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Taxol Inhibits Opioid Binding on T47D Human Breast Cancer Cells

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In the T47D human breast cancer cell line, Taxol was found to compete for ethylketocyclazocine opioid binding (IC_{50} 3.3 pM). In contrast, no interaction of the drug with [^{3}H]diprenorphine binding occurred. Binding was multiphasic, in the absence of colchicine (10^{-6} M), but monophasic in its presence, indicating an involvement of the cytoskeleton in this process. Alignment of Taxol binding domains on alpha and beta tubulin with the kappa opioid site revealed homology of these sites with the first extracellular loop of the receptor. These results indicate a possible new action of Taxol, indicating for the first time a membrane action of the agent.

Taxol, an anticancer drug of natural-product origin from *Taxus Brevifolia* bark (1), enhances microtubule polymerization (2), stabilizes microtubules (3) and tubulin (4, 5), inhibits cell replication by preventing spindle dynamics (6), and has major antitumor properties (7). It acts by the stabilization or strengthening of lateral interactions between tubulin protofilaments (5, 8-10). In eucaryotic cells, Taxol prooved to be a potent inhibitor of cell replication, blocking cells in the G_2-M phase, and reorganizes the cellular tubulin microsceleton (6). On the other hand, opioid agonists inhibit cellular proliferation of breast cancer (11), prostate (Kampa M., et al, submitted) and renal cells (12). Furthermore, these agents inhibit cell proliferation by blocking the cells in the G_2-M phase (Panagiotou S. et al, in preparation), probably by an interaction with cytosceletal elements (Papakonstanti et al, submitted). In the present work, we provide evidences, about an interaction of Taxol with kappa opioid binding sites on T47D human breast cancer cell membranes, indicative for a membrane action of this agent.

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MATERIALS AND METHODS

Cells. T47D human breast cancer cells (originally isolated from a pleural effusion of breast adenocarcinoma), were obtained at passage 86, and grown in RPMI medium, supplemented with 10% heat inactivated fetal calf serum. They were cultured at 37°C, in a humidified atmosphere of 5% CO_2 in air.

Binding experiments. Ligand binding assays in whole T47D cells were performed as described previously (11), at a density of 10^6 cells/well. Before binding, cells were washed twice with 2 ml of phosphate buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4). Binding was performed in the same buffer, in a total volume of 0.5 ml, containing [^{3}H]ethylketocyclazocine (S.A. 18 Ci/mmol, New England Nuclear Co.) or [^{3}H]diprenorphine (S.A. 29 Ci/mmol, Amersham, UK), without (total binding) or with (non specific binding) a thousand fold molar excess of the same unlabelled agent, and the various concentrations of Taxol, ranging from 10^{-12} to 10^{-6} M. As indicated, colchicine (10^{-6} M) was also introduced in some binding assays, simultaneously with Taxol. The cells were incubated for 2 h at room temperature (18-22°C). At the end of the incubation period, the unbound radioactivity was eliminated by washing the cells twice, with 2 ml cold buffer. Cells were removed from plates with 0.4 ml 2N NaOH, and mixed with 4 ml scintillation cocktail (SigmaFluor, Sigma, St Louis, MI). The bound radioactivity was counted in a scintillation counter (Tricarb, Series 4000, Packard), with a 60% efficiency for Tritium. Binding was repeated at least three times (in duplicate). The results were analysed by the Origin (MicroCal Co.) V 4.1 package.

Alignments. Based on the sequence proposed by Mansson et al. (13) and Simonin et al. (14), for the kappa opioid receptor, we have aligned the four extracellular loops of the kappa opioid binding site, and compared their sequence with the putative Taxol binding sites on the alpha and beta tubulin, (8), using the PIMA program, developed by Smith and Smith (15).

Materials. Ethylketocyclazocine was a gift from Sterling-Winthrop. Diprenorphine was from Reckit and Coleman Co. All other chemicals were either from Merck (Darmstad, Germany) or from Sigma (St Louis MI).

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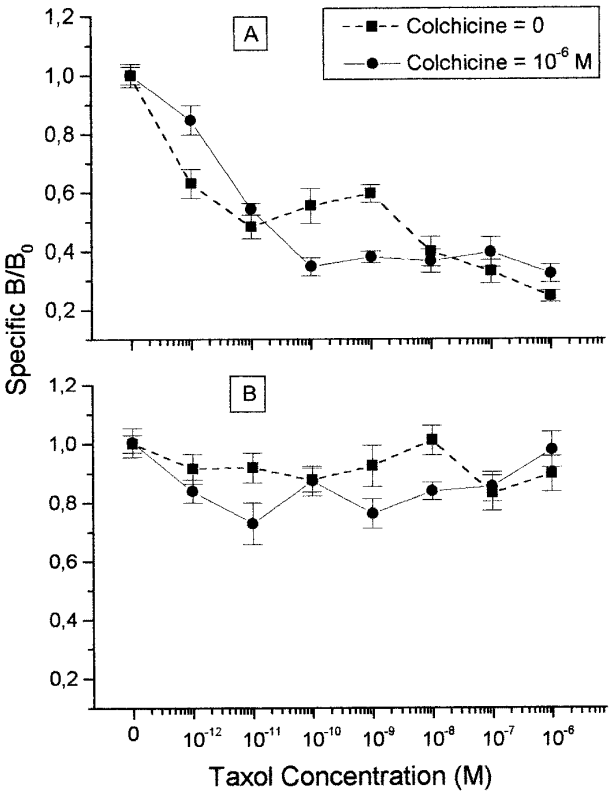

FIG. 1. Displacement of [^{3}H] ethylketocyclazocine (A) and [^{3}H] diprenorphine (B) by Taxol. Results are expressed as a fraction of the specific binding, in either case, in the absence (dotted line, squares) or the presence of 10^{-6} M colchicine (solid line, circles).

RESULTS

T47D human breast cancer cells were incubated with radiolabelled opioid agonists ([^{3}H]ethylketocyclazocine, detecting δ, μ, κ_1, and κ_2 opioid sites) and antagonists ([^{3}H]diprenorphine, detecting δ, μ, κ_2, and κ_3 opioid binding sites). As shown previously (11), specific opioid binding sites are present on membranes of the cells, which, after analysis were found to belong mainly to the kappa$_1$ subtype of the kappa site (~190000 sites/cell) and accessory tot the kappa$_2$ (~19000 sites/cell), and the kappa$_3$ site (~10000 sites/cell). We have further shown that opioid binding could be detected equally well on whole cells or cell membranes (11). Taxol, at concentrations varying from 10^{-12} to 10^{-6} M, was able to compete for [^{3}H]ethylketocyclazocine opioid binding (Figure 1A). On the contrary, no interaction of Taxol was found when diprenorphine was used as a tracer (Figure 1B). These results indicate, in view of the relative selectivities of the tracers used, that Taxol interacts selectively with the kappa$_1$ subtype of the opioid receptor. Ethylketocyclazocine displacement by Taxol was multiphasic with IC$_{50}$s of 0.5×10^{-12} M and 3×10^{-8} M. When colchicine (10^{-6} M) was introduced in the medium, Taxol binds to opioid receptors in a monotonous manner, with IC$_{50}$ 3×10^{-12} M.

Figure 2 shows the putative alignment of Taxol binding domains of alpha and beta-tubulin with the extracellular part of the kappa opioid binding site. As shown, alpha Tubulin (382-402) and (418-436) and beta tubulin (371-391) and (407-426) which are proposed to be the Taxol-tubulin interacting sites (8), are best aligned with the first extracellular loop pf the opioid receptor, implied in the binding of opioids (16), although only an 29% and 14% homology was found with the first and the second pair of alpha-beta tubulin sites. These calculations provide a possible molecular explanation of the results of Taxol-opioids competition.

DISCUSSION

Opioids, are potent inhibitors of different tumor cells, including lung (17), brain (18, 19), and breast tumors (20-22). In this later case, we have shown that this effect is mediated through opioid (mainly kappa) (11) and somatostatin receptors (23). Preliminary, not yet published results, indicate that opioids inhibit cell cycle at the G$_2$-M phase, and that they rearrange the cytosceleton (Panagiotou S., et al. in preparation). Taxol, on the other hand, stabilizes cytosceleton, by binding to tubulin alpha 382-402 and beta 371-391 and to the couple tubulin alpha 418-436 and beta 407-426 (8). In addition of the molecular action on microtubule stabilization, Taxol was found to induce tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta and -8 (24-26) production or expression. Furthermore, through microtubule stasbilization, it inactivates Ca^{++} channels (27, 28). Taxol induced phosphorylation of bcl-2

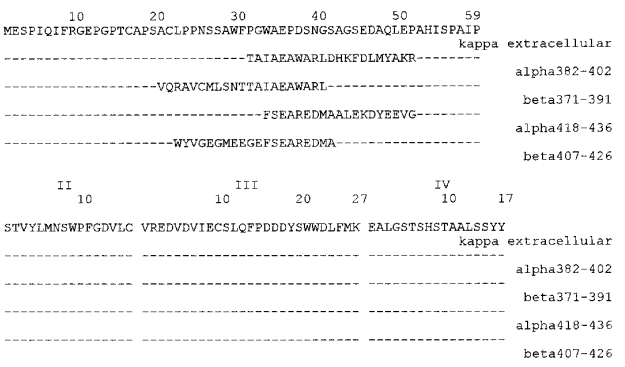

FIG. 2. Alignment of the extracellular domains of the kappa opioid receptor, and the binding sites of Taxol to the tubuline molecules. Using the PIMA program (15), we have aligned the sequences of the alpha tubulin 382-402 and 418-436, and the beta tubulin 371-391 and 407-426 sequences which are found to be the binding domains of Taxol (8) with the proposed sequence of the extracellular domains of the kappa opioid binding site (13, 14). The figure presents these multiple alignments. Extracellular domains of the kappa opioid receptor are designed by roman numbers.

(29) and apoptosis, perhaps through protein tyrosine phosphorylations (30). The drug, at nanomolar concentrations, completely inhibits, when applied chronically, the growth of T47D cells (IC_{50} 20 nM) (31).

As shown in the present work, Taxol interacts with opioid sites in the T47D cell line, at very low concentrations, being able to inhibit selectively ethylketocyclazocine binding. On the contrary, no interaction with diprenorphine was observed. Previous results, on peripheral (32-34) and central nervous tissues (32, 35), as well as on the T47D cell line (11) have shown that a major competitor of the $kappa_1$ and $kappa_2$ sites is ethylketocyclazocine, while diprenorphine (a general opioid antagonist, used as a tracer after the withdrawn of etorphin), interacts mainly, at nanomolar concentrations with $kappa_2$ and $kappa_3$ opioid sites. This result, indicates a selective interaction of Taxol with $kappa_1$ opioid binding sites. This subtype, shares the selectivity of the kappa receptor, with dynorphins being the endogenous ligands (34). The multiphasic displacement of ethylketocyclazocine by Taxol was replaced by a monophasic curve, in the presence of colchicine, indicating a possible involvement of microtubule rearrangement and stabilization on the opioid binding, in the presence of Taxol. Indeed, results from our group showed that opioids modify the cytosceleton, with a not yet identified mechanism (Papakonstanti E. et al, submitted). Comparison of Taxol and benzomorphan opioid alkaloids, did not reveal an extensive similarity in chemical structures, with the exception of the existence of a para-hydroxyl phenethylamine moiety, and the presence (although in a different group) of a nitrogen. In the present state of knowledge we cannot therefore propose a structure-activity explanation of the Taxol opioid interaction.

Opioid receptors have recently been sequenced (see Reisine and Bell (16) for a review). Based on the sequence proposed by Mansson et al. (13) and Simonin et al. (14), we have isolated the four putative extracellular loops of the kappa opioid binding site. We have compared the sequence of the extracellular part of the opioid receptor, with the pairs of alpha- and beta-tubulin sequence, recently proposed as Taxol binding couples (alpha 382-402/ beta 371-391, and alpha 418-436/ beta 407-426) (4). Alignment of the five sequences revealed a certain homology of the putative Taxol binding sites with the first extracellular loop pf the opioid receptor (Figure 2), implied in the binding of opioids (16). These calculations provide a possible molecular explanation of the results of Taxol-opioids competition.

In conclusion, our results show a specific interaction of Taxol with the $kappa_1$ opioid binding site, in the T47D human breast cancer cell line, and indicate a possible new mode of action of this agent. Taking into account the antiproliferative action of opioid drugs, this interaction may indicate a new physiological mode of action of this very potent anticancer drug.

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