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Abstract

3, 11-difluoro-6, 8, 13-trimethyl-8H- quino [4, 3, 2-kl] acridinium methosulfate (RHPS4) is a member of a series of pentacyclic acridines developed at the University of Nottingham, which bind to, and stabilise the structure of G-quadruplex DNA and inhibit the action of telomerase at sub-micromolar concentrations in the cell free TRAP assay and limit cancer cell growth therefore leading to the conclusion that RHPS4 has potential anti-tumour activity. Previous biological studies, however, have suggested that the mechanism of action of RHPS4 may be much more complicated than previously anticipated. Exposure of human melanoma cell lines to low doses of RHPS4 reveal an irreversible cessation of growth and telomere erosion. When used at higher doses, RHPS4 elicits short-term apoptosis/senescence concurrent with an increased number of telomere fusions and this is not a result of telomere shortening suggesting that the mechanism of action of RHPS4 is more than that of a simple telomerase inhibitor. In this study, we have developed a systems biology approach to the analysis and integration of biological data from investigations of the activity of RHPS4. The aim of this approach was to link the emergent properties of the biological studies of RHPS4 to a potential molecular target.

In the laboratory, we are able to quickly derive data with respect to the time- and concentration- effects of RHPS4 on cell cycle distribution, population doublings and senescence levels, however, the kinetics of the cell cycle are neglected. A mathematical model has been derived of adequate complexity to reproduce the biological results of the laboratory and yet sufficient simplicity to yield predictive information and biological insights into the action of this complex molecule has been developed allowing us to integrate the data we can derive. The mathematical model has five compartments, those being X (representative of G_1/G_0 phase), Y (representative of S phase), Z (representative of G_2/M phase), Σ (representative of senescence) and A (representative of apoptosis) with rate and movement between the compartments denoted by the rate parameters k_{xy}, k_{yz}, k_{zx}, $k_{x\Sigma}$ and $k_{\Sigma A}$, respectively. Parameterisation of the mathematical model requires robust data

from a well-characterised cell lines and initially four colorectal cell lines were chosen, namely HCT116, HT-29, KM12 and HCC-2998 to determine the short- and long-term effects of RHPS4 on their cell cycle distributions, population doublings and senescence levels. The aim was to determine which of the cell lines displayed good sensitivity to RHPS4, good growth characteristics in the presence and absence of RHPS4 and tractability in a range of biological assays. Initial studies revealed the HCT116 cell line was the most relevant for the further study of RHPS4. Data regarding the effects of RHPS4 on cell cycle distribution, growth rate and senescence over 21 days was derived and fitted to the five-compartment mathematical model to reveal aspects of the action of RHPS4 that would be difficult to appreciate without it. The model suggests that RHPS4 increases the rate of cells moving into a senescent state (indicated by an increase in $k_{x\Sigma}$ relative to control) and there is inhibition of apoptosis (indicated by a reduction in $k_{\Sigma A}$ relative to control). Data with respect to doxorubicin was also applied to the model and suggested that consistent with RHPS4 there was inhibition of apoptosis relative to control, however, unlike RHPS4, there were initial reductions followed by increases in the rates of cells moving between G_1/G_0 and S by k_{xy}, S and G_2/M by k_{yz} and G_2/M and G_1/G_0 by k_{zx} relative to control in a concentration- and time-dependant manner.

This study has highlighted the important role of mathematics in the understanding of biomolecular processes and this is not the end as we have simply allowed the data to speak for itself. This project is the starting point for further, more focussed studies of RHPS4, and other anti-tumour agents. This approach to the analysis of anti-tumour agents is general, however, allows us preliminary insight into the mode of action of RHPS4 and to relate changes observed to potential underlying molecular targets and further the understanding of this complex molecule.

Abbreviations

^{3}H-Tdr	Tritiated thymidine
ALT	Alternative Lengthening of Telomeres
ATM	Ataxia telangiectasia mutated protein kinase
ATR	ATM and Rad3-related protein kinase
AZT	Azidothymine
bp	Base pairs
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
CDK	Cyclin dependent kinase
CKI	Cyclin dependent kinase inhibitors
CO_2	Carbon dioxide
COPASI	Complex Pathway Simulator
CRUK	Cancer Research UK
dATP	2'-Deoxyadenosine 5'-triphosphate
dCTP	2'-Deoxycytidine 5'-triphosphate
dGTP	2'-Deoxyguanosine 5'-triphosphate
dTTP	2'-deoxythymidine 5'-triphosphate
dH_2O	Distilled water
D-loop	Displacement loop
DMF	N-N' Dimethylformamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ds	Double stranded
ECM	Extracellular matrix
EDTA	Ethylene-diamine-tetra-acetic acid
EGF	Epidermal growth factor
FACS	Fluorescence activated cell sorter
FBS	Foetal Bovine Serum
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
GF	Growth factor
GI_{50}	Concentration of agent at which growth of cells is inhibited by 50%
GJIC	Gap Junctional Intercellular Communication
GS	Mitogenic Growth Signals
HCl	Hydrochloric acid
IC_{50}	Concentration of agent to cause 50% inhibition
IMS	Industrial Methylated Spirits
kb	Kilobases
kDa	Kilodaltons
MAb	Monoclonal antibody
MeOH	Methanol
MPF	M-phase promoting factor

MTT	3-(4, 5- Dimethylthiazol-2-yl)-2, 5-diphenyl-tetarazolium bromide
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
ODE	Ordinary Differential Equation
p53	Tumour protein 53
PARP	Poly (ADP-ribose) polymerase
PBS	Phosphate buffered saline
PBSA	Phosphate buffered saline and 1% bovine serum albumin
PBSAT	Phosphate buffered saline and 1% bovine serum albumin and 0.5% Tween-20
PDE	Partial differential equation
PDGF	Platelet-Derived Growth Factor
PET	Positron emission topography
PFA	Paraformaldehyde
PHA	Phytohaemagglutinin
PI	Propidium iodide
POT 1	Protection of telomere 1 (telomeric DNA binding protein)
Rb	Retinoblastoma
RHPS4	3, 11-difluoro-6, 8, 13-trimethyl-8H-quino [4, 3, 2-kl] acridinium methosulfate
RPM	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RNA	Ribonucleic acid
RNAi	RNA-interference
RNAse A	Ribonuclease A
RNP	Ribonucleoprotein
RT	Room temperature
SA-β-gal	Senescence-Associated β-galactosidase
SD	Standard deviation
SDD	Steady DNA Distribution
ss	Single stranded
SSC	Side scatter
SSD	Steady Size Distribution
siRNA	Silencing RNA
Td	Time taken to complete one population doubling
Tdr	Thymidine
TdT	Terminal Deoxynucleotidyl Transferase
TERC	Telomerse RNA Component
TERT	Telomerase Reverse Transcriptases
TGFα	Tumour Growth Factor α
TIFs	Telomere-dysfunction-induced foci
TIN1	TRF1 interacting protein 1
TIN2	TRF1 interacting protein 2
T-loop	Telomere terminus loop
T_m	Melting point
TPP1	tripeptidyl peptidase I

TRAP	Telomeric repeat amplification protocol
TRF1	Terminal Binding Factor1
TRF2	Terminal Binding Factor 2
UV	Ultraviolet
X-gal	5- Bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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