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MICROTUBULE DISRUPTORS AND THEIR INTERACTION WITH BIOTRANSFORMATION ENZYMES

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Microtubule disruptors, widely known as antimitotics, have broad applications in human medicine, especially as anti-neoplastic agents. They are subject to biotransformation within human body frequently involving cytochromes P450. Therefore antimitotics are potential culprits of drug-drug interactions on the level of activity as well as expression of cytochromes P450. This review discusses the effects of four well-known natural antimitotics: colchicine, taxol (paclitaxel), vincristine, and vinblastine, and a synthetic microtubule disruptor nocodazole on transcriptional activity of glucocorticoid and aryl hydrocarbon receptors. It appears that microtubules disarray restricts the signaling by these two nuclear receptors regardless of cell cycle phase. Consequently, intact microtubules play an important role in the regulation of expression of cytochromes P450, which are under direct or indirect control of the two nuclear receptors.

INTRODUCTION

Developed by plants as a part of defense system, alkaloids display wide variety of biological activities. One of these activities is disruption of microtubules which results in mitotic arrest of any proliferating cell, hence these substances are called antimitotics. Antimitotic substances are often used in current medicine as anti-neoplastic agents.

Biotransformation of antimitotics is an important issue from the point of view of effective dose, and consequent drug-drug interactions, and also development of multiple drug resistance. Antimitotics are primarily substrates of cytochromes P450. Cytochromes P450 (CYPs) constitute a gene superfamily of biotransformation enzymes which are expressed chiefly in hepatocytes, however, extrahepatic expression has been reported as well[1]. Expression of CYPs is regulated by several xenoreceptors: aryl hydrocarbon receptor (AhR) controls CYP1A and 1B subfamilies[2], pregnane X receptor (PXR) and constitutive androstane receptor (CAR) control CYP2B, 2C and 3A subfamilies[3,4]. Furthermore, glucocorticoid receptor (GR), vitamin D receptor (VDR), and short heterodimerization partner (SHP) are involved in CYP enzymes regulation[5]. Down-regulation of CYP enzymes, and consequently decreased detoxification capacity of the organism during inflammation or infection, is a well-known phenomenon. Both up- and down-regulation of a CYP enzyme expression will inevitably affect metabolism of administered drugs, often including the effector (inducer or repressor) substance.

When considering patho-physiological factors, the role of microtubules in receptor transcriptional activity, and consequently CYPs expression, is intriguing. Five well-known microtubule disrupting substances are listed in Table 1. While many other antimitotics were synthesized, either as completely new substances or derivatives of existing compounds, their activity and tubulin binding is compared to the two vinca alkaloids, paclitaxel, and colchicine. Therefore we will limit our attention to these four substances plus nocodazole, a representative synthetic microtubule disrupting compound, and their relationship with CYP expression.

Metabolism of microtubules disruptors by CYPs

All four natural antimitotics discussed in this review (Table 1) are substrates of the major human hepatic CYP3A4, which is responsible for metabolism of approximately 50% of all xenobiotics. In case of vinca alkaloids this fact has been a concern because of drug-drug interactions[6] and multiple drug resistance[7]. Of vinblastine and vincristine metabolites only desacetylvinblastine, the active metabolite of vinblastine, has been structurally characterized. Two colchicine metabolites arising due to CYP3A4 activity have been identified: 2-demethylcolchicine and 3-demethylcolchicine[8]. 2-Demethylcolchicine is much less potent whereas 3-demethylcolchicine is comparable to its parental drug in terms of microtubule disruption. Paclitaxel is interesting in that it is metabolized by two CYP isoenzymes, CYP 2C8 and CYP 3A4, which are responsible for the formation of 6α-hydroxytaxol and 3'-(p-hydroxyphenyl)taxol, respectively[9]. 6α-Hydroxytaxol, formed by CYP2C8, is the major metabolite in humans hence only moderate influence of CYP3A4 substrates was noted on paclitaxel metabolism[10]. Nocodazole, although used in many studies as a potent microtubule disrupting

Table 1. Microtubule disrupting substances

Substance	Source	Biological activity	Medical use	Metabolised by CYP
Colchicine	Colchicum autumnale	inhibits tubulin polymerization	acute gout attack, familial mediterranean fever, Behcet's disease	CYP3A4
Taxol (Paclitaxel)	Taxus brevifolia	inhibits microtubules depolymerization	breast and ovary carcinoma, bronchogenic carcinoma	CYP3A4, CYP2C8
Vincristine	Vinca rosea	inhibits tubulin polymerization	acute lymphoblastic leukemia, lymphomas, multiple myeloma	CYP3A4
Vinblastine	Vinca rosea	inhibits tubulin polymerization	Hodgkin's disease, testicular carcinoma	CYP3A4
Nocodazole	synthetic	inhibits tubulin polymerization	currently not in use	–

Four well-known natural antimitotic substances and one synthetic are listed. Original source plant only is noted, of a large number of medical applications only a few are noted. Metabolism of nocodazole has not been investigated to date.

substance, has not been investigated in respect to its biotransformation. The reason is likely the lack of nocodazole use in clinical applications.

Because these substances are CYP substrates, they are likely to be inducers or repressors of CYP genes expression.

Effects of microtubules disruptors on CYP expression

Regulation of CYP genes expression is governed by nuclear receptors, which may be affected either directly by ligands or indirectly by multiple mechanisms. Of these disruption of microtubules, the characteristic property of antimitotic substances, is likely to influence cytosol-to-nucleus trafficking of receptors. Indeed, two major nuclear receptors, glucocorticoid receptor (GR) and aryl hydrocarbon receptor (AhR), are affected by disruption of microtubules. This is true in both proliferating and non-proliferating cells.

Reiners et al.[11] studied short and long term effects of cytoskeleton-disrupting drugs on CYP1A1 induction in murine hepatoma 1c1c7 cells. Induction of CYP1A1 was unaffected by short-term disruption of the microfilament or microtubule network whereas long-term exposure to microtubule inhibitor nocodazole caused inhibition of CYP1A1 inducible expression[11]. In a follow-up article by the same group the steady-state CYP1A1 mRNA contents was shown to be reduced in TCDD treated cultures arrested in G2/M phase of the cell cycle as a consequence of exposure to microtubule disrupters (demecolcine, estramustine, vinblastine) or the microtubule stabilizer paclitaxel, relative to TCDD-treated asynchronous 1c1c7 cultures[12]. Suppression of CYP1A1 reflected neither changes in AhR protein content nor a hindrance of AhR activation and translocation to the nucleus[12]. The author concluded, that the transcriptional activation of members of the Ah receptor battery by TCDD is cell cycle-dependent, and markedly suppressed in G2/M stage of the cell cycle[11, 12].

Our findings that colchicine and nocodazole suppress TCDD-inducible CYP1A1 expression in both HepG2 cells and primary cultures of rat hepatocytes lend further support to the direct involvement of cytoskeleton in this phenomenon (Dvořák et al., unpublished results).

Recently, we reported the glucocorticoid receptor-mediated down-regulation of CYP2B6, CY2C8, CYP2C9, and CYP3A4 in primary cultures of human hepatocytes treated with colchicine or nocodazole[13, 14]. Microtubules interfering agents (MIAs) decreased both basal and rifampicin- and phenobarbital-inducible expression of these CYPs, whereas colchicine derivative colchiceine (10-O-demethylcolchicine), which lacks tubulin-binding capability, had no effect[13, 15]. A parallel down-regulation of CAR and PXR mRNA and tyrosine aminotransferase (TAT) was observed. MIAs affected neither GR mRNA levels nor glucocorticoid binding to GR. Transcriptional activity of GR in stably transfected HeLa cell line was inhibited by MIAs treatment. We found that colchicine restricted nuclear import of GR in human hepatocytes and in human embryonal kidney cells (HEK293) transiently transfected with GR-GFP chimera[13, 14]. We concluded that alteration of the signal transduction mediated through the GR-CAR/PXR-CYPs cascade by MIAs is responsible for the down-regulation of above listed CYPs, implicating cytoskeleton as necessary for correct functioning of this cascade under physiological conditions. Furthermore, the suppression of TAT, a prototypic gene directly regulated by GR, was observed in human hepatocytes treated with colchicine or nocodazole but not with inactive derivative colchiceine[13–15]. Similarly, strong decrease of TAT activity was observed in primary cultures of rat hepatocytes incubated with colchicine, while inactive analogue lumicolchicine had not effect[16].

Interestingly, microtubules stabilizing agent paclitaxel induced CYP3A in mice, when the functional PXR was found to be essential for this induction[17]. Because paclitaxel is a PXR ligand[18], this direct effect may be decisive for CYP3A4 induction, rather than down-regulation due to antimitotic effects. It is in agreement with GR having a role of transcriptional enhancer in case of CYP3A4 (ref.[19]).

In addition to the GR and AhR inhibition by MIAs, the intact microtubules were found to be essential for 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) dependent modulation of gene transcription[20]. The genomic actions of 1,25(OH)2D3 are mediated by the nuclear receptor – vitamin D receptor (VDR). Microtubules disruption in normal human monocytes totally abolished the ability of exogenous 1,25(OH)2D3 to suppress its own synthesis and to induce 25-hydroxyvitamin D(3)-24-hydroxylase (CYP24) mRNA and activity. Thus, the integrity of microtubules determines 1,25(OH)2D3 synthesis.

Collectively, microtubules disarray restricts the signaling by nuclear receptors involved in CYPs regulation, i.e. GR and AhR. Although the majority of studies attributed the inhibition of AhR and GR transcriptional activities by MIAs to the synchronization of the cells in G2/M phase of the cell cycle, several studies indicated that MIAs inhibit GR and AhR transcriptional activities in non-proliferating cells as well. For instance, hepatocytes, which are non-proliferating cells mostly in the quiescent G0 state, suffer the loss of GR and AhR activities as the consequence of microtubules disarray.

CONCLUSION

The level of CYP enzymes is given by genetic factors such as polymorphism and it is further regulated by transcriptional and post-translational mechanisms. Several studies indicated that microtubules network perturbation alters transcriptional activities of AhR and GR receptors. As the expression of important human drug metabolizing CYPs is under the direct or indirect (via PXR and CAR receptors) control of AhR and/or GR receptors, the role of microtubules network in the expression of CYP1A, CYP2B6, CYP2C, and CYP3A enzymes seems imminent.

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