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COMMENTARY

Tubulin-targeting agent combination therapies: dosing schedule could matter

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Tubulin-binding agents are potent cytotoxic drugs that are largely used to treat haematological malignancies and solid tumours. In this issue of *British Journal of Pharmacology*, doxorubicin was shown to decrease the activity of vincristine when administered simultaneously, unless cell cycle arrest mechanisms were disrupted. This observation emphasizes the need to better explore schedule-dependent antagonist effects in anticancer drug combination therapies.

LINKED ARTICLE
This article is a commentary on the research paper by Ehrhardt *et al.*, pp. 1558–1569 of this issue. To view this paper visit http://dx.doi.org/10.1111/bph.12068

Abbreviation
ABC, ATP-binding cassette

In this issue of *British Journal of Pharmacology*, Harald Ehrardt and colleagues re-evaluated drug regimens that combine vinca-alkaloids with anthracyclines. Using a panel of solid tumour cell lines, they demonstrated that the most common schedule in which the drugs are given simultaneously is not optimal, unless cell cycle arrest mechanisms are disrupted (Ehrhardt *et al.*, 2012). The results from this study confirm those from their previous proof-of-concept study conducted in leukaemia cell lines, which showed that, in most cases, vincristine and doxorubicin are more effective when applied on different days (Ehrhardt *et al.*, 2011). In these times of targeted therapy development aimed at personalizing cancer treatment, such a debate on how to administer old-fashioned chemotherapeutics could appear to be a rearguard action. However, tubulin is a validated target for efficacious cancer therapy and the development of new tubulin-targeting chemotherapeutics remains a very active area of investigation (Dumontet and Jordan, 2010). Therefore, to revisit the use of these compounds in the setting of commonly applied drug combinations is more than appropriate.

Microtubules are cytoskeletal structures whose basic structural components are heterodimers made of α- and β-tubulin. Natural product, small molecule inhibitors with diverse structures that bind the β-tubulin subunit of these heterodimers are widely used in the clinic, mostly in combination with other chemotherapeutic compounds (Dumontet and Jordan, 2010). They have been classified into microtubule-destabilizing drugs such as vinca-alkaloids that inhibit the polymerization of microtubule subunits and microtubule-stabilizing agents such as taxanes and macrolide epothilones that accelerate the polymerization of tubulin heterodimers into microtubules. At high concentrations, these compounds affect the microtubule polymer level. At lower doses, they only disrupt the microtubule dynamics (Kavallaris, 2010). Eribulin mesylate, which is a synthetic derivative of the macrolide halochondrin B, is a microtubule dynamics inhibitor that has recently been approved for the treatment of patients with metastatic breast cancer (Cortes *et al.*, 2011). In all cases, the lack of a functional spindle impedes mitotic progression, leading to the death of cells with an altered, inefficient mitotic checkpoint. Recently, some tubulin-binding agents have been demonstrated to have an additional mechanism of action: when binding to the endothelial β-tubulin, these compounds potently increase vascular permeability and induce vascular disruption, thus leading to tumour necrosis (Dumontet and Jordan, 2010).

Various mechanisms of tumour cell resistance to tubulin-binding agents have been identified, some of which have

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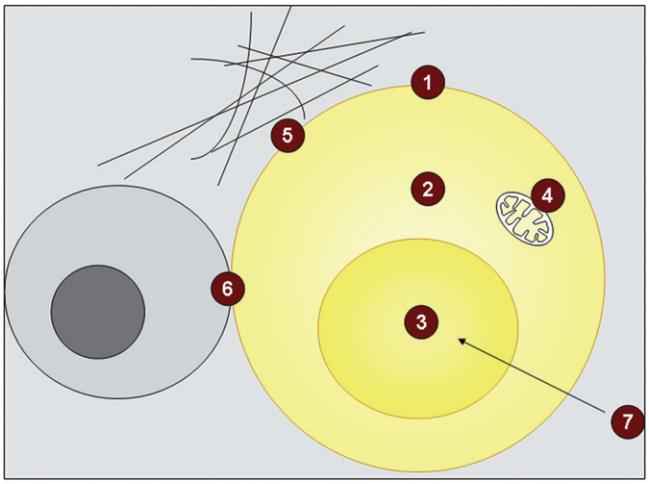

Figure 1

Intrinsic and extrinsic mechanisms of tumour cell resistance to tubulin-binding agents. Intrinsic resistance can be related to overexpression of an ABC transporter (1), to alterations in the β-tubulin target, for example, through either mutation, increased expression of the βIII tubulin isotype, or modification of microtubule-associated proteins (2), to an inactive mitotic checkpoint (3), and to defects in the cell death pathway, for example, through overexpression of Bcl-2 proteins at the mitochondrial level (4). Extrinsic resistance can be due to interactions with extracellular matrix proteins (5) or with neighbouring cells at high cellular density (6). Simultaneous exposure to a cytostatic compound such as doxorubicin (7) is the additional mechanism discussed in this issue of *British Journal of Pharmacology*.

been validated in the clinical setting. They include a decreased accumulation of these agents, owing to overexpression of the membrane-bound drug efflux P-glycopotein (encoded by the *ABCB1* gene) and possibly other ABC transporters, a direct alteration of the drug target through mutation, an increased level of expression of the βIII tubulin isotype, alterations in the expression or post-translational modifications of microtubule-associated proteins, irregular interactions between tubulin and the actin cytoskeletons, an intrinsically inactive mitotic checkpoint and defects in the cell death pathway through deregulation of the expression of the Bcl-2 family of proteins (Figure 1) (Kavallaris, 2010).

Extracellular mechanisms also modulate the response of tumour cells to tubulin-binding agents. For example, in breast and ovarian tumours, the presence of the extracellular matrix protein transforming growth factor β induced in the microenvironment signals through integrins and focal adhesion kinase to stabilize the microtubules and sensitize the tumour cells to taxanes (Ahmed *et al.*, 2007). Cell density is another parameter that affects the response of tumour cells to tubulin-targeting drugs, and one of the proposed mechanisms for this effect is a decrease in the proportion of actively cycling cells at high density (Figure 1) (Dorsey *et al.*, 2010).[7] Harald Ehrardt and colleagues identified a cell resistance mechanism induced by the simultaneous administration of a compound that inhibits cell cycle progression at the G_2/M phase. Several hundreds of clinical trials are currently investigating a tubulin-binding agent/anthracycline combination as a therapeutic for various cancers (source http://www.clinicaltrials.gov). Surprisingly enough, the antagonistic effects of these combinations have not been really explored, as clinical trials mostly focus on dose-dependent toxicity as the main limiting effect of these regimens.

Intrinsic and extrinsic resistance mechanisms to tubulin-binding agents have been explored mostly in tumour cells or xenografted tumours treated with the drug tested alone. Any resistance resulting from the combined administration of these drugs with another anticancer agent, which is, in most cases, established empirically, is much less explored. A rationale design of drug combinations, based on molecular correlates, remains a difficult task as a number of parameters can affect the drug response. For example, crizotinib, an oral tyrosine kinase inhibitor that inhibits c-Met and ALK is also a P-glycoprotein inhibitor that may improve the efficacy of tubulin-binding agents in *ABCG1* expressing tumour cells (Zhou *et al.*, 2012), but this beneficial effect will be negated if crizotinib has a cytostatic effect (Bendall, 2011). Since the schedule-dependent antagonism between vincristine and doxorubicin depends on the status of p53, the simultaneous administration of vinca alkaloids and anthracycline might be more effective in patients with a p53-mutated compared to those with a p53-wild-type tumour; this could easily be checked in ongoing combination trials. If this hypothesis holds true in the clinical setting, disruption of cell cycle arrest may potentiate the tubulin-binding agent/cytostatic drug combination efficacy in tumour cells with efficient cell cycle control mechanisms, pending a simultaneous increase in toxic effects on normal cells.

The immune system also plays a role in a tumour's response to chemotherapy. Tumour cells that succumb *in vitro* to doxorubicin or oxaliplatin can elicit tumour-specific immune responses when inoculated into syngeneic mice, that is, a spatiotemporally defined combination of signals renders the tumour cells capable of eliciting a long-term, protective anti-tumour immune response. This is not the case with vincristine or other tubulin-targeting agents, which are non-immunogenic compounds, but the combination of a protein kinase C agonist with vincristine renders dying chronic lymphocytic leukaemia cells highly immunogenic (Shaha *et al.*, 2009). Thus, a drug combination can modulate the immunogenicity of cell death, which requires the use of syngeneic models to be detected. The exciting re-evaluation of the effects of combining anthracycline with a tubulin-binding agent initiated by Harald Ehrardt and colleagues has now to be completed in an immunocompetent setting. This should also take into account the vulnerability of a particular tumour cell to have its cell cycle arrested, which can be affected, for example, by its p53 status.

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