CITY UNIVERSITY OF HONG KONG

香港城市大學

Molecular and Pharmacological Effects of Schisandrol A and Gomisin A on Multidrug Resistant Cancer Cells

五味子醇甲與五味子醇乙作用於多藥耐性癌細胞的分子機理

Submitted to
Department of Biology and Chemistry
生物及化學系
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
哲學博士學位

by

Wan Chi Keung
尹志強

January 2007
二零零七年一月

ABSTRACT

Multidrug resistance (MDR) is defined as the chemotherapeutic resistant of cancer cells to a broad spectrum of structurally unrelated drugs. Overexpression of membrane transporter P-glycoprotein (P-gp) is the most extensively studied MDR pathway. P-gp modulator screening program in our laboratory has shown that Fructus Schisandrae (the dried fruit of *Schisandra chinensis* (Turcz.) Baill.) modulates P-gp-mediated MDR. Through bioassay guided fractionation two dibenzocyclooctadiene lignans schisandrol A (SCH) and gomisin A (GOM) were identified and we studied their modulatory mechanisms on P-gp-mediated MDR.

In P-gp overexpressing subline HepG2-DR cells SCH or GOM was relatively non-toxic (IC_{50} > 150μM after 72 h incubation) but without altering P-gp expression they restored the cytotoxic actions of anticancer drugs such as vinblastine and doxorubicin that are P-gp substrates. The toxicity of SCH or GOM itself was not altered by P-gp competitive modulator verapamil suggesting that they are P-gp modulators rather than substrates. Although SCH and GOM share similar chemical structure their P-gp modulatory mechanisms are not exactly the same. They showed differential effects on the P-gp-associated ATPase activity. SCH activated while GOM inhibited the P-gp-associated ATPase activity suggesting that SCH contained some substrate-like properties but GOM acted as pure P-gp inhibitor. Consistent with this observation SCH showed a mixed-type inhibition while GOM showed an uncompetitive inhibition on progesterone- or verapamil-stimulated P-gp-associated ATPase activity. In HepG2-DR cells both SCH and GOM were able to increase cellular retention of the P-gp substrate rhodamine 123 (Rh-123) and GOM showed more effective than SCH. The combined effect of verapamil with SCH or GOM was basically additive, implying that SCH or GOM might simultaneously bind on P-gp

with verapamil. Moreover, binding of transport substrates with P-gp would result in a P-gp conformational change favoring UIC-2 antibody reactivity but SCH or GOM impeded UIC-2 binding, suggesting that binding of SCH or GOM altered P-gp conformation in a manner distinct from P-gp-substrate binding. These results suggested that SCH or GOM modulates the P-gp-dependent MDR in HepG2-DR cells possibly by simultaneously binding with substrates onto P-gp and altering the P-gp-substrate interaction. We also investigated the effect of SCH or GOM on the drug metabolizing activity of CYP3A4, which usually show cross substrate/inhibitor specificity with P-gp. SCH or GOM inhibited CYP3A4 activity and GOM (IC_{50} = 1.39μM) showed higher potency than SCH (IC_{50} = 32.02μM). Therefore the pharmacokinetic interaction of SCH or GOM with chemotherapeutic agents should be carefully considered when combined use *in vivo*.

Apart from their modulation on P-gp-mediated MDR SCH or GOM showed vincristine sensitizing effect on both 2008 ovarian cancer cells and its MRP1-transfected subline. Addition of GSH could not reverse this effect suggesting that it was unrelated to the modulation of MRP1-mediated MDR. No sensitizing effect was observed when SCH or GOM was combined with doxorubicin or taxol indicating that the action mechanism was P-gp-independent. We studied the mechanism for this vincristine sensitizing effect. SCH or GOM enhanced vincristine-induced mitosis arrest, apoptosis and Cdc2 dephosphorylation/activation. Combined drug treatments regulated Wee1 and Cdc25C through phosphorylation/dephosphorylation and nuclear/cytoplasmic translocation in favor of Cdc2 activation. Olomoucine inhibited phosphorylation of Wee1 and Cdc25C suggesting the presence of a Cdc2-mediated positive feedback loop. The enhanced apoptosis was associated with increases of caspases 8 activation. The actions of SCH or GOM were independent from p53 as shown firstly that SCH or GOM suppressed

vincristine-stimulated p53 up-regulation. Direct inhibitory interaction of p53/Cdc2 was not detected and SCH or GOM did not affect the expression levels of p53 transactivation targets. Lastly transient transfection and expression of wild-type and dominant negative p53 had no effect on SCH- or GOM-mediated vincristine sensitizing effect. Taken together, our results suggested that besides their modulatory effect on P-gp-mediated MDR, SCH or GOM is able to potentiate vincristine-mediated mitotic arrest, Cdc2 activation and apoptosis in a P-gp- and p53-independet manner.

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LIST OF ABBREVIATIONS

Å	angstrom unit
AML	acute myelogenous leukaemia
BBB	blood-brain barrier
BCRP	breast cancer resistance protein
BSO	DL-buthionine (S,R)-sulfoximine
CDKI	cyclin-dependent kinase inhibitor
CNS	central nervous system
CO-IP	co-immunoprecipitation
CSF	cerebrospinal fluid
CYP	cytochrome P-450
EA	ethyl acetate
FBS	fetal bovine serum
G6PD	glucose-6-phosphas dehydrogenase
GI	gastrointestinal
GOM	gomisin A
GSH	glutathione
GST	glutathione S-transferase
HPLC	high performance liquid chromatography
IAAP	iodoarylazidoprazosin
kD	kilodalton
JNK1	c-jun N-terminal kinase 1
LC-MS	liquid chromatography-mass spectroscopy
MAPK	mitogen-activated protein kinase
MDR	multidrug resistance

MIA	microtubule-interfering agent
MRP	multidrug resistance-related protein
MTS	methanethiosulfonate
MTX	methotrexate
NBD	nucleotide binding domain
NFAT	nuclear factor of activated T cells
NMR	nuclear magnetic resonance
PBS	phosphate buffered saline
PE	petroleum ether
P-gp	p-glycoprotein
Plk	polo-like kinase
QSAR	quantitative structure-activity relationship
rGSH	reduced glutathione
Rh-123	rhodamine 123
RT-PCR	reverse transcription-polymerase chain reaction
SRB	sulforhodamine B
SCH	schisandrol A
SDS-PAGE	SDS-polyacrylamide gel
SXR	steroid xenobiotic receptor
TBS	tris buffered saline
TCA	trichloroacetic acid
TMD	transmembrane biding domain
Y15	tyrosine 15