ABSTRACT

Previous *in vitro and in vivo* studies have reported that 1'S-1'-acetoxychavicol acetate (ACA) and its analogue, 1'S-1'-acetoxyeugenol acetate (AEA) isolated from rhizomes of the Malaysian ethno-medicinal plant *Alpinia conchigera* Griff (Zingiberaceae) induces apoptosis-mediated cell death in tumour cells via dysregulation of the NF-κB pathway and reduced physiological side effects on non-transformed cells. Nevertheless, there were some clinical development drawbacks such as poor solubility *in vivo*, depreciation of biological activity and non-specific targeting of tumour cells. In collaborative study with Institute of Engineering Immunology Russia, all the problems above were addressed using their novel drug conjugation technology involving a recombinant human alpha fetoprotein (rhAFP). However, in terms of future cost effectiveness, only ACA which is a major compound was selected for conjugation with rhAFP because AEA being a minor analogue, requires extensive purification steps with very low yield. As examined, thermodynamic studies showed that water soluble rhAFP was successful in retaining non-soluble forms of ACA within its hydrophobic pockets, hence acting as a chaperone which specifically targets tumour cells containing AFP surface receptors. This study also takes advantage of coupling ACA chemopotentiating effect and extrinsic pathway induction together with rhAFP's specificity and intrinsic pathway induction of apoptosis to increase the efficacy of drugs whilst maintaining a lower dose *per se*. To study the synergistic effect of both agents on human cancer xenografts, nude athymic (*Nu/Nu)* mice were used and treated with various combination regimes subcutaneously. It was found that mice exposed to combined treatments displayed higher reductions in tumour volume compared to standalone agents. In addition to this, combined drug treated mice also demonstrated milder signs of systemic toxicity, such as loss in body weight and inflammation of vital organs compared to

standalone treatments. The immunohistochemistry, ELISA and western blotting results also provided evidence that rhAFP/ACA was not only able to downregulate NF-κB activation, but also reduced the expression of NF-κB regulated genes and inflammatory biomarkers. Therefore, this drug conjugation technology shows great therapeutic potential and a pioneer for the basis of future combination anti-neoplastic drugs development.

ABSTRAK

Kajian *in vitro* dan *in vivo* terdahulu telah melaporkan bahawa 1'S-1'-acetoxychavicol acetate (ACA) dan analog nya, 1'S-1'-acetoxyeugenol acetate (AEA) yang diperolehi dari rizom tumbuhan etno-perubatan *Alpinia conchigera* Griff (Zingiberaceae) telah mendorong kematian sel-pengantara apoptosis dalam sel-sel tumor melalui penyahaktifan laluan signal NF-κB dan mengurangkan kesan sampingan fisiologi pada sel-sel normal. Walau bagaimanapun, mereka mempunyai beberapa kelemahan dalam pengujian klinikal seperti kelarutan yang rendah dalam *in vivo*, penyusutan aktiviti biologi dan tidak mensasar secara spesifik kepada sel-sel tumor. Dalam kajian bersama Institut Kejuruteraan Immunologi Russia, semua masalah tersebut telah diatasi dengan menggunakan teknologi konjugasi yang melibatkan rekombinan alfa fetoprotein manusia (rhAFP). Walau bagaimanapun, dari segi keberkesanan kos pada masa hadapan, hanya ACA yang merupakan sebatian utama telah dipilih untuk dikonjugasi bersama rhAFP kerana AEA adalah analog kecil, memerlukan langkah-langkah pembersihan yang menyeluruh dengan hasil yang sangat rendah. Setelah diuji, kajian termodinamik menunjukkan bahawa rhAFP yang bersifat larut air telah berjaya mengekalkan bentuk tidak larut ACA di dalam poket hidrofobiknya yang bertindak sebagai 'chaperone' yang mensasar secara spesifik kepada sel-sel tumor yang mempunyai reseptor pada permukaan AFP. Kajian ini juga menggunakan kelebihan dari kesan gabungan ACA kimo-potensi dalam induksi laluan ekstrinsik bersama dengan rhAFP yang spesifik dalam rangsangan laluan intrinsik apoptosis untuk meningkatkan keberkesanan ubat, sementara mengekalkan dos '*per se'* yang lebih rendah. Untuk mengkaji kesan sinergi kedua-dua agen dalam 'xenografts' kanser manusia, tikus athymic (*Nu/Nu*) telah digunakan dan dirawat dengan pelbagai rejim gabungan secara suntikan 'subcutaneous'. Hasil kajian mendapati bahawa tikus yang dirawat secara rawatan

gabungan telah mempamerkan penurunan dalam jumlah pertumbuhan tumor berbanding dengan rawatan secara persendirian. Di samping itu, tikus yang dirawat secara rawatan gabungan juga menunjukkan tanda-tanda yang lebih rendah dalam ketoksikan sistemik, seperti kehilangan berat badan dan keradangan pada organ-organ penting, berbanding dengan rawatan secara persendirian. Keputusan immunohistochemistry, ELISA dan Western blot juga menunjukkan bahawa gabungan rhAFP/ACA bukan sahaja dapat menyahaktifan laluan signal NF-κB, tetapi juga mengurangkan ekspresi gen yang dikawal oleh NF-κB serta 'inflammatory biomarkers'. Oleh yang demikian, teknologi konjugasi ini telah menunjukkan potensi teraputik yang besar dan menjadi asas perintis bagi pembangunan gabungan ubat-ubatan 'anti-neoplastic' pada masa hadapan.

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LIST OF ABBREVIATION

%	Percentage
(v/v)	Volume per Volume
(w/v)	Weight per Volume
±SD	Mean Standard Deviation
×	Times
®	Registered
µM	Micromolar
5-FU	5-Fluorouracil
5-LOX	5-Lipoxygenase
ACA	1'S-1'-acetoxychavicol acetate
ADC	Antibody Drug Conjugates
AEA	1'S-1'-acetoxyeugenol acetate
AFP	Alpha-fetoprotein
AFPR	Alpha-fetoprotein Receptor
APS	Ammonium Persulfate
AR	Androgen Receptor
ATP	Adenosine 5'-Triphosphate
AVMA	American Veterinary Medical Association
Bax	Bcl-2 Associated X Protein
Bcl-2	B-cell Lymphocyte 2
Bcl-X_L	B-cell Lymphocyte X_L
BD	Becton Dickenson
C	Carboxyl

CA	California
CAPE	Caffeic Acid Phenethyl Ester
CARIF	Cancer Research Initiative Foundation
Caspase	Cysteine Aspartate Protease
CBP	CREB Binding Protein
CBP	CBP-CREB Binding Protein
CDDP	Cisplatin
CDI	Coefficient Drug Interaction
CDK	Cyclin Dependent Kinases
CDK4	Cyclin Dependent Kinase 4
CEA	Carcinoembryonic Antigen
CI	Combination Index
cIAP-2	Cellular Inhibitor of Apoptotic Protein-2
CO_2	Carbon Dioxide
COX-2	Cyclooxygenase-2
CTA	Cytotoxic activity
DAB	Diaminobenzidine
DASM	Differential Adiabatic Scanning Microcalorimetry
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DR	Doxorubicin
E3	E3 Ubiquitin Ligase
EBV	Epstein–Barr virus

EDTA	Ethylenediaminetetracetic Acid
EGFR	Epidermal Growth Factor Receptors
ELISA	Enzyme Linked Immunosorbent Assay
ERK	Extracellular-Signal Regulated Kinase
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin Embedded
GEM	Genetically Engineered Mice
GSH	Glutathione Synthetase
H2O2	Hydrogen Peroxide
HCl	Hydrocloric Acid
HDAC-2	Histone deacetylase
HEPA	High-Efficiency Particulate Air
HMEC	Human Mammary Epithelial Cells
HPLC	High- Performance Liquid Chromatography
HRP	Horseradish Peroxidase
Hrs	Hour
HSC-4	Human Squamous Carcinoma- Variant 4
i.p.	Intraperitoneal
IACUC	Institutional Animal Care and Use Committee
IAP	Inhibitors of Apoptotic Proteins
IC_{50}	50% Inhibitory Concentration
IGF-IR	Insulin-Like Growth Factor I Receptors
IHC	Immunohistochemistry

IKK	IκB Kinase
IKKα	IκB Kinase Alpha
IKKβ	IκBα Kinase Beta
IKKγ	IκBα Kinase Gamma
IKKε	IκBα Kinase Epsilon
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
ILAR	Institute of Laboratory Animal Resources
iNOS	Inducible Form of Nitric Oxide Synthase
IκBα	Inhibitor of Nuclear Factor Kappa B Alpha
kDa	Kilodalton
Kg	Kilograms
LPS	Lipopolysaccharides
MAP3K	MAP Kinase Kinase Kinases
MAPK	MAP Kinase
MDR	Multiple Drug Resistance
MEKK3	MAP Kinase/ERK Kinase Kinase Kinase Kinase
MeOH	Methanol
mg	Miligrams
mg/ml	Miligrams per Mililitre
Mins	minutes
ml	Mililitre
mm	Millimeter

mM	Milimolar
mm^3	Milimeter Cube
MMP-9	Matrix Metalloproteinase-9
MRP	Multidrug Resistance Protein
MTT	(3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide)
MW	Molecular Weight
NaCl	Sodium Chloride
NBF	Neutral Buffered Formalin
NCI	National Cancer Institute
NCR	National Cancer Registry
NEMO	NF-Kb Essential Modulator
NF-κB	Nuclear Factor Kappa B
NMR	Nuclear Magnetic Resonance
NPC	Nasopharyngeal Cancer
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NSCLC	Non-Small Cell Lung Cancer
^oC	Degree Celsius
p	*p*-value of Data Statistical Significance
PAGE	Polyacrylamide Gel Electrophoresis
PAMAM	Poly(amidoamine)
PARP	Poly [ADP-ribose] Polymerase
PBS	Phosphate Buffered Saline
PEC	Peritoneal Exudate Cells

PEC	Peritoneal Exudate Cells
PGP	P-glycoprotein
PGP	P-glycoprotein
pH	Potential of Hydrogen
PKB	Protein Kinase B
PLA_2	Phospholipase A_2
PSA	Prostate-Specific Antigen
PSA	Prostate Specific Antigen
PSMA	Prostate-Specific Membrane Antigen Negative
RCA	Replication Competent Adenoviruse
RES	Reticulo-Endothelial System
RES	Reticulo-Endothelial System
rhAFP	Recombinant Human Alpha Fetoprotein
RPMI	Rosewell Park Memorial Institute
s.c.	Subcutaneous
SCC	Squamous Cell Carcinoma
SCLC	Small Cell Lung Cancer
SCNC	Small Cell Neuroendocrine Carcinoma
SDS	Sodium Dodecyl Sulfate
SEM	Standard Error Mean
SPF	Specific Pathogen Free
SSD	Statistical Subdivision
STAT3	Signal Tranducer and Activator of Transcription 3
TAK1	Transforming Growth-Factor-β-Activated Kinase

TBS	Tris-Buffered Sakine
TEMED	N,N,N',N'-Tetramethyl-ethylenediamine
TGF	Transforming Growth-Factor
TGS	Tris-Glysine-SDS
TI	Tumour Implantation.
TMB	3.3', 5.5'-Tetramethylbenzidine
TNF	Tumour Necrosis Factor
TRAF	TNF Receptor–Associated Factors
USA	United State of America
UV	Ultraviolet
V	Volts
VEGF-A	Vascular Endothelial Growth Factor A
VGEF	Vascular Endothelial Growth Factor
Vol	Volume
WHO	World Health Organization
XIAP	X-linked Inhibitor of Apoptosis Protein
β	Beta
γ	Gamma
μ	Micro
μg/ml	Micrograms per Mililitre
μl	Microlitre