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Preclinical Pharmacology of Docetaxel

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Docetaxel is a taxoid cytotoxic agent which promotes tubulin assembly into microtubles and inhibits their depolymerisation. *In vitro*, docetaxel reduces murine and human tumour cell survival by 50% at concentrations of 4–35 ng/ml, with a greater cytotoxic effect on proliferating than on non-proliferating cells. *In vivo*, docetaxel is schedule-independent. Over 80% of murine transplantable tumours were found to be very docetaxel sensitive, with complete regression of advanced stage tumours. Activity was also observed in >90% of advanced stage human tumour xenografts in mice. In combination therapy studies, synergism with 5-fluorouracil, cyclophosphamide and etoposide was observed *in vivo*. Docetaxel exhibited linear pharmacokinetics and long tumour retention in tumour-bearing mice; plasma protein binding ranged from 76 to 89%. In toxicological studies in mice and dogs, docetaxel produced haematological, gastrointestinal and neuromotor toxicity. The dog was found to be the most sensitive species to the toxic effects of docetaxel.

Key words: docetaxel, mechanism of action, antitumour efficacy, resistance, pharmacokinetics, toxicology
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INTRODUCTION

IN THE LATE 1960s, the National Cancer Institute (NCI) screening programme revealed that a crude alcohol extract from the bark of the Western (Pacific) yew, *Taxus brevifolia* L., was cytotoxic *in vitro*. The extract's principle, paclitaxel, was subsequently isolated and characterised in 1971 [1, 2].

Paclitaxel belongs to a class of compounds known as the taxoids, mitotic spindle poisons that stabilise microtubules and inhibit their depolymerisation to free tubulin [3]. Although phase I clinical studies suggested that paclitaxel was active against several tumours, further development was impeded by the difficulties in obtaining an adequate supply of the drug from natural resources [4].

In 1981, Rhône-Poulenc and the Institut de Chimie des Substances Naturelles, France concluded a co-operative research agreement which led to the discovery of docetaxel (Taxotere®), a new taxoid structurally similar to paclitaxel. Docetaxel was obtained by a semisynthetic process from an inactive precursor (10-deacetylbaccatin III), extracted from a renewable resource, the needles of the European yew, *Taxus baccata* L. The chemical structure of docetaxel is shown in Figure 1 [5].

MECHANISM OF ACTION AND CELLULAR EFFECTS

Microtubules are long, hollow cylinders composed of 13 protofilaments, assembled from the 100 kDa protein, tubulin, with which they exist in dynamic equilibrium. As ubiquitous components of eukaryotic cells, microtubules have important cellular functions, including chromosome movement during mitosis. Indeed, the shift of equilibrium with free tubulin towards microtubule assembly or disassembly is controlled by various biochemical signals, and can be modified by different reagents and drugs [6].

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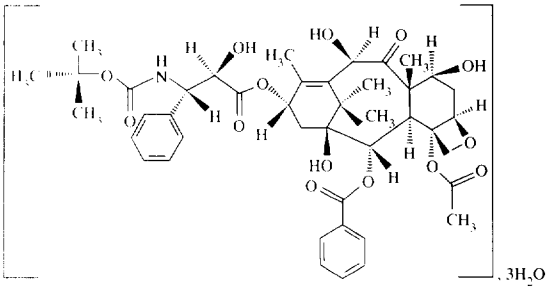

Figure 1. Structure of docetaxel ((2R,3S)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5β,20-epoxy-1,2a,4,7β,10β, 13a-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate).

Effects of docetaxel on the microtubule/tubulin system

Docetaxel has been shown to promote both the rate and extent of tubulin assembly into stable microtubules, which were not depolymerised by cold exposure [7]. In comparison to paclitaxel, docetaxel is slightly more active as a tubulin assembly promoter and microtubule stabiliser, and approximately 2-fold more potent as an inhibitor of microtubule depolymerisation [8]. Furthermore, docetaxel promotes the assembly of tubulin under conditions in which polymerisation would not normally occur, such as the polymerisation of guanosine diphosphate (GDP) tubulin [9], and tubulin assembly in the absence of guanosine triphosphate (GTP) [10]. The thermodynamic process of taxoid-induced assembly of tubulin is similar for both docetaxel and paclitaxel.

Studies have shown that docetaxel and paclitaxel share the same microtubule binding site. Indeed, both taxoids are able to induce the assembly of GDP-tubulin and bind to the assembled tubulin with a stoichiometry approaching 1 ligand per $\alpha\beta$ dimer.

However, the effective affinity of docetaxel for the microtubule binding site is 1.9-fold greater than that of paclitaxel [9]. Although the binding of either docetaxel or paclitaxel appears to reduce the minimum concentration of tubulin required before microtubule assembly can occur, the "critical concentration" which shifts the equilibrium between tubulin and microtubules in favour of the latter, docetaxel induces a 2-fold greater decrease of the critical concentration of GTP-tubulin required for tubulin assembly than paclitaxel [7, 9].

Further differences between docetaxel and paclitaxel relate to their efficiency in disassembling tubulin polymers, which is more apparent if the assembly is promoted by the microtubule-associated protein (MAP) Tau, rather than MAP2 [11]. Such results suggest that the tubulin polymers generated by paclitaxel may differ structurally from those generated by docetaxel. At the structural level, taxoids also possess the capacity to induce the formation of abnormal tubulin polymers, such as sheets or waved microtubules. Compared to normal microtubules (which contain 13 protofilaments), paclitaxel induces the formation of microtubules containing 12 protofilaments, whereas docetaxel does not alter the number of protofilaments per microtubule [12].

Cellular effects of docetaxel

In cells, docetaxel produces an alteration of the cytoskeleton morphology and induced microtubule bundle formation, probably as a result of arrested microtubule-mediated mitosis [7]. In experiments which investigated the effects of taxoids on the cell cycle of human and rodent cell lines, there was an accumulation of cells in mitosis regardless of the cell line investigated [13]. Moreover, HeLa cells remained in 4*n* mitosis, whereas all other cells were able to synthesise DNA, giving rise to highly polyploid cells containing at least 4*n* DNA [13].

Uptake and efflux studies of docetaxel and paclitaxel have been evaluated in P388 leukaemia cells *in vitro*. The studies demonstrated that these cells could accumulate three times more docetaxel than paclitaxel. Furthermore, efflux studies have shown that the retention of docetaxel is longer, with a half-life of efflux three times longer than that of paclitaxel [14].

ANTITUMOUR ACTIVITY OF DOCETAXEL *IN VITRO*

Cytotoxicity in long-term tumour cell cultures

Several studies have shown that docetaxel is generally between 1.3 and 12-fold more cytotoxic than paclitaxel [15–17], a result that could be explained by its higher affinity for microtubules [7, 8]. Docetaxel was found to be cytotoxic against both murine and human tumour cell lines *in vitro*, with the latter being the most drug sensitive. The concentration of docetaxel required to reduce cell survival by 50% (IC_{50}) ranged from 4 to 35 ng/ml (Figure 2), and the cytotoxic effects were greater on proliferating cells than on non-proliferating cells [15], being both time- and concentration-dependent [15, 16]. Interestingly, a study in mice bearing colon adenocarcinoma revealed that the area under the plasma (AUC_p) and tumour (AUC_t) concentration–time curves were much higher than in the most sensitive human cell lines [18]. The AUC values required for 50% kill of tumour cells *in vitro* were much lower (0.4–3.4 μg/ml h) than the pharmacologically achievable AUC values in mice at non-toxic dosage both in plasma (17 μg/ml h) and in tumour (44 μg/g h) [18]. Such results demonstrate that docetaxel achieves concentrations in plasma and tumours which are much greater than is required for 50% kill of tumour cells.

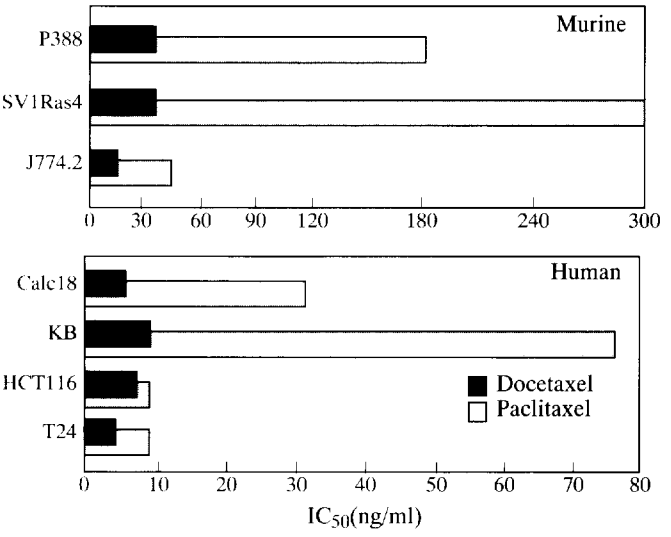

Figure 2. Comparative cytotoxicity of docetaxel and paclitaxel on murine and human cell lines *in vitro* [15].

Cytotoxicity in freshly explanted cells

Using a soft agar colony formation assay, the antiproliferative action of clinically relevant concentrations of docetaxel and paclitaxel have been compared against a variety of freshly explanted human tumour cells [19]. Both agents exhibited cytotoxic effects against tumour colony-forming units from breast, lung, ovarian and colorectal cancer and melanoma. Overall, 29 tumour specimens were more sensitive to docetaxel than to paclitaxel, whereas only 13 were more sensitive to the latter [19]. Furthermore, cross-resistance between these two agents was incomplete [19], in agreement with the results of other studies which showed that docetaxel remained effective in paclitaxel resistant cells [7].

ANTITUMOUR ACTIVITY OF DOCETAXEL *IN VIVO*

Several studies have evaluated the antitumour activity of docetaxel against a variety of murine tumour models *in vivo*, including murine transplantable tumours and human tumour xenografts.

Murine tumour models

Docetaxel possesses a demonstrably wide spectrum of efficacy against syngeneic murine transplantable tumours [5, 20, 21]. For example, of 15 tumour models tested, 13 responded to docetaxel (Table 1). Docetaxel was found to be highly active against the fast-growing B16 melanoma. A direct comparison with paclitaxel showed clear superiority of docetaxel at equitoxic dosages; the tumour growth delay was 12.2 days in mice treated with docetaxel, and 4.7 days in those treated with paclitaxel. Hence, the log cell kill was 2.6 times greater for docetaxel than for paclitaxel, as shown in Figure 3. In other subcutaneous (s.c.) tumour models, docetaxel produced 100% cure rates of early stage pancreatic ductal adenocarcinoma (PO3) and colon adenocarcinoma (C38), respectively. For both tumours, complete regressions of advanced stage disease were noted in >80% of mice (Table 1) [20]. In addition, docetaxel was active against s.c. early and advanced stage colon adenocarcinoma (C51), and against 3/4 of the mammary tumours evaluated (MA13/C, MA16/C, MA44) with complete regressions of advanced stage tumours in the cases of MA16/C and MA13/C [21]. Other s.c. solid tumours, including Lewis lung (3LL), Glasgow osteogenic

Table 1. *Antitumour activity of docetaxel against murine and human tumours* in vivo [20–24]

	Early stage	Advanced stage
Murine tumour		
B16	+ + + +	+ + +
MA16/C	+ + +	+ + +
MA13/C	+ + + +	+ + +
MA44	+	
C38	+ + + +	+ + +
C51	+ + +	+ +
C26	+	
PO2	+	
PO3	+ + + +	+ + +
3LL	+ +	
GOS	+	
M5076		
Human		
MX-1		+ + + +
Calc18		+ + +
LX-1		+ +
CX-1		+ + +
KM20L2		+ + +
SKMEL-2		+ + + +
OvPe		+ + +
OvSh		+ +
F.Ma		+ + +
F.Ko		
MRI-H-207		+ + +
OVCAR-3*	+ + + +	

+ + + +, >2.8 log cell kill; −, <0.7 log cell kill.

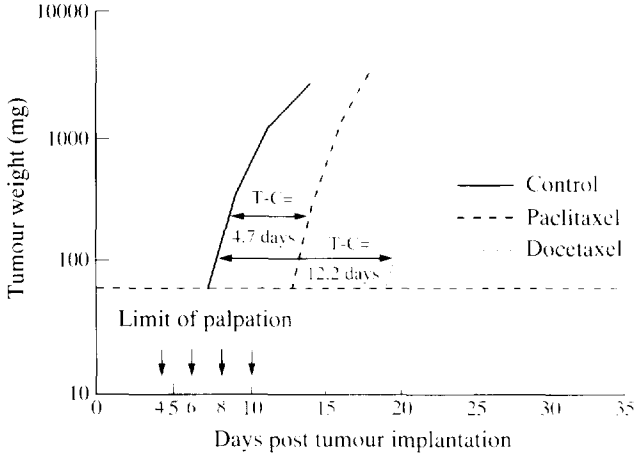

Figure 3. ***In vivo*** **comparison of i.v. docetaxel and paclitaxel against growth of s.c. B16 melanoma B6D2F$_1$ in mice. Tumour fragments (30–60 mg) were implanted on day 0. The results are presented at the highest non-toxic dose: docetaxel, 13.4 mg/kg/injection; paclitaxel 21.7 mg/kg/injection. Arrows indicate the days when treatment was administered. T-C, tumour growth delay (after Bissery** ***et al.*** **[20]).**

sarcoma (GOS), pancreatic ductal adenocarcinoma (PO2) and colon carcinoma (C26) were sensitive to docetaxel according to the NCI criteria, as were the intraperitoneal (i.p.) leukaemias, L1210 and P388. However, docetaxel was inactive against histiocytosarcoma (M5076) and mammary adenocarcinoma (MA17) [20, 21].

A review of all studies performed showed that the mean optimal total dose of docetaxel, 80 mg/kg, produced an average 13% loss of body weight at nadir, and was without delayed toxicity. On average, the host recovery time occurred 8 days postnadir [20].

Although intravenous (i.v.) and i.p. docetaxel was found to be active against s.c. implanted tumours, indicating that it effectively crosses physiological barriers, it was found to be inactive by the oral route, probably as a result of de-esterification and cleavage of the molecule at the acidic pH of the stomach [20].

Docetaxel appears to be a schedule-independent drug, since its administration schedule does not markedly influence the total dosage that can be administered. In contrast, schedule-dependent agents, such as 1β-D arabinofuranosylcytosine, require a minimum 10-fold lower dosage on the split-dose schedule compared to the intermittent schedule. Although the antitumour activity of docetaxel correlated with the total dosage administered, dose-splitting did not appear to alter efficacy [20].

Human tumour xenografts

A broad spectrum of activity was also obtained against human tumour xenografts in mice; 13/14 tumour models were responsive to docetaxel [22–24]. At non-toxic drug levels (22–33 mg/kg for three administrations), there were long-term survivors among mice bearing early stage OVCAR-3 ovarian carcinoma (Table 1) [22]. Impressive delays in tumour growth were obtained in advanced tumours, including LX-1 lung carcinoma, Calc18 mammary adenocarcinoma [21], and two colon carcinomas, KM20L2 and CX-1 [22]. Although tumour regression was apparent in the latter model, no cures were observed. In contrast, complete regression in 100% of tumour-free survivors was seen against advanced MX-1 (a mammary tumour xenograft) and advanced SKMEL-2, a melanoma [22].

At the maximum tolerated i.v. dose of 15–20 mg/kg administered at weekly intervals for two injections, docetaxel was found to be active in 4/5 human ovarian cancer lines grafted s.c. in mice (Table 1) [23]. Docetaxel was active against the cisplatin-insensitive OvPe tumour, although activity against MRI-H-207, OvSh and F.Ma tumours was comparable to cisplatin, cyclophosphamide and doxorubicin. However, docetaxel was found to be inactive against F.Ko tumours, in concurrence with other antitumour agents [23]. The activity of docetaxel against three other ovarian carcinoma xenografts, HOC8, HOC18 and HOC22, has been documented in other studies [24].

RESISTANCE

Acquired resistance

The emergence of drug resistant tumour cell clones is often cited as the cause of treatment failure. Acquired resistance to taxoids *in vitro* has been described, possibly due to either overproduction of P-glycoprotein (Pgp) [25–27], or tubulin alteration [27, 28].

In order to further elucidate the mechanism of docetaxel resistance *in vitro*, various docetaxel resistant cell lines have been developed. For example, human breast adenocarcinoma cells resistant to docetaxel (Calc18/TXT), which overexpress the *MDR1* gene and have decreased β-tubulin mRNA levels compared with parental cells, are 15 times less sensitive to docetaxel and are also highly cross-resistant to vinblastine [27]. However, cross-resistance to other agents is either moderate (doxorubicin and cisplatin) or absent (camptothecin or 5-fluorouracil) [27]. In other docetaxel resistant cell lines, such as CHO/Doce-R, low or

no levels of cross-resistance were found with vincristine and etoposide, and with cisplatin, respectively [16]. Importantly, although docetaxel and paclitaxel are structurally similar, docetaxel is still able to inhibit tumour cell replication of paclitaxel resistant J774.2 murine macrophage cells [7].

Innate resistance

Innate resistance or "intrinsic insensitivity", relates to tumours which are insensitive to antitumour agents having never been exposed to the agent before. Although the underlying mechanisms responsible for such resistance have not been extensively investigated, it has been shown that determinants of the innate resistance may occur at the level of MAPs. Differences in MAPs may account for the differences in docetaxel tumour sensitivity and intrinsic insensitivity in murine mammary tumours *in vivo* [29].

Cross-resistance with other antitumour agents

In terms of patterns of cross-resistance with other antitumour agents, cross-resistance to docetaxel has been observed in several multidrug resistant sublines [16]. However, the absence of cross-resistance to docetaxel was observed in cells that expressed low levels of vincristine or etoposide resistance but were Pgp-positive, such as CHO/AUX-10E, CHO/DXR-101, Susa/VPC3, Susa/VPC4 [16]. Such results suggest that cross-resistance to docetaxel is not automatically observed in cell sublines that express the MDR phenotype. Furthermore, a lack of cross-resistance to docetaxel was noted with 5-fluorouracil in colon COLO/5FU-R and LOVO/5FU-R, and with cisplatin in ovarian 41McisR, CH1cisR, and OVCAR-3carboR [16, 17].

Reversal of resistance

Reversal of resistance to docetaxel was examined in human myeloma cell lines, in which the effects of eight chemosensitisers on Pgp-associated docetaxel resistance were evaluated. The results suggest that oral quinidine could prove useful for clinical reversal of Pgp-associated resistance to docetaxel [30]. Furthermore, the dihydropyridine dexniguldipine was found to reverse multidrug resistance and enhance the sensitivity to docetaxel of 2780AD, a doxorubicin resistant variant of the ovarian carcinoma cell line A2780 [31].

POTENTIAL SYNERGISM WITH OTHER ANTITUMOUR AGENTS

In vitro *studies*

In studies of SKBR-3 human breast cells, synergistic effects were noted after cell pretreatment with edatrexate followed by docetaxel. However, antagonism was evident when the schedule was reversed [32].

Furthermore, the results of recent studies suggest that docetaxel may have a radiosensitising effect in human leukaemia HL-60 cells, probably due to its ability to arrest cells in the G2 and M phases of the cell cycle, the most radiosensitive phases [33].

In vivo *studies*

In mice bearing s.c. transplanted tumours, 6/9 combinations of docetaxel with a second antitumour agent produced a modest to marked synergistic effect [21, 34, 35]. The favourable combinations included: docetaxel–vincristine, docetaxel–navelbine, docetaxel–etoposide, docetaxel–cyclophosphamide, docetaxel–mitomycin C and docetaxel–5-fluorouracil. Although good activity was obtained in combination with either vinblastine or doxorubicin, the combinations were not more effective than docetaxel alone.

In terms of toxicity, the combination toxicity index (CTI; the sum of the fractions of the LD_{10} of each agent) ranged from 0.75 for the most toxic combination (docetaxel–cisplatin), indicating complete overlap in dose-limiting toxicity, to a CTI index of 2 for the least toxic combination, docetaxel–vincristine (simultaneously administered). Such results suggest that for the latter combination, the maximum tolerated dose of each agent could be administered without additional toxicity. A CTI index of approximately 1.2 was apparent for all other combinations, indicating that approximately 60% of the full dose of each agent can be used in combination without additional toxicity [21, 34, 35].

PHARMACOKINETICS

In mice bearing colon adenocarcinoma, the disposal of docetaxel was biphasic, with half-lives of 7 min and 1.2 h, respectively [18]. Furthermore, docetaxel displayed linear pharmacokinetics; peak plasma concentration and AUC_p increased in proportion to the administered dose [18]. At the optimal single i.v. dose of 37 mg/kg, the plasma total body clearance averaged 2.2 l/h/kg with an apparent volume of distribution at a steady state of 2.2 l/kg. The AUC_p at doses of 13–62 mg/kg ranged from 4.5 to 29.6 μg/ml h.

Distribution and excretion

Following rapid tissue uptake (especially in liver and kidneys) [18], the elimination of docetaxel in normal tissues was found to be biphasic with terminal half-lives ranging from 2.2 to 4.5 h [36]. However, the elimination half-life of docetaxel from tumour sites is approximately 22 h [36], suggesting long tumour site retention. Indeed, at a dose of 37 mg/kg, AUC_t and plasma AUC in the tumour values were 84 μg/g h and 17 μg/ml h, respectively [18].

Several studies have also investigated the kinetics of radiolabelled docetaxel disposal in mice. Docetaxel was rapidly distributed with an apparent distribution half-life of 10 min [37]; plasma protein binding ranged from 76 to 89% [38]. Furthermore, tissue uptake of radiolabelled docetaxel was rapid, especially in the liver, bile, intestine and gastric contents, as well as in haematopoietic tissues, muscle, the salivary glands and pancreas; however, docetaxel did not penetrate the central nervous system [37].

The primary elimination route of docetaxel is hepatobiliary. Faecal excretion accounted for more than 80% of the administered dose, and less than 10% was eliminated by the urinary route [37, 38].

Metabolism

In the mouse, docetaxel is almost completely metabolised. Only a minor fraction (<20%) of the parent drug is eliminated unchanged in the mouse, dog, and rat excreta, rat bile and human faeces [37, 38]. Urine presents a more complex profile of very minor metabolites [38]. Structural analysis has shown that docetaxel is predominately metabolised by successive oxidation of the tert-butyl ester group on the side chain, with cyclisation occurring for the aldehyde and acid derivatives [39, 40].

Four major metabolites of docetaxel have been identified. One of the metabolites was found to be 30-fold less cytotoxic than docetaxel against P388 leukaemic cells *in vitro*, whereas the others were all at least 140-fold less cytotoxic. Furthermore, the metabolites were found to be inactive against B16 melanoma in mice [39].

TOXICOLOGY

In toxicological studies performed in mice and drugs using either single i.v. doses of docetaxel (X1), or once daily i.v. dosing for 5 days (X5), the dog was found to be the most sensitive species to the toxic effects of docetaxel [41]. The dose-limiting toxicities were digestive tract lesions, and myelosuppression associated with peripheral leucopenia. Hypotension was also observed in the dog, although this was attributed to the injection vehicle (polysorbate 80). Cumulative and reversible neurotoxicity was only observed in mice. When the X1 and X5 schedules were compared, similar cumulative haematopoietic toxicity was observed with the 5 day schedule.

Taking into consideration the NCI guidelines, 1/10 of the murine LD_{10} (approximately 30 mg/m^2), was tested in dogs and was found to be highly toxic. Hence, the proposed phase I clinical starting dose of docetaxel was 5 mg/m^2, corresponding to one-third of the low toxic dose in dogs (15 mg/m^2) [41].

CONCLUSION

Docetaxel is a novel antitumour agent with a unique mechanism of action and a broad spectrum of activity, which has been confirmed in phase II clinical trials [42]. The preclinical data for docetaxel, much of which have been generated *in vivo*, in models including murine and human advanced stage tumours, suggest that the drug represents a major advance in the treatment of cancer.

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