Anti-Cancer Drugs 1996, **7** (suppl 2):33–36

The scientific rationale for developing taxoids

M Aapro
European Institute of Oncology, I-20141 Milan, Italy.

The taxoid drugs, docetaxel (Taxotere®) and paclitaxel (Taxol®), represent a new class of antitumour agents which act by promoting the assembly and inhibiting the disassembly of microtubules. Docetaxel has been shown to be more potent than paclitaxel with regard to the formation and stabilization of microtubules *in vitro*. Docetaxel also has a higher cell uptake than paclitaxel and a longer intracellular retention time. Docetaxel is a more potent antitumour agent than paclitaxel in most model systems. The observation that the cytotoxic concentration for docetaxel is lower than that for paclitaxel in cultures of human haematopoietic cells supports the clinical observation that dose-limiting neutropenia is seen at a lower dose of docetaxel than paclitaxel. The concentration of docetaxel required to kill tumour cells *in vitro* is well within the plasma concentrations recorded in clinical studies, and docetaxel has shown extensive clinical activity against a variety of solid tumours. Most drugs are used in combination regimens in the clinic and combinations of docetaxel with other agents are under active investigation. The agents to be combined with docetaxel include those which showed synergism with docetaxel *in vitro* and can be delivered at optimal doses without additive toxicity.

Keywords: Docetaxel (Taxotere®), preclinical studies, microtubules, haematopoietic cells, plasma concentration.

Introduction

New classes of antitumour agents are not often introduced into preclinical and clinical research. Many new drugs represent improvements over previously available analogues, but do not offer new modes of action. The taxoids, docetaxel (Taxotere®) and paclitaxel (Taxol®), are a rare example of an entirely new class of anticancer agents which act in a unique way, by promoting the assembly and stability of microtubules.

New compounds are extensively tested in preclinical systems before the medical oncologists decide whether they offer sufficient advantage over existing therapies to be tested clinically. Few of these novel compounds are developed beyond limited phase I trials, often because preclinical promise does not translate into clinical advantage. The taxoids have a novel mechanism of action, and as an example the preclinical activity of docetaxel will be briefly reviewed here.

Correspondence to M Aapro

Mode of action of taxoids

Microtubules are essential to normal cell function and are an established target for cytotoxic drugs. The vinca alkaloids prevent the formation of microtubules by inhibiting the polymerization of tubulin. Cells affected by vinca alkaloids cannot divide because microtubules cannot be assembled to form the mitotic spindle. Taxoids act in the broadly opposite direction to the vinca alkaloids. Taxoids enhance the polymerization of tubulin and inhibit the breakdown of microtubules. The disassembly of microtubules is as much an essential aspect of cell function as their assembly. Cells affected by taxoids cannot divide because the dynamics of mitotic spindle formation have been disrupted.

The effect of taxoids on tubulin polymerization can be studied by measuring the optical density of a solution containing tubulin. As microtubules form, the solution becomes turbid and the optical density increases. Given the appropriate constituents in solution, microtubules form spontaneously when the temperature of the solution is raised from 3 to 37°C. Adding a taxoid to this solution will greatly increase the rate of formation of microtubules. The effect of docetaxel on microtubule formation in solution is illustrated in Figure 1 [1]. Taxoids also inhibit the breakdown of microtubules, which occurs as a part of normal cell dynamics and can be initiated in solution by decreasing the temperature to 3°C. Figure 1 illustrates that microtubules formed in the presence of docetaxel depolymerize very little on exposure to cold. In the physiological environment this means that microtubules form easily, at very low concentrations of tubulin, and become long-lived structures in the presence of taxoids instead of the occurrence of the normal dynamic equilibrium between microtubule formation and destruction.

Although both paclitaxel and docetaxel act in this way, there are differences in the potency of these closely related compounds with regard to microtubule

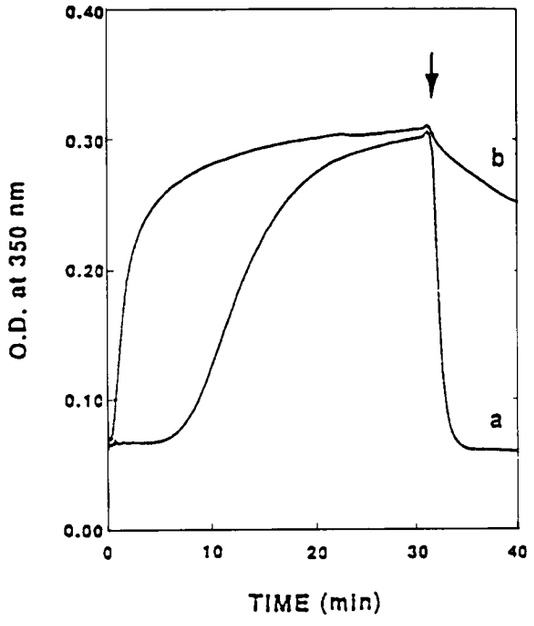

Figure 1. Effect of docetaxel on polymerization of tubulin and depolymerization of microtubules. Tubulin was polymerized by heating from 3 to 37°C. Depolymerization of microtubules was obtained by cooling from 37 to 3°C (arrow). Polymerization or depolymerization was monitored by following the turbidimetry at 350 nm. Line a, 10 μmol/L porcine brain tubulin; line b, 10 μmol/L tubulin and 3 μmol/L docetaxel. With permission from [1].

dynamics. Docetaxel is about twice as potent as paclitaxel as an inhibitor of cold-induced microtubule disassembly [2] and about twice as potent as paclitaxel in reducing the concentration of tubulin which is necessary before microtubule formation can take place [3]. There are also differences in the structure of the microtubules which are formed in the presence of either docetaxel or paclitaxel [4,5].

The two taxoid drugs bind to the same site on tubulin, but the affinity for the binding site for docetaxel is twice that of paclitaxel [3]. Docetaxel also accumulates in cells to a greater extent than paclitaxel and its efflux from cells is slower than that for paclitaxel. Figure 2 illustrates experiments in which [^{3}H]-paclitaxel or [^{3}H]-docetaxel were incubated with P388 cells and the influx and efflux of radioactivity were studied [1,6]. When the extracellular concentration of the drug was the same (0.1 μmol/L), the maximum intracellular concentration of docetaxel achieved was about three times as great as the maximum intracellular concentration of paclitaxel. When the efflux of radioactivity from cells was studied, the time taken for the intracellular concentration of docetaxel to halve was more than three times as long as the time taken for the intracellular concentration of paclitaxel to halve.

Drugs tested in *in vitro* systems may be classed as antiproliferative, cytostatic or cytotoxic, depending on the *in vitro* system used. Paclitaxel and docetaxel have been compared against panels of tumour-derived human and murine cell lines and against cell clones derived from human tumour biopsies. Both taxoid drugs have been shown to be antiproliferative, cytostatic and cytotoxic against cell lines derived from breast, colon, ovarian and bladder cancers, and neuroblastoma and sarcoma [7–9]. Docetaxel has been found to be two to four times as potent as paclitaxel

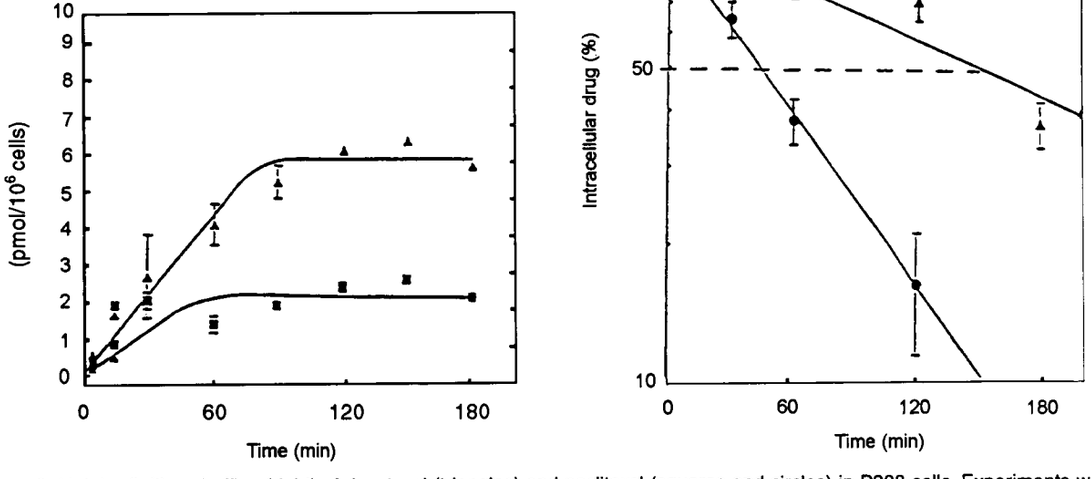

Figure 2. Uptake (left) and efflux (right) of docetaxel (triangles) and paclitaxel (squares and circles) in P388 cells. Experiments were done according to the methods of Riou *et al.* [6]. From [1] with permission.

in these systems. Docetaxel and paclitaxel have also been shown to be cytotoxic against cell lines derived from ovarian cancers with intrinsic or acquired resistance to cisplatin [8]. Docetaxel was approximately twice as potent as paclitaxel against these cisplatin-resistant ovarian cell lines. When the taxoids were compared in cell cloning assays using tissue from biopsies, with drug concentrations similar to those found in plasma in patients in clinical trials [9–11], the division of cells from breast, lung, ovarian and colon tumours and sarcomas was inhibited by the taxoids. Docetaxel was found to be more potent than paclitaxel in 29 tumours, and paclitaxel was more potent than docetaxel in 13 tumours [10].

When docetaxel and paclitaxel were tested against human haematopoietic stem cells from bone marrow samples, docetaxel was found to be 3.7 times more cytotoxic than paclitaxel, at concentrations similar to those attained in plasma in clinical trials [9]. This *in vitro* finding corresponds well with clinical observations that dose-limiting neutropenia is attained at a lower dosage of docetaxel than paclitaxel.

Preclinical testing

The taxoids have been tested in animal models *in vivo* to assess their systemic antitumour activity. Systemic activity is very important for successful clinical development of a drug, as is activity against advanced, as well as early, tumours. The *in vivo* activity of drugs can be assessed against murine tumours implanted into otherwise healthy mice and against human tumours by using xenografts implanted into the nude mouse. The nude mouse is a mutant with no T-cell-based immune responses, which, because of this mutation, does not reject foreign tissue. The *in vivo* activity of docetaxel has been found to be impressive, with strong activity against early and advanced tumours, and complete regression of the tumour seen in several instances.

Docetaxel has a broad spectrum of activity against murine tumours with an activity rating of greater than $\log_{10}$ cell kill 2.0 against melanoma (B16), colon carcinomas (C38, C51), pancreatic ductal carcinoma (PO3), and breast carcinomas (MA16/C, MA13/C), with some complete cures against colon, pancreatic and breast tumours [12,13]. Antitumour activity has also been recorded against early and advanced stage xenografts of human colon (CX-1, KM20L2), lung (LX-1), breast (MX-1) and ovarian (OVCAR-3) cancers and melanoma (SK-MEL2), with cures obtained with breast and ovarian cancers and melanoma [14–16].

Docetaxel in combination

In clinical oncology, most drugs are used in combinations rather than as single agents. Docetaxel has been tested in combination regimens in animal studies in order to ascertain those drug combinations with acceptable toxicity and advantageous activity. A drug combination is said to be synergistic when the antitumour activity with the combination is greater than that found with the most active drug as a single agent. Combinations of docetaxel with cyclophosphamide, 5-fluorouracil, vinorelbine, methotrexate and etoposide have all been shown to be synergistic in various tumour models [1]. Synergy is not, however, the only consideration when combining drugs. The toxicity of drug combinations can result in dose reductions for some, or all, constituents of the combination, such that the optimal dose is not delivered. Combinations of docetaxel with mitomycin-C and cisplatin were found to require significant dose reductions. Full doses could be delivered with docetaxel and vinca alkaloids, and the combination of docetaxel and vinorelbine was found to be very active [1]. Clinical studies are now ongoing to explore drug combinations with docetaxel.

Anticancer drugs are often also combined with other classes of drug in order to ameliorate side-effects. It is recommended that docetaxel is given with prophylactic corticosteroids. Corticosteroids have been reported to reduce antitumour activity in some models directly, but not in others [17,18]. Studies with docetaxel and dexamethasone have demonstrated that dexamethasone does not reduce the antitumour effects of docetaxel [12].

Conclusion

Preclinical studies predicted that docetaxel would be an active anticancer agent, possibly with some advantages over paclitaxel. Clinical studies have confirmed the broad spectrum of antitumour activity of docetaxel and this drug is one of the few that has merited development beyond phase I studies.

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