Computer modelling of nocodazole exposure on cell cultures *in vitro*

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1 - Introduction

Life science is an extremely fertile field with regard to interdisciplinarity and technology transfer. Physiomics, a company specialised in oncology modelling, and the Institute of Life Science at Swansea University (ILS) have entered into a collaborative research program. The goal of this collaboration was to validate Physiomics models and technology on supercomputers, such as the High Performance Computer "Blue C" available at the ILS, and provide new biological insights in the dynamic of antimitotic agents.

The genetic toxicology group of Dr Shareen Doak at the ILS has produced experimental data on the dose-response relationship of nocodazole exposure with mitotic arrest and aberrant mitosis. Nocodazole (Fig. 1) is an anti-neoplastic agent which reversibly impairs tubulin assembly, resulting in blocking cells in mitosis[1].

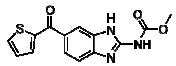

Fig. 1. Nocodazole molecule

In order to simulate the effect of drugs on a cell culture, Physiomics has used these data to build a model of nocodazole exposure and ported its cell population simulator called SystemCell® to "Blue C".

Supercomputing now plays a key role in medical science, and this simulation platform will be particularly useful to design optimal delivery schedules for anti-mitotic agents.

2 - Experimental results

Material and Methods

Human prostate epithelial PNT2 cells were cultured in the presence of nocodazole for a defined number of cell cycles (Fig. 2).

• Mitotic index was calculated as a proportion of interphase cells compared to mitotic cells. 1000 cells scored per treatment, carried out in triplicate.

• Aberrant metaphase cells are shown in Fig. 2 and quantified in Fig. 3 A. One hundred metaphase cells scored per treatment, carried out in triplicate.

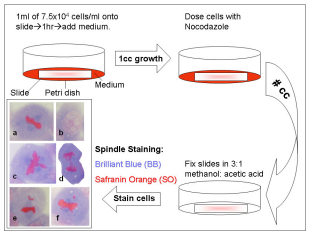

Fig. 2. Staining technique used for image analysis of mitotic machinery following treatment with nocodazole. Abbreviations: cc, cell cycle; V79 cells shown here for representation; a, metaphase; b, interphase; c-f, aberrant; c, tripolar; d, bridge late anaphase; e, lagging chromosome; f, dislocated chromosome.

Results

Dose-response nocodazole data-set
The data illustrated in Fig. 3 A demonstrates that nocodazole induced a significant ($p<0.05$) increase in mitotic index (MI) in PNT2 cells, with a no-observed effect level (NOEL) at 50nM and lowest-observed effect level (LOEL) at 60nM. With respect to aberrant metaphase cells, a NOEL was observed at 40nM nocodazole, while the LOEL was 50nM. Thus, a non-linear dose response was observed for both cell viability and cell cycle aberrations after treatment with nocodazole.

Time-series measurements
As can be seen in Fig. 3 B, the 80nM nocodazole dose causes over a 4-fold increase in mitotic index up to 24h exposure, due to metaphase arrest. With longer exposure periods up to 4 cell cycles, the MI is still substantially higher than in the control (0nM) treatments, but there is a steady decline from the 24h time-point.

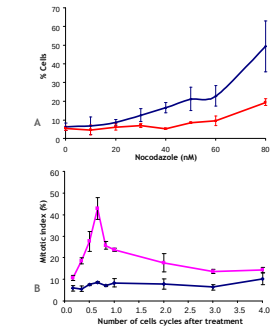

Fig. 3. Experimental effect of nocaodazole on cell culture. A: Cell division aberrations (blue line) and mitotic index (red line) induced by nocodazole in the PNT2 cell line. B: Cell division aberrations induced by nocodazole in the PNT2 cell line. Control (blue line) and 80nM nocodazole (pink line). Data presented are means and standard deviations (1SD) calculated from raw data with each dose point representing an experiment carried out in triplicate.

3 - Core Cell Cycle Model

The **eukaryotic cell cycle** is usually divided into four phases (→ G1 → S → G2 → M →): the S-phase (synthesis -- DNA replication), the M-phase (mitosis, cytokinesis) and two gaps phases G1 and G2 (Fig. 4).

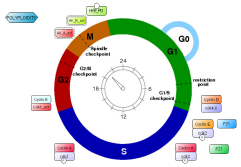

Fig. 4. Schematic representation of the cell cycle and its main players.

Our **agent-based cell cycle model** breaks down the cell cycle progression into the 4 phases: G1, S, G2, and M where transition depends on mathematical functions (Fig. 5). Apoptosis can occur stochastically at any phase (basal rate) but can also be phase-specific and be enhanced by the effect of drugs.

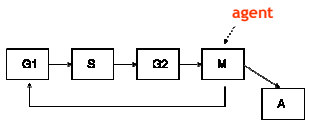

Fig. 5. Schematic representation of the agent-based cell cycle model

4 - SystemCell® cell population simulator on "Blue C" High Performance Computer

SystemCell® is a **cell population simulator**. Each cell is an instance of the agent-based model which determines the cell's fate including proliferation, polyploidy, or apoptosis. Several implementations of SystemCell® have been developed, one of them on a High Performance Computer "Blue C" available at Institute of Life Science, University of Swansea[2].

SystemCell® allows the monitoring of any kind of variable in the population, reproducing typical experiments such as FACS (monitoring the overall DNA content), the phosphorylation state of biomarkers or the mitotic index. It is also possible to simulate the "injection" of a drug concentration-time profile into the cell population, providing a **fully integrated PK/PD simulation framework** (Fig. 6).

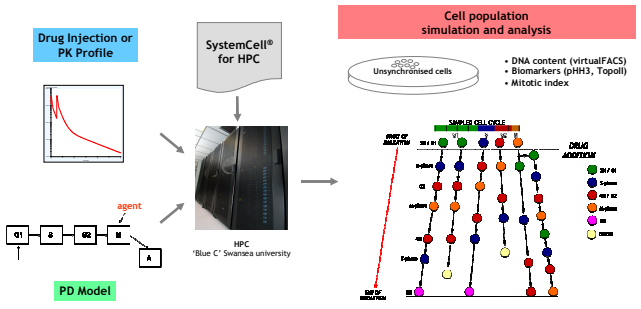

Fig. 6. PK/PD simulations using the SystemCell® cell population simulator. The continuous infusion or the PK profile of a drug can be injected into the PD model and the effect of the agent on the cell population monitored through time.

The HPC facility "Blue C" is currently based on IBM's pSeries Power5+ technology and has been regularly upgraded through a rolling upgrade scheme. It is configured with a high performance high bandwidth and low latency interconnect with disk and tape storage to exploit parallel programming methods in the Life Science environment. Based on Linpack Test performance, in June 2005 it ranked highly in the top500 list (1.7 teraflops). Code parallelism and execution on two 16-CPU nodes confer an increase in performance of SystemCell® by a factor ~30 compared with single-processor implementation (Fig. 7).

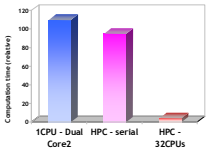

Fig. 7. SystemCell® cell population simulator parallel version performance on HPC. Left: comparative cell population simulation times on a single processor desktop computer and on a 32-CPU node on "Blue C".

5 - Model building, calibration and simulations

Modelling the pharmacodynamic effect of nocodazole
In our model, nocodazole delays M phase progression in a concentration-dependent manner (Fig. 8 A & B). At the start of a cell population simulation, cells are unsynchronised (Fig. 8 C). When nocodazole is present, the M phase duration of each cell is increased, leading to a partial synchronisation of cells in the population (Fig. 8 D). This also leads to an increase of the mitotic index, as more cells are stopped in M phase at the same time. The non-linearity of this effect, which was observed experimentally (Fig. 3 A), has been included in the mathematical model (Fig. 9 C). In simulations, the mitotic index dramatically increases between 60nM and 80nM.

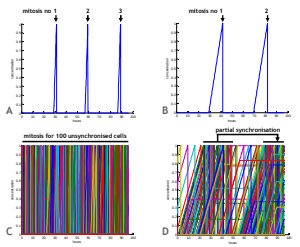

Fig. 8. Nocodazole-mediated M phase delay by activation of spindle checkpoint in cell population simulations. A & B: M phase progression for 1 cell in population in control (A) or in presence of 80nM nocodazole (B). C & D: superimposition of M-phase progression for 100 unsynchronised cell culture in control (C) or in presence of nocodazole (D). M phase delay in population leads to cell synchronisation and mitotic index increase.

Nocodazole effect on mitotic arrest
The model is parameterised to reproduce the mitotic index increase that is observed immediately after addition of nocodazole (Fig. 3 B). Interestingly, the M phase delay caused by nocodazole conditions the amplitude of the mitotic index increase, but not the slope of that increase (Fig. 9 A). In absence of any nocodazole-mediated apoptosis, the mitotic index can theoretically reach a level of 100%, which corresponds to the entire cell population arrested in M phase. The mitotic index then decreases again when cells finally exit mitosis and start a new cell cycle (Fig. 9 A). On the other hand, the total cell cycle duration will influence the slope of the mitotic index increase (Fig. 9 B). This allows the fine tuning of the doubling time of cells to fit experimental values.

Apoptosis parameterisation
Apoptosis is a function of the mitotic arrest duration and nocodazole concentration, and is also calibrated according to experimental values (Fig. 3 B). When cells go into apoptosis, they are removed from the cell culture, leading to a sharp decrease of surviving cells after the first cell cycle in presence of nocodazole. This is shown in Fig. 10: in order to accurately simulate the effect of nocodazole, it is necessary to combine both the mitotic arrest effect and the apoptosis. Fig. 10 C overlays both mitotic arrest and apoptotic effects and shows how the combination between M phase arrest and cell death leads to the experimental observations.

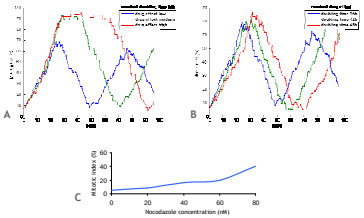

Fig. 9. Mitotic index calibration in absence of nocodazole-mediated apoptosis. A: mitotic index timecourse for three levels of nocodazole effect on M phase delay ; B: mitotic index timecourse for three cell cycle duration values using constant nocodazole effect. Total cell cycle duration influences the slope of the mitotic index increase whereas agent-mediated M phase delay only conditions the amplitude of the mitotic index increase. C: non-linear response of nocodazole as observed in simulations, mitotic index at 48h after nocodazole addition;

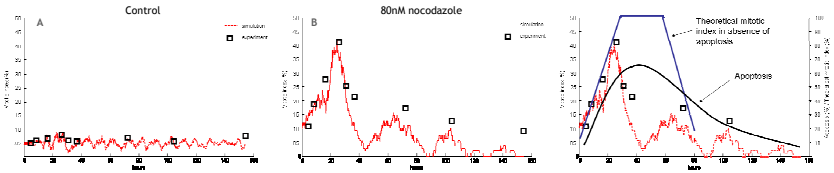

Fig. 10. Simulation of nocodazole in a cell culture. A: control, mitotic index stays constant. B: effect of 80nM nocodazole. C: Breakdown of the two overlapping effects of nocodazole: a mitotic index increase caused by spindle checkpoint delay in M phase and drug-mediated apotosis.

6 - Conclusion

The collaborative research project between Physiomics and the ILS has fulfilled several objectives. Firstly, we designed a novel *in silico* model of the effect of nocodazole, an anti-mitotic agent widely used in cell cycle research. This model could be validated using the experimental data provided by the genetic toxicology group at the ILS. Secondly, we tested the High Performance Computer "Blue C" available at the ILS, which allows performing calculation-intensive simulations. This technological platform allows the simulation of *in* vitro cell populations, such as cell cultures, or *in vivo* neoplastic cells, such as tumours. Since nocodazole belongs to the wider class of anti-mitotic agents, its accurate representation will allow simulating the effect of other similar molecules used as anticancer drugs, such as taxol or docetaxel. This massive computational capability will play a key role in designing optimal therapeutical schedules of antimitotic drugs, in isolation or in combination with other anticancer drugs.

References:
[1] Hoebeke *et al.*, Biochemical and Biophysical Research Communications 69 (1976), 319-324.
[2] http://www.ils.swan.ac.uk/supercomputing.html