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 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 and multiple drug resistance. 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. 2-Demethylcolchicine is much less potent whereas 3-demethylcolchicine is comparable to its parental drug in terms of microtubule disrubption. 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, 6α-Hydroxytaxol, formed by CYP2C8, is the major metabolite in humans hence only moderate influence of CYP3A4 substrates was noted on paclitaxel metabolism. Nocodazole, although used in many studies as a potent microtubule disrupting substance, is 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 and multiple drug resistance. Vincristine and vinblastine 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. 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, 6α-Hydroxytaxol, formed by CYP2C8, is the major metabolite in humans hence only moderate influence of CYP3A4 substrates was noted on paclitaxel metabolism.
substance, has not been investigated in respect to its bio-
transformation. The reason is likely the lack of nocoda-
zole use in clinical applications.

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

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 nu-
clear 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. 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. In a follow-up article by
the same group the steady-state CYP1A1 mRNA contents
was shown to be reduced in TCDD treated cultures ar-
rested 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. Suppression of CYP1A1 reflected neither
changes in AhR protein content nor a hindrance of AhR
activation and translocation to the nucleus. The author
concluded, that the transcriptional activation of members
of the Ah receptor battery by TCDD is cell cycle-depend-
ent, and markedly suppressed in G2/M stage of the cell
cycle.

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-me-
diated down-regulation of CYP2B6, CY2C8, CYP2C9,
and CYP3A4 in primary cultures of human hepatocytes
treated with colchicine or nocodazole. Microtubules inter-
fering agents (MIAs) decreased both basal and ri-
fampicin- and phenobarbital-inducible expression of
these CYPs, whereas colchicine derivative colchicine
(10-O-demethylcolchicine), which lacks tubulin-binding
capability, had no effect. 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 re-
stricted nuclear import of GR in human hepatocytes and
in human embryonal kidney cells (HEK293) transiently
transfected with GR-GFP chimera. 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
expression of TAT, a prototype gene directly regulated
by GR, was observed in human hepatocytes treated with
colchicine or nocodazole but not with inactive derivative
colchicine. Similarly, strong decrease of TAT activ-
ity was observed in primary cultures of rat hepatocytes
incubated with colchicine, while inactive analogue lum-
icolchicine had not effect.

Interestingly, microtubules stabilizing agent paclitaxel
induced CYP3A in mice, when the functional PXR was
found to be essential for this induction. Because pacli-
taxel is a PXR ligand, this direct effect may be decisive
for CYP3A4 induction, rather than down-regulation due

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

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.
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 Mías, the intact microtubules were found to be essential for 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) dependent modulation of gene transcription20. 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 Mías to the synchronization of the cells in G2/M phase of the cell cycle, several studies indicated that Mías 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 CYP1A1, CYP2B6, CYP2C, and CYP3A enzymes seems imminent.

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