

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

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Wan Chi Keung
尹志強

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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 ($IC_{50} > 150\mu\text{M}$ 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 ($IC_{50} = 1.39\mu\text{M}$) showed higher potency than SCH ($IC_{50} = 32.02\mu\text{M}$). 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-independent 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 binding domain
Y15	tyrosine 15