An Integrative Biological Approach to the Analysis of Tissue Culture Data: Application to the Antitumour agent RHPS4

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Supplementary Data

Development of the Mathematical Model

The five-compartment model that we study is presented in figure 2 in the main paper. We assume that cycling cells flow between compartments G, S and M at rates k_{gs}, k_{sm} and k_{mg} and exit the cell cycle from compartment G by entering the senescent compartment, Σ at rate $k_{g\Sigma}$. Cells in compartment Σ either remain in this compartment or undergo apoptosis at rate $k_{\Sigma c}$ when they enter compartment C. In practice, cell death can occur at any stage of the cell cycle if conditions are unfavourable or if DNA damage has been sustained. However, for simplicity, we assume that the dominant mechanism for cell death is due to senescent cells undergoing apoptosis. The data available from the cell cycle data enables us to determine $\frac{G}{N}, \frac{S}{N}, \frac{M}{N} and \frac{C}{N}$ (i.e. the proportions of cells in each phase of the cell cycle) whilst the senescence data gives us $\frac{\Sigma}{N}$. The population doublings data also gives α (the overall population growth rate). We now explain how to derive formulae which relate the unknown rate parameters (k_{gs}, k_{sm}, etc) to the known experimental data. The system of ordinary differential equations (ODEs) associated with the five-compartment model depicted in figure 2 can be written as follows:

$$\frac{dG}{dt} = 2k_{mg}M - (k_{gs} + k_{g\Sigma})G \qquad \textbf{(4.3.1)}$$

$$\frac{dS}{dt} = k_{gs} G - k_{sm} S \quad \textbf{(4.3.2)}$$

$$\frac{dM}{dt} = k_{sm} S - k_{mg} M \quad \textbf{(4.3.3)}$$

$$\frac{d\Sigma}{dt} = k_{g\Sigma} G - k_{\Sigma c} \Sigma \quad \textbf{(4.3.4)}$$

$$\frac{dC}{dt} = k_{\Sigma c} \Sigma \quad \textbf{(4.3.5)}$$

Since the ODES are linear they admit solutions of the form:

$$G(t) = \hat{G} e^{\alpha t} \text{ etc} \quad \textbf{(4.3.6)}$$

for some constants $\hat{G}, \hat{S}, \hat{M}, \hat{\Sigma}, \hat{C}$ and α.

From equations (4.3.1) – (4.3.6), we have the following relationships:

$$\frac{dG}{dt} = \alpha G, \frac{dS}{dt} = \alpha S \quad \textbf{(4.3.7)}$$

And so on. Substituting (4.3.7) into equations (4.3.1) - (4.3.5) and cancelling by a non-zero factor of $e^{\alpha t}$, we obtain the following algebraic equations for the constants $\hat{G}, \hat{S}$ and $\hat{M}$, together with expressions for $\hat{\Sigma}$ and $\hat{A}$ in terms of $\hat{G}, \hat{S}, \hat{M}$ and system parameters.

$$\alpha \hat{G} = 2 k_{mg} \hat{M} - (k_{gs} + k_{g\Sigma}) \hat{G}, \quad \textbf{(4.3.8)}$$

$$\alpha \hat{S} = k_{gs} \hat{G} - k_{sm} \hat{S}, \quad \textbf{(4.3.9)}$$

$$\alpha \hat{M} = k_{sm} \hat{S} - k_{mg} \hat{M}, \quad \textbf{(4.3.10)}$$

$$\alpha \hat{\Sigma} = k_{g\Sigma} \hat{G} - k_{\Sigma c} \hat{\Sigma}, \quad \textbf{(4.3.11)}$$

$$\alpha \hat{C} = k_{\Sigma c} \hat{\Sigma}. \quad \textbf{(4.3.12)}$$

We remark that for the five-compartment model, compartments G, S and M control the overall system dynamics since their evolution is independent of Σ and C. Further, an expression relating α to system parameters can be obtained by combining equations (4.3.8) – (4.3.12) and noting that for a nontrivial solution, we require $\hat{G}, \hat{S}, \hat{M} > 0$. After some algebra, we obtain the following cubic relating the growth rate to the kinetic rate parameters.

$$0 = \begin{vmatrix} \alpha + k_{gs} + k_{g\Sigma} & 0 & -2k_{mg} \\ -k_{gs} & \alpha + k_{sm} & 0 \\ 0 & -k_{sm} & \alpha + k_{mg} \end{vmatrix} = (\alpha + k_{gs} + k_{g\Sigma})(\alpha + k_{sm})(\alpha + k_{mg}) - 2k_{mg}k_{gs}k_{sm}$$

The five-compartment model contains five rate parameters. We now explain below how it is possible to derive expressions relating these parameters to the experimental data (i.e. $\frac{G}{N}, \frac{S}{N}, \frac{M}{N}, \frac{\Sigma}{N}, \frac{C}{N}$ and α, where $\hat{N} = \hat{G} + \hat{S} + \hat{M} + \hat{\Sigma} + \hat{C}$).

Addition of equations (4.3.8) - (4.3.12) yields:

$$\alpha\hat{N} = k_{mg}\hat{G} \text{ or } k_{mg} = \frac{\alpha}{\hat{M}/\hat{N}} \qquad \textbf{(4.3.13)}$$

With k_{mg} specified in terms of α and $\frac{\hat{M}}{N}$, equation (4.3.10) supplies $\alpha\ (\hat{N} + \hat{M}) = k_{yz}\hat{Y}$ or

$$k_{sm} = \frac{\alpha(1 + \hat{M}/\hat{N})}{\hat{S}/\hat{N}}. \qquad \textbf{(4.3.14)}$$

In a similar manner, equation (4.3.9) supplies the following equation for the rate parameter k_{xy}:

$$k_{gs} = \alpha \frac{\left\{1 + (\hat{S}/\hat{N}) + (\hat{M}/\hat{N})\right\}}{\hat{G}/\hat{N}}. \qquad \textbf{(4.3.15)}$$

Equations (4.3.13) - (4.3.15) specify k_{mg}, k_{gs} and k_{sm} in terms of the experimental data. Equations for $k_{x\Sigma}$ and $k_{\Sigma A}$ are derived by referring to equations (4.3.8) and (4.3.9) which supply:

$$k_{g\Sigma} = \frac{\alpha(\hat{C}/\hat{N})}{\hat{\Sigma}/\hat{N}} \text{ and } k_{g\Sigma}\hat{G} = (\alpha + k_{\Sigma c})\hat{\Sigma} \qquad \textbf{(4.3.16)}$$

or equivalently,

$$k_{\Sigma c} = \frac{\alpha(\hat{C}/\hat{N})}{\hat{\Sigma}/\hat{N}} \text{ and } k_{x\Sigma} = \frac{\alpha(\hat{C}/\hat{N} + \hat{\Sigma}/\hat{N})}{\hat{G}/\hat{N}} \quad \textbf{(4.3.17)}$$

Equations (4.3.13) - (4.3.17) enable us to specify the five rate parameters, k_{gs}, k_{sm}, k_{mg}, $k_{g\Sigma}$ and $k_{\Sigma c}$ from the available data and in doing so, predict the effects of RHPS4, and other anti-tumour agents on the HCT116 cell line that would be difficult to determine otherwise.

Doxorubicin data

Day		Senescence		Doublings		Pre-G1 phase		G1/G0 phase		S phase		G2/M phase	
		Average	±SD	Average	±SD	Average	±SD	Average	±SD	Average	±SD	Average	±SD
4	**Control**	12.3	5.5	8.9	4.8	6.4	2.2	4.5	2.1	2.9	1.3	2.1	0.8
4	**3.15nM**	12.9	5.9	9.4	4.9	6.7	2.3	4.7	2.2	3.1	1.4	2.2	0.8
4	**6.3nM**	20.5	7.4	14.0	9.3	10.2	3.4	7.6	3.7	4.9	2.4	3.7	1.3
4	**31.5nM**	17.7	1.8	9.7	11.3	7.6	5.1	8.0	3.1	5.4	2.5	3.7	1.5
4	**63nM**	39.7	18.3	29.0	15.2	20.8	7.3	14.4	6.8	9.5	4.3	6.9	2.6
6	**Control**	14.2	4.7	9.4	6.7	6.9	2.4	5.4	2.6	3.4	1.7	2.6	0.9
6	**3.15nM**	18.8	3.9	11.3	10.6	8.6	4.1	7.7	3.3	5.1	2.4	3.6	1.4
6	**6.3nM**	33.1	9.4	21.2	16.8	15.8	6.0	12.8	6.0	8.3	4.0	6.1	2.1
6	**31.5nM**	36.7	13.7	25.2	16.2	18.4	6.0	13.6	6.6	8.7	4.2	6.5	2.3
6	**63nM**	44.9	16.8	30.8	19.9	22.5	7.4	16.6	8.1	10.7	5.1	8.0	2.8
10	**Control**	18.0	5.6	11.8	8.7	8.7	3.1	6.9	3.3	4.4	2.1	3.3	1.1
10	**3.15nM**	21.5	7.2	14.3	10.1	10.5	3.6	8.1	3.9	5.2	2.5	3.9	1.3
10	**6.3nM**	30.1	4.6	17.4	18.0	13.3	7.5	12.9	5.2	8.6	4.0	5.9	2.4
10	**31.5nM**	49.7	18.8	34.3	21.9	25.0	8.2	18.3	8.9	11.8	5.7	8.8	3.1
10	**63nM**	50.3	14.3	32.3	25.4	24.0	9.1	19.5	9.1	12.5	6.0	9.2	3.3
14	**Control**	18.5	5.6	12.1	9.2	8.9	3.3	7.1	3.4	4.6	2.2	3.4	1.2
14	**3.15nM**	27.3	6.1	16.7	14.9	12.6	5.7	11.1	4.8	7.2	3.4	5.1	1.9
14	**6.3nM**	32.5	2.9	17.7	20.9	13.8	9.6	14.8	5.7	10.0	4.6	6.8	2.9
14	**31.5nM**	43.6	12.7	28.2	21.9	20.9	7.8	16.8	7.9	10.8	5.2	8.0	2.8
14	**63nM**	51.7	14.6	33.2	26.3	24.7	13.1	21.4	7.2	13.9	7.1	9.4	3.9
21	**Control**	18.8	4.9	11.9	9.8	8.9	3.6	7.4	3.4	4.8	2.3	3.5	1.3
21	**3.15nM**	30.9	10.8	20.9	14.2	15.3	5.1	11.5	5.6	7.4	3.6	5.5	1.9
21	**6.3nM**	37.1	5.8	21.4	22.1	16.4	9.2	15.9	6.5	10.5	4.9	7.3	2.9
21	**31.5nM**	47.3	9.4	28.3	26.8	21.5	10.5	19.6	8.3	12.8	6.0	9.0	3.5
21	**63nM**	59.1	8.7	33.9	35.7	26.1	15.1	25.6	10.3	17.0	7.8	11.7	4.7

RHPS4 data

Day		Senescence		Doublings		Pre-G1 phase		G1/G0 phase		S phase		G2/M phase	
		Average	±SD	Average	±SD	Average	±SD	Average	±SD	Average	±SD	Average	±SD
4	Control	18.7	2.9	3.4	1.2	4.6	0.8	56.8	7.6	20.9	5.3	15.1	6.08
4	50nM	37.4	2.6	3.3	1.1	5.3	4.5	59.9	7.5	16.2	4.7	15.7	1.83
4	100nM	36.6	1.9	3.1	0.9	6.9	5.7	61.5	6.2	17.4	3.8	16.7	3.79
4	1uM	39.2		2.5	0.9	6.7	5.5	58.4	10.7	20.2	3.6	15.3	2.40
6	Control	18.8	6.8	4.5	1.1	6.2	1.6	55.6	9.7	22.1	4.0	18.9	3.22
6	50nM	35.1	10.3	4.0	1.1	0.9	0.6	61.1	3.6	16.8	2.2	17.0	5.23
6	100nM	43.8	5.1	3.7	0.7	1.8	0.9	61.3	0.4	16.7	3.2	15.7	5.16
6	1uM	53.1	4.9	2.6	1.1	1.2	1.7	60.7	12.1	16.7	3.5	13.1	2.81
10	Control	14.8	3.0	7.7	0.9	7.1	4.4	59.0	7.9	19.4	5.1	14.8	5.17
10	50nM	31.4	9.9	7.0	1.1	6.6	3.1	67.8	0.3	15.8	3.9	9.2	2.00
10	100nM	40.8	7.4	6.4	0.9	2.9	2.8	74.3	6.3	11.9	2.7	6.0	0.57
10	1uM	42.9		3.7	1.4	1.1	0.1	70.1	6.4	11.5	3.7	15.3	5.66
14	Control	19.8	4.5	9.8	1.0	4.3	3.5	51.1	5.7	20.5	2.1	22.1	
14	50nM	47.4	3.3	8.9	1.0	1.2	0.9	66.7	6.2	18.3	0.5	17.8	4.03
14	100nM	52.4	5.0	8.2	1.1	2.5	2.4	73.1	3.5	7.3	1.5	15.2	9.12
14	1uM	66.3		3.3	1.5	2.9	1.2	69.1	8.5	13.1	1.6	9.9	1.98
21	Control	18.0	3.1	13.2	1.0	4.4	2.7	46.9	13.3	22.7	3.6	21.3	8.24
21	50nM	43.6	4.8	11.5	1.0	3.0	2.4	63.2	9.9	17.6	4.3	14.6	5.85
21	100nM	45.8	2.8	10.7	0.8	5.1	3.0	63.5	17.4	15.5	10.3	16.1	10.31
21	1uM	100.0	0.0	5.1	3.3	4.4	2.6	76.8	8.4	9.6	4.3	9.8	5.16