Article Title: The Dynamics of Cell Proliferation

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Abstract

The article provides a mathematical description based on the theory of differential equations, for the proliferation of malignant cells (cancer). A model is developed which enables us to describe and predict the dynamics of cell proliferation much better than by using ordinary curve fitting procedures. By using differential equations the ability to foresee the dynamics of cell proliferation is in general much better than by using polynomial extrapolations. Complex time relations can be revealed. The mass of each living cell and the number of living cells are described as functions of time, accounting for each living cell's age since cell-birth. The linkage between micro dynamics and the population dynamics is furnished by coupling the mass increase of each living cell up against the mitosis rate. A comparison is made by in vitro experiments with cancer cells exposed to digitoxin, a new promising anti cancer drug. Theoretical results for the total number of cells (living or dead) is found to be in good agreement with experiments for the cell line considered, assuming different concentrations of digitoxin. It is shown that for the chosen cell line, the proliferation is halted by an increased time from birth to mitosis of the cells. The delay is probably connected with changes in the Ca concentration inside the cell. The enhanced time between the birth and mitosis of a cell leads effectively to smaller mitosis rates and thereby smaller proliferation rates. This mechanism is different from the earlier results on digitoxin for different cell lines where an increased rate of apoptosis was reported. But we find it reasonable that cell lines can react differently to digitoxin. A development from enhanced time between birth and mitosis to apoptosis can be furnished, dependent of the sensitivity of the cell lines. This mechanism is in general very different from the mechanism appealed to by standard chemotherapy and radiotherapy where the death ratios of the cells are mainly affected. Thus the analysis supports the view that a quite different mechanism is invoked when using digitoxin. This is important, since by appealing to different types of mechanism in parallel during cancer treatment, more selectivity in the targeting of benign versus malignant cells can be invoked. This increases the probability of successful treatment. The critical digitoxin level concentration, i.e. the concentration level where the number of living cells is not increasing, is approximately 50 ng/ml for the cell line we investigated in this article. Therapeutic plasma concentration of digitoxin when treating cardiac congestion is about 15-33ng/ml, but individual tolerances are large. The effect of digitoxin during cancer treatment is therefore

very promising. The dynamic model constitutes a new powerful tool, supported by empirics, describing the mechanism or process by which the number of malignant cells during anticancer treatment can be studied and reduced.

1 Introduction

A considerable amount of experiments has been carried out to test the growth and proliferation of cells. Although this literature is quantitative and technical in nature, with suggested polynomials describing the cell growth and cell proliferation, lacking is to our knowledge a mathematical differential equation describing the process. By using differential equations the ability to foresee the dynamics of cell proliferation is much better than by using polynomial extrapolations. Complex time relations can be revealed.

Of fundamental importance for all living cells is the ability to divide (mitosis) or to die by apoptotic or necrotic death. During treatments of living organisms possessing malignant cells (cancer), chemotherapy and/or radiotherapy are/is frequently used. Cells are very sensitive to cytostatics or radiation during the G2-M phase in the cell cycle. Therefore the increased relative amount of deaths caused by the treatment during a short time interval is proportional with the mitosis rate. The increased death rate caused by chemotherapy and/or radiotherapy for cell lines with large mitosis rates will then lead to a large negative value of the mitosis rate minus the death rate, i.e. a strongly negative proliferation rate. Disappointingly, this is also an obstacle to treatment since benign cells with mitosis rates of the same or higher order will be strongly attacked and reduced in number and functional quality by such a treatment. This is due to the non-selective action of most of the standard chemotherapeutic drugs or radiation. If the mitosis rate is larger than the death rate, and both are small during cancer development, the corresponding negative proliferation rate caused by treatment is small, and the number of cells are therefore almost unaffected during “treatment”. By using small amounts of cytostatics over long time spans, the benign cells with high mitosis/deaths rates are more affected than the malignant cells with low proliferation.

It is well known that increased Ca ions concentrations in the interior of cells strongly enhance the probability of so called apoptotic deaths of the cells (Russo 1982). Inhibition of the Na-K pump can indirectly increase the apoptotic death rates of cells since the reduced effect of the Na-K pump leads to higher Na concentration in the cells’ interior. Therefore the effect of the NA-Ca exchanger which pumps Na ions in and Ca ions out of the cell by use of the Na gradient over the cell membrane, is reduced. This leads to higher Ca concentration in the cells’ interior and increased probability of apoptotic deaths. Recently, analysis of herbal extracts used in alternative medicine has revealed that some of these also contain cardiac

glycosides, which have been known for a long time to inhibit proliferation of cancer cells by inhibition of the Na-K pump across the cell membrane. Inspired by the results presented on the anti-cancer effects of cardiac glycosides, Haux et al. (1999) examined the effect of the cardiac glycoside digitoxin, and more specifically the effect of digitoxin on different but typically malignant cell lines. They showed that digitoxin inhibited the proliferation of cells for most of the malignant cell lines by increasing the number of apoptotic cells. However, and interestingly, the normal cell lines consisting of lines with large mitosis/deaths rate and slow mitosis/death rates, were not affected by the digitoxin treatment. This means that a new sort of selectivity in the targeting of benign versus malignant cells, which is not directly linked to the proliferation rates of cell lines, is invoked when using digitoxin. In the experiments the concentration of digitoxin was not higher than in standard treatments of cardiac diseases. Therapeutic digitoxin concentration does not seem to give any bad side effects in persons with or without cardiac diseases (Grossmann 1998). Further, Stenkvist et al (1979) found that five years after mastectomy the recurrences among breast cancer patients not taking digitalis were ten times that in patients taking digitalis. Also, Moxnes and Hausken (2003) provided a mathematical dynamic description of the interaction between the organism and the drug, analysing the dynamics by using ordinary differential equations.

Inspired by these very promising results, this article builds a mathematical model in order to study the proliferation of cells in a more fundamental way. For the cell line the model's intrinsic predictive power is used to analyze the effect of using digitoxin. Of special interest is the examination of whether the cell proliferation when using digitoxin follows from very simple analytical relationships where the effect of digitoxin can be captured by a simple re-scaling of the death coefficient of the cells. This mechanism is typically invoked during standard chemotherapy and radiotherapy. The analysis supports the view that a quite different mechanism is invoked when using digitoxin. This is important, since by appealing to different types of mechanism in parallel during cancer treatment, more selectivity in the targeting of benign versus malignant cells can be invoked. This increases the probability of successful treatment. By using the mathematical model together with the experimental results we find for our cell line that the proliferation is halted by an increased time from birth to mitosis of the cells. The enhanced time between the birth and mitosis of a cell leads effectively to smaller proliferation rates. This mechanism is somewhat different from the earlier results on digitoxin for different cell lines where an increased rate of apoptosis was

reported. But we find it reasonable that cell lines can react differently to digitoxin. A development from enhanced time between birth and mitosis to apoptosis can be furnished, dependent of the digitoxin sensitivity of the cell lines.

Further, when introducing digitoxin the simulations are run 30-40 days into the future, and model is used to pinpoint the exact critical digitoxin level where the number of living cells is decreasing as a function of time.

Simulations are run over 40 days with varying levels of digitoxin to pinpoint that exact critical digitoxin level where the number of living cells is decreasing as a function of time.

Section 2 provides the theoretical model. Section 3 presents analytical solutions for simple scenarios. Section 4 compares the model with results from in vitro experiments with cancer cells exposed to digitoxin. Section 5 concludes.

2 The theoretical model

Central for the theoretical model are two quantities, the number of cells at different ages, and the mass of cells of different ages. Let $N(t,\tau)$ be the number of living cells at time t of ages less than or equal to τ in a volume V[1]. Define the age density $\rho(t,\tau)$ by

$$\rho(t,\tau) \overset{def}{=} \partial N(t,\tau)/\partial\tau, \quad i.e. \quad N(t,\tau) = \int_0^\tau \rho(t,u)du, \tag{2.1}$$

where "def" means a definition. $\rho(t,\tau)d\tau$ is the number of living cells with ages in the interval from τ to τ+dτ. At time t the total number of living cells of all ages is given by

$$N_T(t) \overset{def}{=} N(t,\infty). \tag{2.2}$$

where the subscript "T" is used to indicate the total number of living cells of all ages.

Let $M(t,\tau)$ be the total mass of all living cells at time t of ages less than or equal to τ. Define the age mass density $m(t,\tau)$ by

$$m(t,\tau) \overset{def}{=} \partial M(t,\tau)/\partial\tau, \quad i.e. \quad M(t,\tau) = \int_0^\tau m(t,u)du \tag{2.3}$$

where $m(t,\tau)d\tau$ is the total mass of all living cells at time t of ages in the interval from τ to τ+dτ. At time t the total mass of all living cells of all ages, i.e. total living biomass in the sample volume, is given by

$$M_T(t) \overset{def}{=} M(t,\infty). \tag{2.4}$$

We now define the important ratio

$$m_c(t,\tau) \overset{def}{=} \frac{m(t,\tau)}{\rho(t,\tau)} \quad when \quad \rho(t,\tau) \neq 0, \quad m_c(t,\tau) \overset{def}{=} 0 \quad when \quad \rho(t,\tau) = 0, \tag{2.5}$$

which can be interpreted as the average mass of one living cell at time t of age τ.

The rest of this article focuses on the construction of mathematical models of the two main quantities; the age density $\rho(t,\tau)$ and the average mass of a cell $m_c(t,\tau)$.

For the age density $\rho(t,\tau)$ in (2.1) the following equation follows directly from the conservation of cells

The number of living cells at time t+dt of ages between τ+dτ and τ+2dτ equals the number $\rho(t,\tau)d\tau$ of living cells at time t of ages from τ to τ+dτ, plus the number $r(t,\tau)d\tau dt$ of living

[1] This article applies expected values for all variables and we suppress the word expectation.

cells reinforced from t to t+dt of ages between τ and τ+dτ, minus the number $l(t,\tau)d\tau dt$ of living cell lost from t to t+dt of ages between τ and τ+dτ. This gives

$$\rho(t+dt,\tau+d\tau)d\tau = \rho(t,\tau)d\tau + r(t,\tau)d\tau dt - l(t,\tau)d\tau dt. \tag{2.6}$$

Taylor expansion of (2.6) up to order O(h), where time t and age τ is coupled such that dt=dτ, applying that dt=dτ tends to zero, yields the conservation equation

$$\frac{\partial\rho(t,\tau)}{\partial t} + \frac{\partial\rho(t,\tau)}{\partial\tau} = r(t,\tau) - l(t,\tau),\ \rho^0(\tau) \overset{def}{=} \rho(0,\tau) \tag{2.7}$$

$\rho(0,\tau)$ is the initial age density. The following differential equation follows directly from mass conservation of each living cell

$$m_c(t+dt,\tau+d\tau) = m_c(t,\tau) + r_c(t,\tau)dt - l_c(t,\tau)dt,\quad m_c^0(t) \overset{def}{=} m_c(t,0) \tag{2.8}$$

(2.8) expresses that the mass of a living cell at time t+dt of ages τ+dτ equals the mass of the living cell at time t of age τ plus the mass increase $r_c(t,\tau)dt$ from t to t+dt, minus the mass loss $l_c(t,\tau)dt$ from t to t+dt. $m_c(t,0)$ is the mass of an average cell at birth. This mass can vary with time due to different external or internal conditions.

Taylor expansion of (2.8) up to order O(h), where time t and age τ is coupled such that dt=dτ, applying that dt=dτ tends to zero, yields

$$\frac{\partial m_c(t,\tau)}{\partial t} + \frac{\partial m_c(t,\tau)}{\partial\tau} = r_c(t,\tau) - l_c(t,\tau),\quad M_c(t) \overset{def}{=} \int_0^\infty m_c(t,\tau)d\tau, \tag{2.9}$$

where $M_c(t)$ is the total mass of a living cell during the life cycle.

Let us now move on to the specific relations that are connected to the life cycle of the cells. Moving back to equation (2.7), the loss and reinforcement are given as

$$l(t,\tau) \overset{mod}{=} \mu(t,\tau)\rho(t,\tau), \quad r(t,\tau) \overset{mod}{=} 0, \tag{2.10}$$

where "*mod*" means that this is a testable model assumption. $\mu(t,\tau)$ is the death rate coefficient as a function of time (dependent on external conditions) and age[2]. A living cell's death rate usually increases with age τ but also depends on growth mechanisms and whether the living cell is located in a nutritionally optimal environment at time t. The cell death coefficient $\mu(t,\tau)$ can generally be divided in two parts, one from apoptotic cell death and one from necrotic cell death. We do not separate those two events in this article. $r(t,\tau)$ is the general reinforcement. This term will be set to zero in this article since all reinforcements will be given through a boundary condition for the age density at age zero $\rho(t,0)$.

We assume that the loss and reinforcement of mass for an average cell follow the equations

$$r_c(t,\tau) \overset{mod}{=} c_1 m_c(t,\tau)^n, \quad l_c(t,\tau) \overset{mod}{=} c_2 m_c(t,\tau) - l_{mit}(t,\tau). \tag{2.11}$$

The mass increase $m_c(t,\tau)^n$ is due to anabolism, where n is a parameter[3], the loss term $l_c(t,\tau)$ is divided in two parts. $c_2 m_c(t,\tau)$ is due to catabolism, and $l_{mit}(t,\tau)$ is due to mitosis loss of a living cell, where c_1 and c_2 are parameters which depend on the temperature and the supply/availability of oxygen and other nutrients.

Crucial for cell division is that a living cell can generally only divide when it has reached a certain mass size and a specific age. Also the population densities of cells inhibit mitosis due to contact inhibition. The following mathematical construction is descriptive

[2] Strictly speaking, without a more specific relation for this coefficient this equation is a pure definition.
[3] For the distinct process of anabolism there is some discussion in the literature (see e.g. Bartilanffy 1955, 1962) of whether n=1 or n=2/3. We find empirical support for n=1 which is used in section 3 and thereafter.

$$
\begin{aligned}
l_{mit}(t,\tau) &\overset{mod}{=} \left(r_c(t,\tau) - c_2 m_c(t,\tau)\right) g(\tau) \\
g(\tau) &\overset{mod}{=} \begin{cases} 0, \ when \ \left(r_c(t,\tau) - c_2 m_c(t,\tau)\right) \le 0 \quad or \quad N_T(t) \ge N_{max} \\ \chi(1 - e^{-\tau^2/\beta^2}) \ \ otherwise, \quad 0 \le \chi \le 1. \end{cases}
\end{aligned}
\tag{2.12}
$$

(2.12) states that a living cell divides only if the mass tends to increase, i.e. a part of the mass increase is converted to offspring. Due to contact inhibition, the cell divides only if the population density is below a certain value N_{max}. The cell starts to divide at the age of approximately β. The χ value expresses the mass fraction of the cell growth that is used to mitosis. $\chi = 1$ means that the cell converts all its potential mass growth into mitosis.

In order to close the system a connection between the mass loss due to mitosis and the number of offspring must be established. One simple relation is

$$
m_c^b(t,\tau) \overset{mod}{=} \alpha \, m_c(t,\tau), \tag{2.13}
$$

where $m_c^b(t,\tau)$ is the mass of a newly born cell at time t, born from parents of age τ. Different age classes have different average mass for cells, and large cells in general give larger offspring than smaller cells. The offspring are a constant fraction α of the cells mass.

The number of cells born is now given by the boundary condition

$$
\rho(t,0) \overset{mod}{=} \int_{\varepsilon}^{\infty} \left(l_{mit}(t,\tau) \rho(t,\tau) / \, m_c^b(t,\tau) \right) d\tau, \quad \varepsilon > 0, \tag{2.14}
$$

where ε is arbitrarily small but positive (to indicate that the integration does not include zero). Equation (2.14) expresses that the reinforced (born) number of cells of age zero is equal with the mass loss $l_{mit}(t,\tau)$ pr time t of cells of age τ, multiplied with the number $\rho(t,\tau)$ of cells of age τ, divided with the mass $m_c^b(t,\tau)$ of the newly born cells from cells of age τ, integrated over

all cell ages τ , i.e. from zero to infinity.

A more simple relation than (2.14) will be used in this article. By assuming that all cells are born with the same mass m_c^0 at all times, it follows from (2.12) and (2.14) that

$$\rho(t,0) \overset{mod}{=} \int_{\varepsilon}^{\infty} \left[\left(c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau) \right) g(\tau) \rho(t,\tau) / m_c^0 \right] d\tau, \quad m_c^b(t,\tau) \overset{mod}{=} m_c^0 \tag{2.15}$$

The equation set is now closed. A stepwise algorithm is stated in appendix A.

3 Analytical solutions

This section presents some analytical solutions when the cells starts to divide immediately at time zero, the death coefficient $\mu(t,\tau)$ is constant through time and independent of age, and the mass $m_c^b(t,\tau)$ of a newly born average cell is constant through time and independent of age, i.e.

$$\mu(t,\tau) = \mu, \quad m_c^b(t,\tau) = m_c^0, g(\tau) = 1, \alpha = 1, \beta = 1, \chi = 1. \tag{3.1}$$

Using (2.10) and (2.12), integrating (2.7) with respect to τ gives

$$\begin{aligned} \dot{N}_T(t) &= \int_0^\infty r(t,\tau) d\tau - \mu N_T(t) = (1/m_c^0) \int_0^\infty (c_1 - c_2) m_c(t,\tau) \rho(t,\tau) d\tau - \mu N(t) \\ &= (c_1 - c_2) N_T(t) - \mu N_T(t), \end{aligned} \tag{3.2}$$

which has the exponential growth solution

$$N_T(t) = N_T(t_0) Exp[(c_1 - c_2 - \mu)(t - t_0)], \tag{3.3}$$

where a dot above a variable means time derivation. $N_T^d(t)$ is the number of dead cells, and $N_T^{ad}(t)$ is the total number of cells (dead or alive), i.e. $N_T^{ad}(t) = N_T(t) + N_T^d(t)$. The following solutions follows directly from (3.3)

$$\dot{N}_T^d(t) = \mu N_T(t) \Rightarrow N_T^d(t) = \int_{t_0}^{t} \mu N_T(t')dt' = \frac{\mu}{c_1 - c_2 - \mu}[N_T(t) - N_T(t_0)],$$
$$N_T^{ad}(t) = N_T(t) + N_T^d(t) = \frac{(c_1 - c_2)N_T(t) - \mu N_T(t_0)}{c_1 - c_2 - \mu} \tag{3.4}$$

The fraction of dead cells of the total number of cells is then given as

$$\frac{N_T(t)}{N_T^{ad}(t)} = \frac{\mu(N_T(t) - N_T(t_0))}{(c_1 - c_2)N_T(t) - \mu N_T(t_0)}, lim_{t\to\infty} \frac{N_T(t)}{N_T^{ad}(t)} = \frac{\mu}{c_1 - c_2}, when\ c_1 - c_2 - \mu > 0 \tag{3.5}$$

Observe from the solution in (3.5) that increasing the death coefficient μ increases the fraction of dead cells $\mu/(c_1 - c_2)$ and vice versa. This does not mean that the proliferation of cells is stopped, since to stop the proliferation one must achieve that $(c_1 - c_2 - \mu) < 0$ as equation (3.3) shows.

The analytical solution of the total number $N_T^{ad}(t)$ of cells will be compared with simulation results using the more general model in section 2, and with experimental results, in the next section. The solutions given in (3.3)-(3.5) are typical during standard cancer treatments, where use of chemotherapy and/or radiotherapy simply means to increase the death coefficient μ by some fraction. We will show that the analytical solution can not in general be used to describe the number of cells. This means that using digitoxin does not correspond to a simple increase of the death coefficient μ.

4 Simulations

This section illustrates typical simulations of the model compared with in vitro experiments with cancer cells exposed to digitoxin (Haux 1999). We show that the model matches the experiments very well.

For the cell type we set m_c^0 to be a constant, which means that all cells at all times t are born with the same mass. We further assume $\chi = 1$, which means that the mass growth of a cell goes entirely into mitosis. We furthermore set that the death coefficient μ and the growth

parameter c_1 are constants through time, i.e. that there is no change in temperature, oxygen supply, and nutrino supply during a specific test. More specifically, without loss of generality we set c_2=0, since we can always redefine c_1-c_2 when n=1 in (2.12).

We first simulate the case without digitoxin. The numerical value of the parameters are found by estimating values of the parameters c_1 and μ such that the total number of cells (dead or alive) are in agreement with the measurements. Thereafter we make the crucial assumption that introducing digitoxin at subsequently higher concentration levels only changes the numerical values of the parameters. For each subsequently higher digitoxin concentration we estimate new values for the parameters, but change the values for as few as possible. The number of cells (dead or alive) are again compared with the experiments.

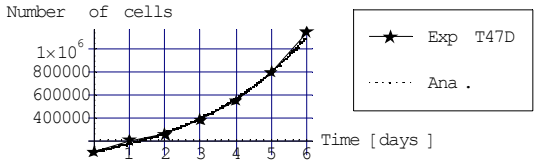

Fig. 1: Analytical and experiments results for the number of cells (dead or alive) without digitoxin, $\beta = 0/day, \chi = 1, c_1$*=0.6/day,*$\mu$*=0.3/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

Fig. 1 shows the experimental and simulated results without digotoxin. The analytical and experimental results correspond. The very good agreement between the analytical model and the experimental results strongly simplifies the analysis, implying that the more general model in section 2 is not necessary to invoke so far.

We now make the crucial step of introducing digotoxin such that the concentration is 25ng/ml. digitoxin. Searching for values of the parameters which facilitate correspondence between empirics and simulations for the number of cells (dead or alive), suggest that the analytical solution given in section 3 does not fit the data. Fig. 2 and Fig. 3 show two attempts to fit the data, but neither of them are good. But by using the general model in section with $\beta = 1.3/day$, while keeping the other parameters constant as for the case without digitoxin, gives much

better fit to the data, as shown in Fig. 4. This is an important tentative observation; *The introduction of digitoxin gives an increased delay between the time of birth of a cell to the time of mitosis. The values of the other parameters do not change!*

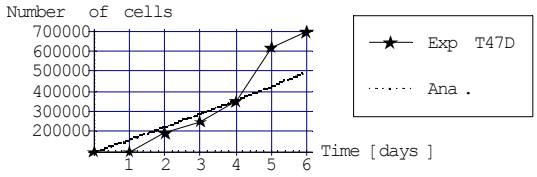

Fig.2: Analytical and experimental results for the number of cells (dead or alive) using 25 ng/ml digitoxin, $\beta = 0/day, \chi = 1$, c_1*=0.6/day,* μ*=0.57/day.*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

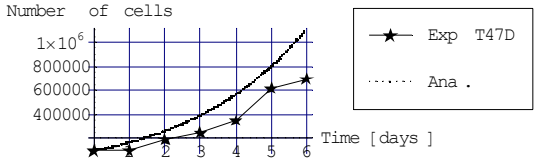

Fig.3: Analytical and experimental results for the number of cells (dead or alive) using 25 ng/ml digitoxin, $\beta = 0/day, \chi = 1$, c_1*=0.6/day,* μ*=0.3/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

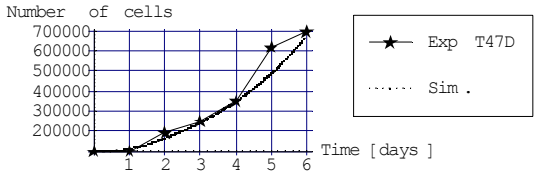

Fig.4: Simulation and experimental results for the number of cells (dead or alive) using

25ng/ml digitoxin, $\beta = 1.3/day, \chi = 1$ *,* c_1*=0.6/day,* μ*=0.3/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

Fig. 5-7 show the number of cells when the digitoxin concentration is 50ng/ml. Figs. 5 and 6 show two attempts to fit the data with the analytical solution. The fits are not so good. Fig. 7 shows the simulation results when the delay from birth to mitosis is increased to $\beta = 5/day$, while the other parameters have the same values as in the case without digitoxin. Observe now the very good agreement with the experimental results.

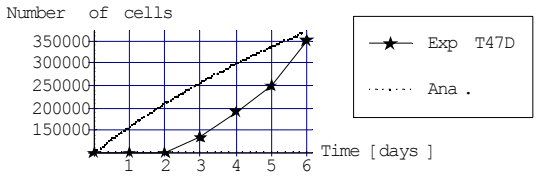

Fig.5:Analytical and experimental results for the number of cells (dead or alive) using 50 ng/ml digitoxin, $\beta = 0/day, \chi = 1$*,*c_1*=0.6/day,*μ*=0.7/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

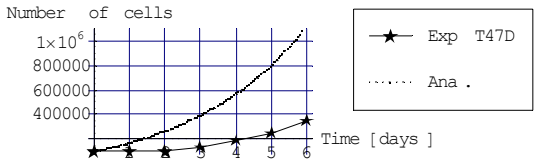

Fig.6:Analytical and experimental results for the number of cells (dead or alive) using 50 ng/ml digitoxin, $\beta = 0/day, \chi = 1$*,*c_1*=0.6/day,*μ*=0.3/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

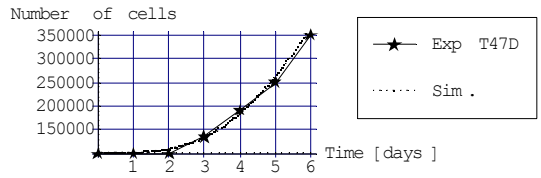

Fig. 7: Simulation and experiments results for the number of cells (dead or alive) using 50 ng/ml digitoxin, $\beta = 5/day$ *,* c_1*=0.6/day,* μ*=0.3/day,*

$$m_c^0 = 4.2\,10^{-12} kg, N_T(0) = \int_0^\infty \rho_c^0 d\tau \approx \rho_c^0 \Delta\tau = 10^5 / ml$$

The tentative hypothesis based on the results for 25ng/ml digitoxin is strongly supported, i.e. *the introduction of digitoxin only enhance the time delay from birth to mitosis of the cells, and higher values of digitoxin leads to larger time delays*.

Fig. 8 assumes no exposure to digitoxin for the first 29 days, with a concentration of 50ng/ml digitoxin at day 30 and for each day thereafter. Observe how the number of living cells starts to flatten out at day 30 and thereafter.

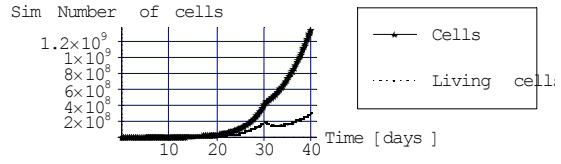

Fig. 8: The number of cells when the digitoxin level is 50ng/ml after day 30.

A closer inspection shows that the critical concentration level is around 50 ng/ml digitoxin for this cell line, which is not very sensitive to digitoxin (Haux (1999)).

4 Conclusion

The article provides a mathematical description based on the theory of differential equations, for the proliferation of malignant cells (cancer). A model is developed which enables us to

describe and predict the dynamics of cell proliferation much better than by using ordinary curve fitting procedures. Theoretical results for the total number of cells (living or dead) is found to be in good agreement with experiments for the cell line considered, assuming different concentrations of digitoxin. The numerical results show that by introducing amounts of digotoxin, the time from birth to mitosis increases, which effectively gives a mitosis delay. The delay is probably connected with changes in the Ca ions concentration inside the cell. The enhanced time between the birth and mitosis of a cell leads effectively to smaller proliferation rates. This mechanism is very different from the mechanism appealed to by standard chemotherapy and radiotherapy where the death ratios of the cells are mainly affected. Based on the literature and the present results we have established the following different mechanisms effectively reducing proliferation: a) necrotic death, b) apoptosis and c) enhanced time between birth and mitosis. The last mechanism is as far as we know new.

By systematically analyzing and building models for the mechanisms appealed to by standard treatment, and by use of digitoxin or other drugs which are likely to emerge, we expect that a more specific treatment can be found for a given cell line, which increases the probability of a successful treatment. The critical digitoxin level concentration, i.e. the concentration level where the number of living cells is not increasing, is approximately 50 ng/ml for the cell line we investigated in this article. It is most likely closer to 25 ng/ml for other malignant cell lines (prostate cells for example; Haux 2000). Therapeutic plasma concentration of digitoxin when treating cardiac congestion is about 15-33ng/ml, but individual tolerances are large. The effect of digitoxin during cancer treatment is therefore very promising.

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Appendix A

Assume that all quantities are given at time t. The following algorithm is used for calculating the values at time $t+\Delta t \; and \; \tau+\Delta\tau \; ,where \; \Delta\tau=\Delta t$

$$\begin{aligned}
&\forall \tau \geq 0, \\
&\rho(t+\Delta t,\tau+\Delta\tau)=\rho(t,\tau)-\Delta t\mu\ \rho(t,\tau) \\
&\rho(0,\tau)=0 \quad when \quad \tau\neq 0, \rho(0,0)=N_T(0)/\Delta\tau
\end{aligned} \tag{A1}$$

$$\begin{aligned}
&\forall \tau \geq 0, \\
&If \quad c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau) \leq 0, or \quad N_T(t) \geq N_{\max}, \\
&m_c(t+\Delta t,\tau+\Delta\tau)=m_c(t,\tau)+\Delta t\ [c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau)] \\
&else \\
&m_c(t+\Delta t,\tau+\Delta\tau)=m_c(t,\tau)+\Delta t\,[c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau)][1-\chi(1-Exp[-\tau^2/\beta^2])], \\
&m_c(0,\tau)=0 \quad when \quad \tau\neq 0, m_c(0,0)=m_c^0
\end{aligned} \tag{A2}$$

The boundary condition is given by

$$
\begin{aligned}
&\forall t \neq 0, \Delta t \neq 0, \\
&\textit{If} \quad c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau) \leq 0, \textit{or} \quad N_T(t) \geq N_{\max}, \textit{then} \\
&\rho(t+\Delta t, 0) = 0, m_c(t+\Delta t, 0) = 0, \\
&\textit{else} \\
&\rho(t+\Delta t, 0) = \int_{0'}^{\infty} \rho(t,\tau)\,(c_1 m_c(t,\tau)^n - c_2 m_c(t,\tau))\chi(1 - Exp[-\tau^2/\beta^2])d\tau / m_c^0, \\
&m_c(t+\Delta t, 0) = m_c^0
\end{aligned}
\tag{A3}
$$

The total numbers of living cells are

$$
\begin{aligned}
&N_T(t) = \int_0^{\infty} \rho(t,\tau)d\tau, N_T^d(t) = \int_0^{\infty} \mu\,\rho(t,\tau)d\tau dt, \\
&N_T^{ad}(t) = N_T(t) + N_T^d(t)
\end{aligned}
\tag{A4}
$$