

Studying the Non-Linearity of Tumour Cell Populations under Chemotherapeutic Drug Influence¹

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Abstract: Biological systems are characterized by their potential for dynamic adaptation. Such systems, whose properties depend on their initial conditions and response over time, are expected to manifest non-linear behaviour. In a previous work we examined the oscillatory pattern exhibited by leukemic cells under *in vitro* growth conditions, where the system was simulating the dynamics of growth with disease progression. Our question in the previous study evolved around the nature of the dynamics of a cell population that grows, or even struggles to grow, under treatment with chemotherapeutic agents. We mentioned several tools that could become useful in answering that question, as for example the *in vitro* models which provide information over the spatio-temporal nature of such dynamics, but *in vivo* models could prove useful too.

In the present work we have studied the non-linear effects that arise from cell population dynamics during chemotherapy. The study was performed not only in the sense of cell populations *per se* but also as an attempt of identifying sub-populations of cells, such as apoptotic cells and cells distributed within the cell cycle. The temporal transition from one state to the next was revealed to follow non-linear dynamics. We have managed to approximate the non-linear factor that influences these temporal space transitions. To the best of our knowledge there are not many studies dealing with this topic, which makes it even more interesting. Such approaches could become very useful in understanding the nature of cell proliferation and the role that certain chemotherapeutic drugs play in cell growth, with emphasis given on the underlying drug resistance and cell differentiation mechanisms.

Keywords: Proliferation, oscillations, non-linearity, CCRF-CEM, glucocorticoids.

1. Introduction

Population dynamics have been the subject of study of various groups. It has already been shown that even cells growing under normal conditions can manifest proliferation dynamics of non-linear nature [1, 2]. In addition, other groups have demonstrated that this non-linear behaviour can also exist under the influence of drugs [3], or similarly, under the influence of environmental factors. Any new knowledge on the mechanisms underlying cell proliferation is

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of major importance, and even the smallest of indications towards a certain direction could enable us to further discover differences in the mechanisms distinguishing between health and disease. This issue is especially important in tumors, the incidence of which is approaching that of an epidemic. In the present study we have focused on the dynamics that have been revealed through an *in vitro* cell system and particularly on the dynamics manifested under the influence of a certain type of chemotherapeutic i.e. glucocorticoids. Glucocorticoids (GC) are among the most important alternatives in the treatment of leukemia. Resistance to glucocorticoids represents a crucial parameter in the prognosis of leukemia [4-6], whereas it has been shown that GC-resistant T-cell leukemia cells manifest a biphasic mechanism of action or imply an inherent resistance mechanism of action to glucocorticoids [7]. New questions arise regarding the nature of the dynamics of a cell population under the influence of a drug. If certain physical measures, such as proliferation, are observed on the phenotypic level, how are these translated on the molecular, genomic level? For example, if a cell population increases its rate of proliferation, does it mean that the genes required for this effect transcribe faster than usual? An interesting report by *Mar et al. (2009)* suggested that gene expression takes place in quanta, i.e. that it happens discretely and not continuously [8, 9]. Also, in two other reports it was suggested that gene expression follows oscillatory patterns, which makes things even more complicated with regards to the proliferation rate, be it growth acceleration or deceleration [10, 11]. This means that cells cannot simply transit from one state to another in terms of growth rate. Should the hypothesis of oscillatory modulation of gene expression, which implies non-linearity, stand correct, then a much more complicated regulatory pattern is required by a cell so as to change its state, as a function of environmental stimuli. The present work provides evidence supporting this view, with respect to glucocorticoids.

2. The Model and Simulations

In order to establish a modeling approach to the phenomenon described above, we have discriminated between different cell populations. That is, if at time t a cell population is considered to be N , then this is a mixture of cells in various stages. More specifically, we have discriminated between the cell cycle phases and cell death. The cell cycle is the path through which cells manifest proliferation. The identification of cells in specific cell cycle phases is of critical importance since it will determine cellular proliferation, cessation or cell death. Also, in various systems the detection of cells at specific cell cycle points denotes a mechanism of reaction to an environmental stimulus, as for example in the present case is the glucocorticoid. In Figure 1, we present the model diagrammatically.

The diagram in Figure 1, represents the three phases of the cell cycle. Where, $G_{1,t}$, $G_{1,t+1}$, $G_{1,t+n}$ is the number of cells in G_1 phase at time t , $t+1$ and $t+n$ respectively, S_t , S_{t+1} , S_{t+n} is the number of cells in S phase at time t , $t+1$, $t+n$ respectively, $G_{2,t}$ is the number of cells in G_2 phase at time t , $t+1$, $t+n$ respectively and CD_t , CD_{t+1} , CD_{t+n} is the number of dead cells at time t , $t+1$,

$t+n$ respectively. The arrows connecting the different cell states denote the possibilities that a cell has to transit from one state to another. So, for example, a cell in G_1 phase has three possibilities: to remain in the G_1 phase, to transit to the S phase or to become apoptotic i.e. cell death (CD). This means that it is impossible for the cell to go from the G_1 phase to G_2 phase. A very important factor denoted in Figure 1 is the $K_{factor,t}$, which denotes the rate of transition from one cell state to another. Hence, the factor k will take the following subscripts:

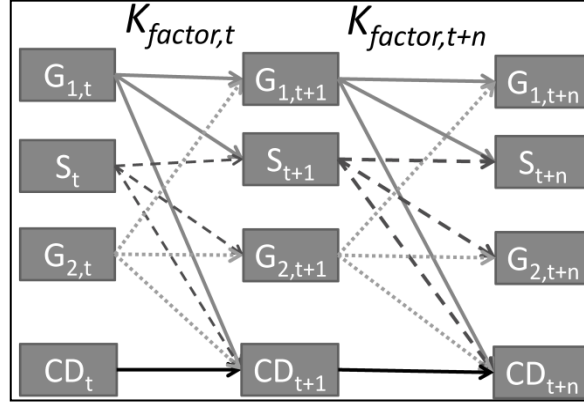


Fig. 1. A schematic representation of the model approach for cell population showing transitions between cell cycle phases and cell death.

$$\begin{aligned}
 G_{1,t} &\rightarrow G_{1,t+1}: k_1, G_{1,t} \rightarrow S_{t+1}: k_2, G_{1,t} \rightarrow CD_{t+1}: k_3, \\
 S_t &\rightarrow S_{t+1}: k_4, S_t \rightarrow G_{2,t+1}: k_5, S_t \rightarrow CD_{t+1}: k_6 \\
 G_{2,t} &\rightarrow G_{2,t+1}: k_7, G_{2,t} \rightarrow G_{1,t+1}: k_8, G_{2,t} \rightarrow CD_{t+1}: k_9 \\
 CD_t &\rightarrow CD_{t+1}: k_{10}
 \end{aligned}$$

The following equations describe the transitions from one state to the next:

$$N_{G_{1,t+1}} = N_{G_{1,t}} \cdot k_1 + N_{G_{2,t}} \cdot k_8$$

$$N_{S_{t+1}} = N_{S_t} \cdot k_4 + N_{G_{1,t}} \cdot k_2$$

$$N_{G_{2,t+1}} = N_{G_{2,t}} \cdot k_7 + N_{S_t} \cdot k_5$$

$$N_{CD_{t+1}} = N_{CD_t} + N_{G_{1,t}} \cdot k_3 + N_{G_{2,t}} \cdot k_9 + N_{S_t} \cdot k_6$$

Where, N denotes the respective cell population at time t . These equations could be formulated in more generalized form since each population at time $t+1$ consists of two other populations at time t . Hence, the generalized form would be:

$$N_{p_x,t+1} = N_{p_y,t} k_y + N_{p_z,t} k_z$$

In other words, our model shows that the next state is defined by the previous one. Each cell subpopulation consists of parts of the other subpopulations.

These equations appear to be of linear form and are simple to solve. Yet, the factor k is a non-linear factor, which can be determined only experimentally. It is dependent upon environmental factors $f(\text{environmental})$, such as nutrient availability and space, and in the present case is a function of glucocorticoid concentration $f(C_p)$. In Figure 2, experimental measurements are presented as an effort to calculate the rate of population change for the total population and data were fitted with Fourier series. We have reported this previously, that cell populations defined experimentally, could be described with Fourier series, with respect to the transition factor k [12].

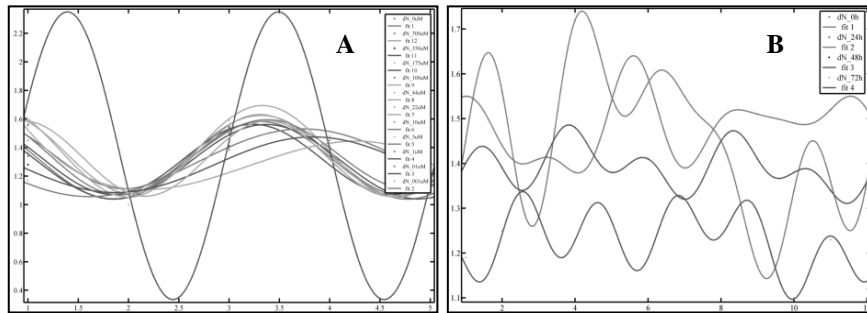


Fig. 2. Simulating the factor k in relation to time (**A**) and glucocorticoid concentration (**B**) showed that both could be fitted with Fourier series. In (**A**) the x-axis corresponds to experimental values from time point measurements of cell numbers, while each curve corresponds to the respective k factor of each glucocorticoid concentration. Similarly, in (**B**) the x-axis corresponds to the glucocorticoid concentrations and each curve corresponds to the time points measured.

The generalized form of the series we have used for our approach was given by:

$$f(x, y) = a_0 + a_1 \cos(xy) + a_2 \sin(xy)$$

Hence, the factor k for each transition, meaning from one cell state to the next would be given by the following system of equations:

$$\begin{aligned}
k_1 &= a_{0,1} + a_{1,1} \cos(N_{G_{1,t}} \cdot N_{G_{1,t+1}}) + a_{2,1} \sin(N_{G_{1,t}} \cdot N_{G_{1,t+1}}) \\
k_2 &= a_{0,2} + a_{1,2} \cos(N_{G_{1,t}} \cdot N_{S_{t+1}}) + a_{2,2} \sin(N_{G_{1,t}} \cdot N_{S_{t+1}}) \\
k_3 &= a_{0,3} + a_{1,3} \cos(N_{G_{1,t}} \cdot N_{CD_{t+1}}) + a_{2,3} \sin(N_{G_{1,t}} \cdot N_{CD_{t+1}}) \\
k_4 &= a_{0,4} + a_{1,4} \cos(N_{S_t} \cdot N_{S_{t+1}}) + a_{2,4} \sin(N_{S_t} \cdot N_{S_{t+1}}) \\
k_5 &= a_{0,5} + a_{1,5} \cos(N_{S_t} \cdot N_{G_{2,t+1}}) + a_{2,5} \sin(N_{S_t} \cdot N_{G_{2,t+1}}) \\
k_6 &= a_{0,6} + a_{1,6} \cos(N_{S_t} \cdot N_{CD_{t+1}}) + a_{2,6} \sin(N_{S_t} \cdot N_{CD_{t+1}}) \\
k_7 &= a_{0,7} + a_{1,7} \cos(N_{G_{2,t}} \cdot N_{G_{2,t+1}}) + a_{2,7} \sin(N_{G_{2,t}} \cdot N_{G_{2,t+1}}) \\
k_8 &= a_{0,8} + a_{1,8} \cos(N_{G_{2,t}} \cdot N_{G_{1,t+1}}) + a_{2,8} \sin(N_{G_{2,t}} \cdot N_{G_{1,t+1}}) \\
k_9 &= a_{0,9} + a_{1,9} \cos(N_{G_{2,t}} \cdot N_{CD_{t+1}}) + a_{2,6} \sin(N_{G_{2,t}} \cdot N_{CD_{t+1}}) \\
k_{10} &= 1
\end{aligned}$$

We could write this system of equations in a more generalized form, which would be:

$$k = a_0 + a_1 \cos(N_{p_{y,z,t}} N_{p_{x,t+1}}) + a_2 \sin(N_{p_{y,z,t}} N_{p_{x,t+1}})$$

Where k is the transition factor, $a_{0,1,2}$ are constants, $N_{p_{1,t}}$ and $N_{p_{2,t+1}}$ are the populations implicated in the transition at time t and $t+1$ respectively.

Substituting the equation describing the generalized k with the equation of the generalized $N_{p,t+1}$ we obtain:

$$\begin{aligned}
N_{p_{x,t+1}} &= N_{p_{y,t}} \left[a_0 + a_1 \cos(N_{p_{y,t}} N_{p_{x,t+1}}) + a_2 \sin(N_{p_{y,t}} N_{p_{x,t+1}}) \right] \\
&\quad + N_{p_{z,t}} \left[a_0 + a_1 \cos(N_{p_{z,t}} N_{p_{x,t+1}}) + a_2 \sin(N_{p_{z,t}} N_{p_{x,t+1}}) \right]
\end{aligned}$$

This equation describes the transition of a cell population from one state to the next but it cannot be solved analytically. Solutions can only be found numerically, since future populations (N_x) depend on the previous ones and on the fraction of other future cell populations ($N_{y,z}$). In Figure 3, we have performed numerical approximations of the function in order to represent it schematically. The function appeared to give interesting dynamics as it manifested a saddle point. Also, these phenomena were time dependent, as clearly seen on the experimental level. Thus, by differentiating with respect to time we could obtain a possible role of the temporal factor in this system. Similarly, we have made numerical approximations in order to design the dynamics of the first derivative for both variables, that is $N_{p,y}$ and $N_{p,z}$. The result is presented in Figure 4.

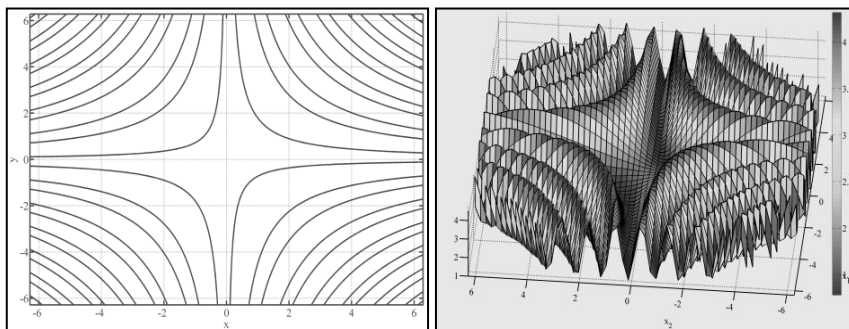


Fig. 3. Using a numerical approximation of the function describing the population transitions manifested interesting dynamics as they formed a saddle.

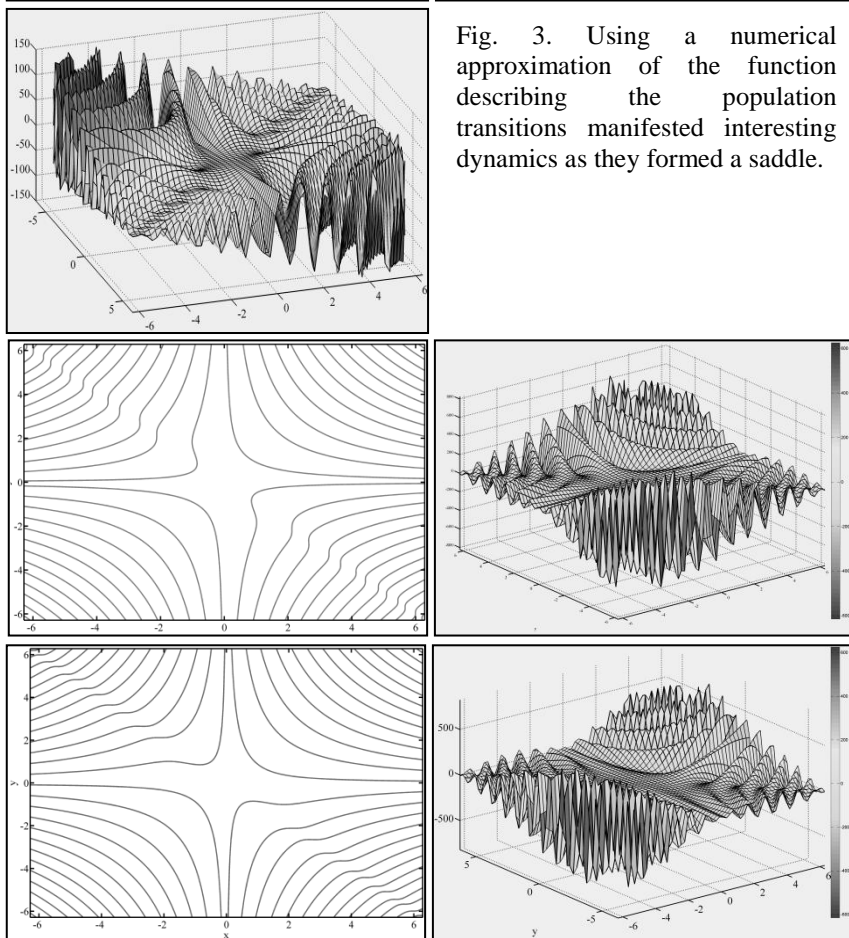


Fig. 4. Numerical representation of the first partial derivative with respect to $N_{p,y}$ (upper left and right) and with respect to $N_{p,z}$ (lower left and right).

3. Conclusions

In the present work we have attempted to identify non-linear factors of cell proliferation under the influence of chemotherapeutics, and more specifically under the influence of the glucocorticoid prednisolone. We have attempted to establish an initial theoretical framework for the analysis of such phenomena and for future considerations. Cell growth appeared to be of a non-linear character. This knowledge could prove useful in the treatment of tumors since understanding the biology of proliferation would lead us to a better understanding of cellular resistance to chemotherapeutics. Biological systems are extremely complicated and they manifest, without doubt, non-linear/chaotic phenomena. Therefore, as we have mentioned in previous works, we believe that the maturity of biological sciences would come through integration with other disciplines, such as mathematics and physics, and the ability to give generalized models for these phenomena. Such an example is the understanding of cell proliferation in which we attempted to contribute with hints.

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