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.