum was from Grand Island Biological Co. (Grand Island, NY) and Eagle's minimum essential medium (MEM) from Nissui Seiyaku Co. (Tokyo).

Preparation of Antisera. Antibodies against rat serum albumin and α_1-protease inhibitor were raised in rabbits as described previously (14-16).

Hepatocyte Culture. Hepatocytes were isolated from adult Wistar rats, weighing 200-250 g, according to the method of Seglen (17). 2 x 10^6 cells were cultured in a 60-mm Falcon dish containing 4 ml of Eagle's MEM (18), supplemented with 5% newborn calf serum, 0.1 μM insulin, 1 μM dexamethasone and 60 μg/ml kanamycin (19). The medium was changed after the first 24 h and then once every two days. All the following experiments were carried out using the cells after 24 h-culture.

Pulse-Chase Experiments. Cells were preincubated with or without taxol at the indicated concentrations at 37°C for 2 h. After two washings with Dulbecco's phosphate buffered saline (PBS) (18), cells were pulse-labeled for 10 min either with [^{35}S]methionine (100 μCi/1.5 ml) or [^{3}H]leucine (20 μCi/1.5 ml) in the medium lacking cold methionine or leucine, respectively. After two washings with Dulbecco's PBS, the cells were cultured in 2 ml of Eagle's MEM. At the chase periods indicated, the cells and medium were taken for determination of trichloroacetic acid-insoluble radioactivity and used for immunoprecipitation of albumin and α_1-protease inhibitor. Taxol was also present in the medium during all the pulse-chase periods.

Immunoprecipitation. After removal of the chase medium, the cells in monolayer culture were washed twice with PBS and lysed in 0.5 ml of distilled water containing 1% sodium deoxycholate and 1% Triton X-100. After centrifugation at 15,000 x g for 10 min, the resulting supernatants were used as cell lysates. Albumin and α_1-protease inhibitor in the cell lysates and chase medium were immunoprecipitated with respective monospecific antibodies as described previously (19). The immunoprecipitates were washed and dissolved in 1% sodium dodecyl sulfate (SDS)-5 mM EDTA-1% β-mercaptoethanol-

Table 1 Dose effects of taxol on protein synthesis in cultured rat hepatocytes

Treatment		Incorporation of [^{3}H]leucine	
		dpm x 10^{-3}/dish	% of Control
Control (0.5% DMSO)		1,535	100
Taxol in 0.5% DMSO	1 x 10^{-7} M	1,494	97.3
	1 x 10^{-6}	1,465	95.4
	1 x 10^{-5}	1,281	83.5
	5 x 10^{-5}	1,195	77.9
	1 x 10^{-4}	1,092	71.1

2 x 10^6 cells/dish were preincubated for 2 h at 37°C in the absence or presence of taxol at the indicated concentrations. Cells were then exposed to 20 μCi of [^{3}H]leucine in the same medium for 10 min and harvested for determination of trichloroacetic acid-insoluble radioactivity.

62.5 mM Tris-HCl (pH 6.8), boiled for 3 min and stored at -20°C until use (19).

Endoglycosidase Treatment. Frozen samples prepared as above were thawed, boiled again for 3 min and diluted to 10-fold with 0.3 M citrate buffer (pH 5.5). Treatment with endo-β-N-acetylglucosaminidase H (final concentration, 0.2 unit/ml) was carried out for 16 h at 37°C. Proteins were concentrated by precipitation in 25% trichloroacetic acid and prepared for gel electrophoresis (20).

Polyacrylamide Gel Electrophoresis. Slab-gel electrofocusing was carried out according to Ames and Nikaido (21) as described previously (19). SDS-gel electrophoresis was performed on 10% polyacrylamide slab gels (22). After electrophoresis, gels were fixed with 30% methanol-10% trichloroacetic acid-7% acetic acid for 1 h and treated with "Enhance", followed by drying and fluorography (23).

RESULTS AND DISCUSSION

In contrast to other antimitotic drugs such as colchicine, podophyllotoxin, and vinblastin, which inhibit microtubule assembly, taxol promotes tubulin polymerization and stabilizes the assembled microtubules (10-13). These microtubules in the taxol-treated cells, however, do not function in cell replication (12,13) and in cell migration (12). It is of interest to know whether taxol exerts its effect on protein secretion in which microtubules are believed to be involved.

Taxol at concentrations less than 10^{-6} M did not significantly inhibit protein synthesis in cultured rat hepatocytes (Table 1). Some inhibition (16-22%) of synthesis was caused by higher doses of the drug (1-5 x 10^{-5}M).

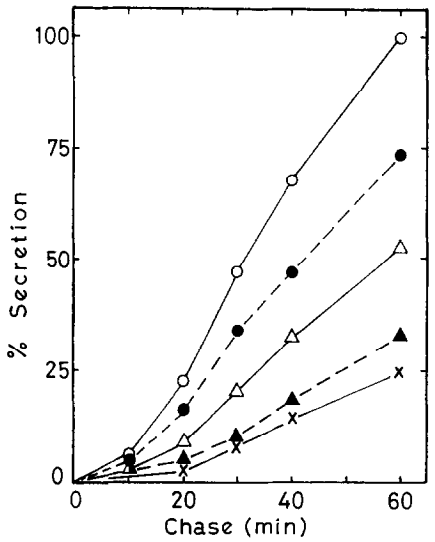

Fig. 1. Dose effect of taxol on secretion in cultured rat hepatocytes. 2 x 10^6 cells/dish were treated with taxol, pulse-labeled with 20 μCi of [^{3}H]leucine for 10 min and chased as described in "Materials and Methods". Aliquots of medium were taken at the indicated times of chase and determined for trichloroacetic acid-insoluble radioactivity. % Secretion was calculated by taking the radioactivity of the control cell medium at 60 min as 100%. Control cells (○); taxol-treated cells, 1 x 10^{-6} M (●), 1 x 10^{-5} M (△), 5 x 10^{-5} M (▲), and 1 x 10^{-4} M (X).

Under the conditions of the experiments in Table 1, the dose effect of taxol on secretion was determined (Fig. 1). The drug at 5 x 10^{-5} M caused about 70% inhibition of secretion at 60 min of chase, as compared with that of the control cells. The inhibitory effect of taxol on secretion was similar to that of colchicine. The secretion of plasma protein by rat liver was inhibited by about 70% in the presence of 5 x 10^{-5} M colchicine (8), which was also confirmed by us using the cultured rat hepatocytes (data not shown). Thus, these results provide strong evidence for involvement of microtubules in the secretory process, in addition to the previous results obtained with depolymerizing agents (5-8). The fact that the same inhibitory effect was caused by drugs with different modes of action, colchicine and taxol, suggests that a dynamic process of polymerization and depolymerization may be required for microtubules to function in the secretory process, as suggested for cell migration (12).

In the previous report (19) we described the effects of the monovalent ionophore monensin on albumin secretion in the same culture system. The

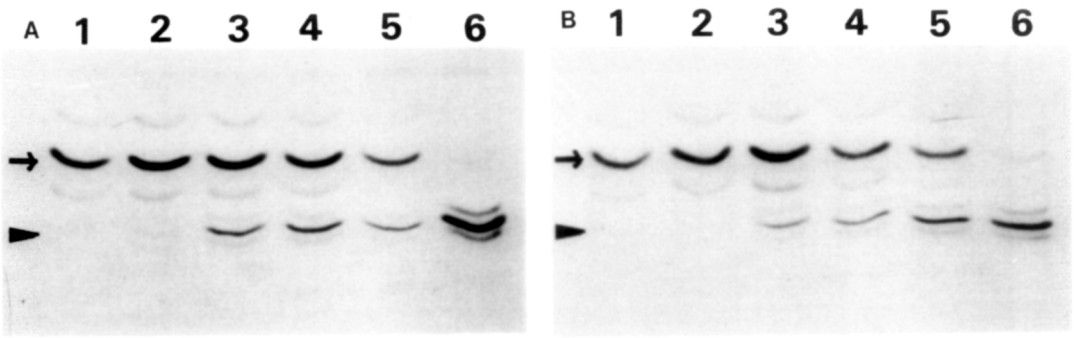

Fig. 2. Effect of taxol on the conversion of proalbumin to serum albumin. Cells were treated with taxol (5×10^{-5} M), pulse-labeled with [^{35}S]methionine (200 μCi/dish) and chased. Cellular and medium albumins prepared by immunoprecipitation were subjected to gel electrofocusing (pH 5-7), followed by fluorography (19). (A) Control cells; (B) taxol-treated cells; lanes 1-5, cellular albumin taken at 0, 10, 20, 30, and 60 min of chase, respectively; lane 6, medium albumin taken at 60 min of chase. Arrows and arrow-heads indicate the major forms of proalbumin and serum-type albumin, respectively.

inhibition of albumin secretion by monensin was accompanied by an intracellular accumulation of proalbumin, suggesting that the drug arrests the intracellular transport of proalbumin before the site where its conversion to serum albumin takes place. It has also been reported that monensin blocks the intracellular transport of IgM and transferrin, serum glycoproteins, at a stage where the glycoproteins are still sensitive to endoglycosidase H (24,25). Taken together with cytochemical observation (24), these results are regarded as evidence suggesting that monensin blocks the transport of these secretory proteins at the proximal (cis) region of the Golgi complex (19,24,25).

For comparison with these results, we examined the effect of taxol on the intracellular processing of proalbumin (selective proteolysis), and of α_1-protease inhibitor (glycosylation) which contains 13% carbohydrate in N-glycosidic linkage (16). The taxol-treated hepatocytes were pulse-labeled with [^{35}S]methionine and chased. The secretion of albumin and α_1-protease inhibitor was inhibited by taxol to the same extent as observed for overall protein secretion (data not shown, see Fig. 1). In contrast to the effect by monensin (19), no significant accumulation of proalbumin was observed in the taxol-treated cells compared with the control cells (Fig. 2). Furthermore, the intracellular processing of α_1-protease

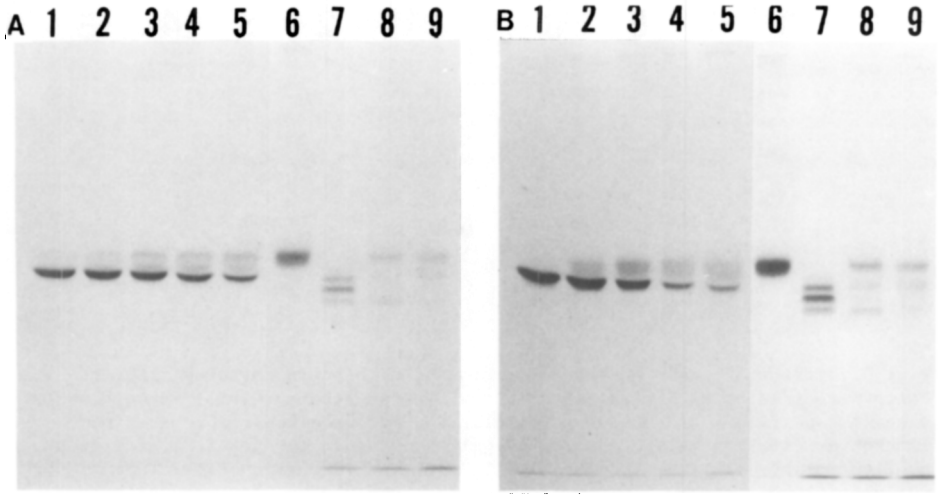

Fig. 3. Effect of taxol on the glycosylation of α_1-protease inhibitor. Cells were treated with taxol, labeled with [^{35}S]methionine and chased as in Fig. 2. Cellular and medium α_1-protease inhibitors prepared by immunoprecipitation were subjected to SDS-gel electrophoresis followed by fluorography. (A) Control cells; (B) taxol-treated cells; lanes 1-5, cellular α_1-protease inhibitor taken at 0, 10, 20, 30, and 60 min of chase, respectively; lane 6, medium α_1-protease inhibitor taken at 60 min; lanes 7-9, endoglycosidase-treated α_1-protease inhibitor which were taken at 0, 30, and 60 min, respectively.

inhibitor was not significantly different in the taxol-treated and the control cells (Fig. 3). In both treated and non-treated cells, the initially labeled form of α_1-protease inhibitor (lane 1 in Fig. 3A & B) decreased in intensity with chase time, while higher molecular form(s) appeared first at 10 min of chase and increased thereafter (lanes 2-5). The latter form in both cells was found to be resistant to endoglycosidase H (lanes 8-9 in A & B) and to correspond to the form secreted into the medium (lane 6). These results demonstrate that taxol did not affect the aquisition of endoglycosidase H resistance by α_1-protease inhibitor. A preliminary experiment revealed that monensin, in contrast, blocks the intracellular transport of the inhibitor in a stage where the glycoprotein is still sensitive to endoglycosidase H, as demonstrated for IgM (24) and transferrin (25).

Based on these results (Figs. 2 and 3), it is concluded that taxol inhibits the transport of proalbumin and α_1-protease inhibitor after the site where these molecules are modified by selective proteolysis or by

addition of terminal sugars. Thus, microtubules appear to be intimately involved in the secretory process beyond the trans region of the Golgi complex including the secretory vesicles, where these modifications are suggested to occur (4,19,24).

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