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Microdialysis: improving local chemotherapy in cancer using a mathematical model

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1. ABSTRACT

Although intratumoral chemotherapy administration has been evaluated in the past, its results have not been frequently comparable to those from systemic administration. We recently described microdialysis as a method for local chemotherapy administration with increasing effectiveness while reducing systemic toxicity. We present a mathematical model which supports the successful application of this procedure in optimizing the administered drug in different cases, using informatics tools and considering several parameters. We also review and discuss important aspects of cancer biology that should be taken into consideration in cancer chemotherapy, such as tumor heterogeneity, drug resistance and metastasis, and how this technique may be used to overcome any set-backs presented by these.

2. INTRODUCTION

Over the past decades, a great effort has been undertaken to evaluate the benefits of chemotherapy intratumoral injection (1-4). A description of past experiments is beyond the scope of this paper and is well reviewed elsewhere by other authors, who have been intensively studying cancer delivery mechanisms (5). Despite this, intratumoral injection is still far from becoming an alternative administration route in clinical practice. Besides the invasiveness of this procedure, the most apposite reasons behind the failure of this local therapy in the past include rapid clearance of the drug from the tumor resulting in drug resistance, surrounding tissues toxicity and inability to target systemic spread. Furthermore, surgery and local radiotherapy have been demonstrating better results.

Table 1. Parameters used to build the mathematical model which supports microdialysis as a local chemotherapy delivery technique

Cd	drug concentration (constant during the administration)
t_d	drug administration time
C_m *and* t_m	required minimal concentration in the whole tumor and minimal time of action, respectively, for good effectiveness
u (x,t)	drug concentration in a cell placed at a distance *x* from the membrane, in the instant *t*
L	maximal distance between tumor periphery and membrane
T	maximal time considered in the model
K	diffusion constant of the drug in the tumor

In the past, a group proposed intratumorally injected carrier-based chemotherapy as an interesting alternative to routinely used chemotherapy regimens and routes of administration (6). It presented a significantly increased antitumor efficacy, as well as an improved therapeutic index, when compared to both intravenous and intratumoral applied free chemotherapy and also to intravenously applied carrier-based chemotherapy. In addition, it was found that this delivery technique substantially improved both the tumor concentrations and the tumor-to-organ ratios, which correlate well to toxicity.

Considering the important role of chemotherapy in cancer and knowing that most limitations are due to the toxicity and resistance mechanisms of tumor cells, we recently proposed microdialysis as a new method for local chemotherapy (7). By increasing local drug concentration and avoiding systemic distribution by locoregional administration, the referred limitations may be overcome.

A microdialysis catheter consists of a double lumen cannula with a semipermeable membrane glued to its end, allowing for a process of continuous diffusion. A chemotherapy perfusion is pumped in a lumen leaving the catheter by the other one (8). As the drug molecules are smaller than the membrane pores, the composition of the intracanulla fluid reflects the equilibrium between the perfusion liquid and the extracellular liquid.

As the success of this technique is dependent on the optimization of several parameters, we will describe a mathematical model which, recurring to informatics tools, explains why this method may produce better results than conventional ones and, at the same time, helps optimize therapies by also limiting toxicity. We will then discuss the implications of this model, correlating it with some of the available knowledge on tumor biology and progression.

3. MATHEMATICAL MODEL WHICH SUPPORTS MICRODIALYSIS AS A CHEMOTHERAPY DELIVERY METHOD

3.1. Assumptions, parameters and basis of the model

It is quite challenging to represent accurately biologic phenomena by using equations. Therefore, most mathematical models are based on generalizations and simplifications of reality. In this model, we assume that, in order to treat the tumor, all tumor cells should be exposed to a minimal concentration of drug for a certain period of time. Surely, there will always be cells that may be killed with lower drug concentrations or if exposed for shorter periods. However, since tumors are heterogeneous and resistant cells can have any location within them, our minimal drug concentration (*Cm*) and the minimal time of action (*tm*), in order to kill all the cells will be quite high.

Tumors may have different shapes. Thus, the microdialysis probe should always be placed in the middle of the tumor, which, in irregularly-shaped tumors, means that the operator should try to minimize the distance from the probe to all the peripheral cells of the tumor. We will consider the maximal length L between the tumor periphery and the probe membrane in our model. All the points which are at the same distance x from the membrane will have the same drug concentration *u(x,t)* , if considered at the same instant t.

A drug with concentration *Cd* is delivered into the tumor during a specific time *td*, through the probe membrane. The parameters used to build this model are summarized in Table 1.

In biological means, if we introduce a drug inside a tumor, it will not diffuse homogeneously cell by cell. Since most tumors have a rich network of vessels, a portion of the drug will enter the blood vessels and easily reach the periphery of the tumor. However, in order to simplify the model, we will consider an average diffusion constant k, which may vary depending on the tumor type (density, vascularization…) and its location.

3.2. Mathematical model – equations and graphs

A simulation of the drug diffusion within the tumor was performed using the following diffusion equation:

$$u_t(x,t) - ku_{xx}(x,t) = 0, x \in \mathrm{IR}, t > 0,$$

with *k* as the diffusion constant in the tumor.

Considering that the whole drug was administered in $t = 0$, intratumoral chemotherapy injections, the concentration of the drug within the tumor would be obtained using the initial condition:

$$u(x,0) = \delta(x), x \in \mathrm{IR},$$

where δ is the Dirac delta function.

A fundamental solution for this problem is:

$$\varphi(x,t) = \frac{1}{\sqrt{4\pi kt}} e^{-\frac{x^2}{4kt}}$$

For each instant $\bar{t} > 0$, $\varphi(x,\bar{t})$ Gaussian curve with a unitary area, demonstrating how the drug is spreading through the tumor. In a similar way, a

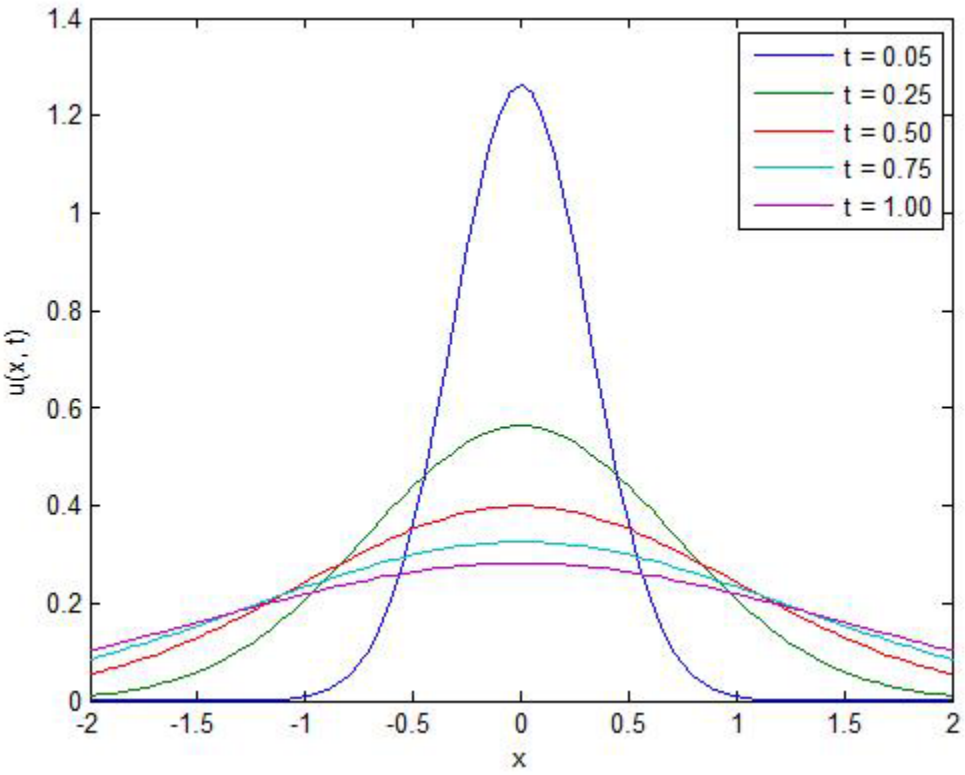

Figure 1. Gaussian distribution of drug concentration when the whole drug is administered in t=0. Different points in the time frame were considered to represent drug distribution within the tumor from the centre (x=0) to the periphery (x=2 or x=-2).

possible solution for the problem i $\alpha\varphi(x,t)$ _initial condition is $u(x,0)=\alpha\delta(x)$ and α represents the initial drug concentration (9,10).

In Figure 1, we represent this Gaussian distribution of drug concentration in the tumor, in different instants, when the whole drug is administered in t=0. In Figure 2, the variation of drug concentration with time is evaluated in different positions within the tumor located at x cm from the membrane. Drug concentration variation, considering the distance to the membrane x and the time t is represented in a three dimensional graph in Figure 3.

In order to represent drug concentration when we perfuse it through microdialysis catheters, we consider now that a drug with concentration Cd is pumped during t_d units, instead of the above presented instant administration. In order to simplify this model, the temporal axis was discretized in the following way $t_0 = 0 < t_1 < \ldots < t_n = T,$ and we assumed that the drug is pumped at all instants $t_i \le t_d$. Considering that t_d is one of the mentioned points in the discretization, we considered the following approximation to the solution of this model:

$$u(x,t)=\sum_{i=1}^{p}\alpha_i\ \overline{\varphi}_i(x,t),$$

where p satisfies $t_d = t_p$ and

$$\overline{\varphi}_i(x,t)=\begin{cases}0, & t<t_i\\ \varphi(x,t+t_1-t_i), & t\ge t_i\end{cases}$$

α_i constants are determined so that $u(0,t_i)=c,\forall i\in\{1,\ldots,p\}$. Therefore, they are the solution of the following triangular linear system is:

$$\sum_{i=1}^{k}\alpha_i\ \varphi(0,t_{k+1-i})=Cd,\quad k\in\{1,\ldots,p\}$$

Considering this model, we optimised the drug concentration C_d and the drug administration time t_d, in order to minimize the total drug administration and subsequently decrease systemic toxicity. C_d and t_d should be the optimal solution of the below stated problem

$$\min 2\int_{L}^{\infty}\int_{0}^{t_d} u(x,t)\ dtdx$$

$$s.a\quad \exists\varepsilon\in[0,T-t_m]:\ u(L,t)\ge c_m,\forall t\in[\varepsilon,\varepsilon+t_m]$$
$$0\le c\le\overline{c}$$
$$0\le t_\alpha\le T$$

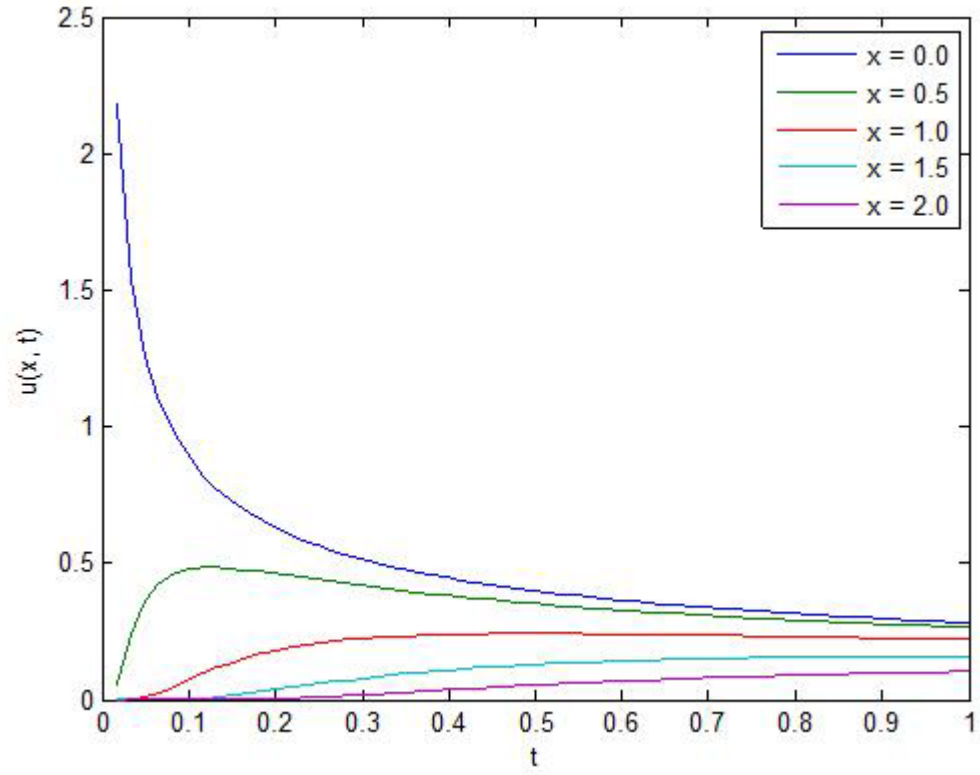

Figure 2. Variation of drug concentration with time when the whole drug is administered at t=0, evaluated in different positions within the tumor located at x cm from the membrane.

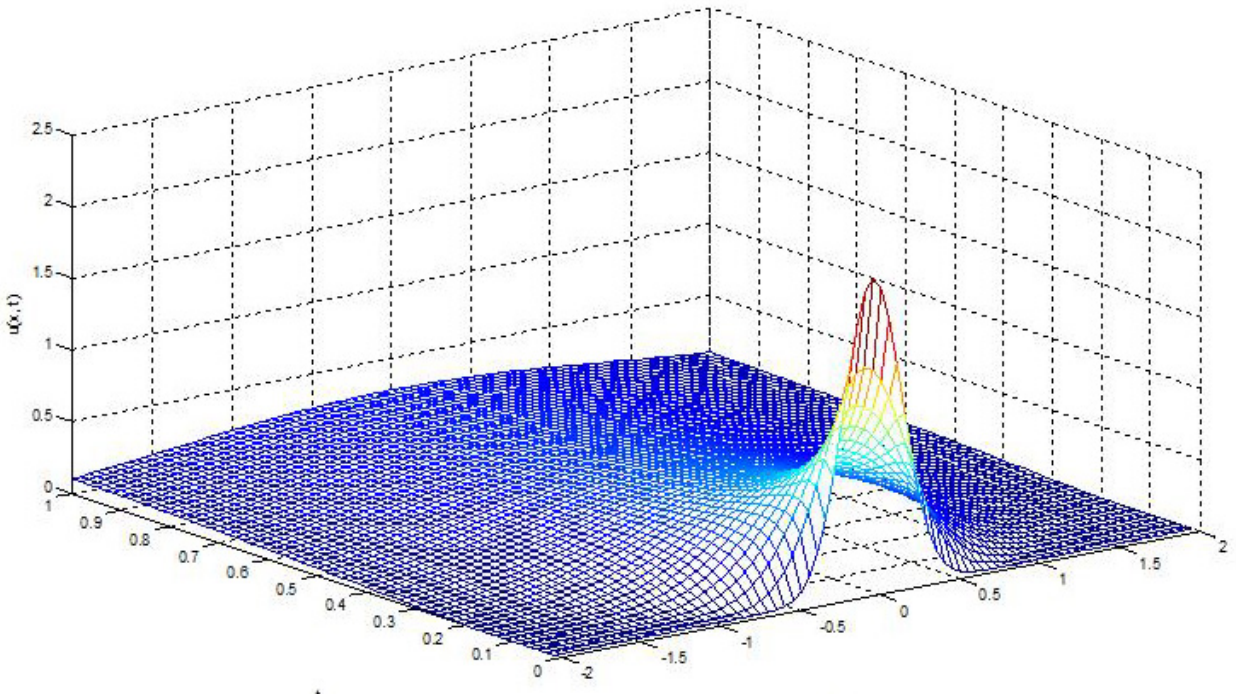

Figure 3. Three dimensional graph representing drug concentration variation within the tumor, considering the distance to the membrane x and the time t, when the whole drug is administered in t=0.

Table 2. Comparison between different chemotherapy delivery methods: perfusion through the proposed method (microdialysis) and intratumoral instantaneous injection

	Perfusion	**Injection**
Cd (mM)	24.3	279.1
Td (min)	57	0.1
drug quantity (mmol)	742.8	2249.8

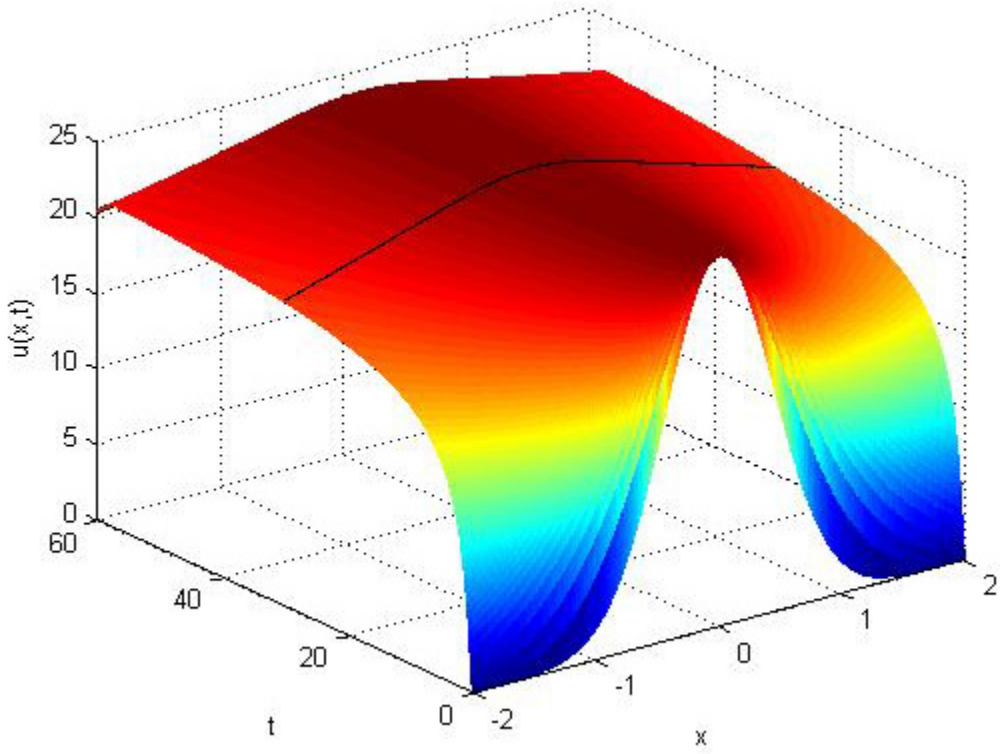

Figure 4. Three dimensional graph representing drug concentration variation within the tumor, when the optimal solution to minimize the quantity of administered drug is applied.

We considered L=2cm, T=60min, k=1, Cm=20mM and tm=30min and used Matlab software to mimic these conditions. In order to minimize the given drug, the optimal solution is achieved when the drug concentration is 24.3 mM and it is administrated during 57 min (Figures 4 and 5). Comparing this optimization of drug perfusion with intratumoral injections, where the whole drug is administered at once, we note that the former implies a 67% reduction in the required drug quantity (Table 2). The concentration used in the perfusion system is also much lower.

This mathematical model supports microdialysis as a means for significant improvement in locoregional chemotherapy as it clearly allows for a decrease in toxicity concurrent to a rise in chemotherapy effectiveness.

3.2. Determining Cm, tm and k in each tumor

In order to apply this model to clinical use, we should know what the sensitivity of the tumor to the drug is. This is reflected in the Cm and tm values. Small pieces of tumor collected during the biopsies may be used to analyse *ex vivo* responses to different drugs and concentrations, before starting treatment. By doing this, we will not only be selecting the most suitable therapies, but also analyzing what the most effective drug concentrations to use are. After analyzing the drug-response curves, we can approximately deduce Cm and tm. Five times the IC50 (concentration needed to kill half of the cell population in a determined therapeutic time) or the IC95 (concentration that leads to 95 percent of tumor cell death) may be a good empirical approximation to Cm, since if we kill the bulk mass of the tumor, remaining cells may also lose their viability. These values should certainly be refined with clinical experience.

A limitation of these pre-therapeutic tests is related to tumor heterogeneity, further discussed below. We may kill most of the tumor cells, but not the most resistant ones, which may cause relapses in the future. This limitation is, however, also common to peripheral blood chemotherapy administration. On the other hand, if we minimize the administered drug and consequently its toxicity, we can perform more frequent treatments and combine different drugs, reducing cell survival probability.

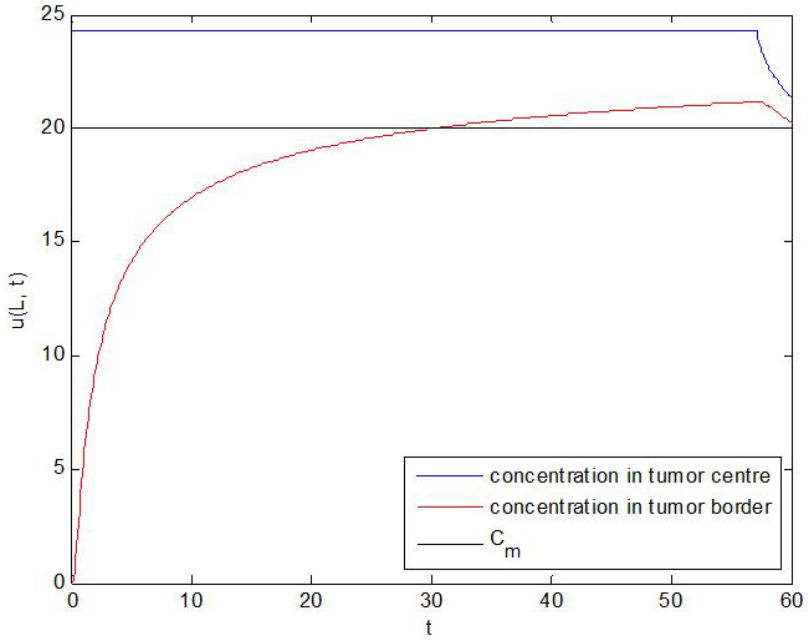

Figure 5. Drug concentration variation in the centre and border of the tumor, when the optimal solution to minimize the quantity of administered drug is applied.

The diffusion constant may be harder to determine. There are two different ways to get an approximation of its value. A rough estimation may be obtained from *ex vivo* studies performed in big cohorts of tumors analyzed after their surgical removal. However, the constant which is determined *ex vivo* may be different from the real one, mostly because tumors placed in culture do not have a dynamic vascularisation. Although further studies should be performed, radiologic and pathologic characteristics of the tumor together with its location might be correlated with its diffusion constant. Again, diffusion constants will become more close to the reality along with an increasing clinical experience.

A more accurate way of determining the diffusion constant is to place, together with the chemotherapy administration probe placed in the middle of the tumor, another probe in the periphery, which will detect drug concentration at the edges of the tumor in a dynamic way. Although less convenient for the patient, it provides a more accurate approximation of the diffusion constant and allows for adjustments during treatment.

4. DRUG KINETICS USING DIFFERENT ADMINISTRATION ROUTES AND TOXICITY MINIMIZATION

In a previous section, we demonstrated that, if we use microdialysis equipment and consequently maintain a constant drug concentration in the middle of the tumor, we may achieve better therapeutic results with less toxicity than intratumoral injections. However, we should also compare these results with the ones obtained using clinical practice state-of-the-art (intravenous perfusions – systemic administration), by evaluating drug kinetics.

Despite knowing that blood vessels can cross the tumor, we considered a blood drug concentration similar to the concentration in the periphery of the tumor, in order to use our model. Considering the same parameters values we used before, we realize that a lower drug concentration is needed in the blood (around 22mM – see Figure 6 and 7), when we compare to the concentration needed in the middle of the tumor through the microdialysis probe (around 24mM – Figure 5). However, we should not forget that, during systemic administration, the whole body blood will have the same drug concentration and, as such, most tissues may reach the Cm and be submitted to an excessive toxicity. This consequence is never attained when we use locoregional therapies because the overall administered drug is reduced. Besides overall body diffusion, drugs in the blood are also directly eliminated by kidney clearance or liver metabolism, and so we have to add an extra amount of drug in order to compensate this loss.

5. TUMOR HETEROGENEITY AND DRUG RESISTANCE

Spontaneous tumors originate almost always from a single cell. However, at the time of diagnosis they already exhibit great cell heterogeneity. Thus, tumor heterogeneity, both phenotypic and genotypic, is a widely accepted concept. Both clonal evolution and stem cell theories of cancer state that certain cells are more prone to acquire resistance to drug therapies (11). The former considers that certain cells may acquire mutations and give

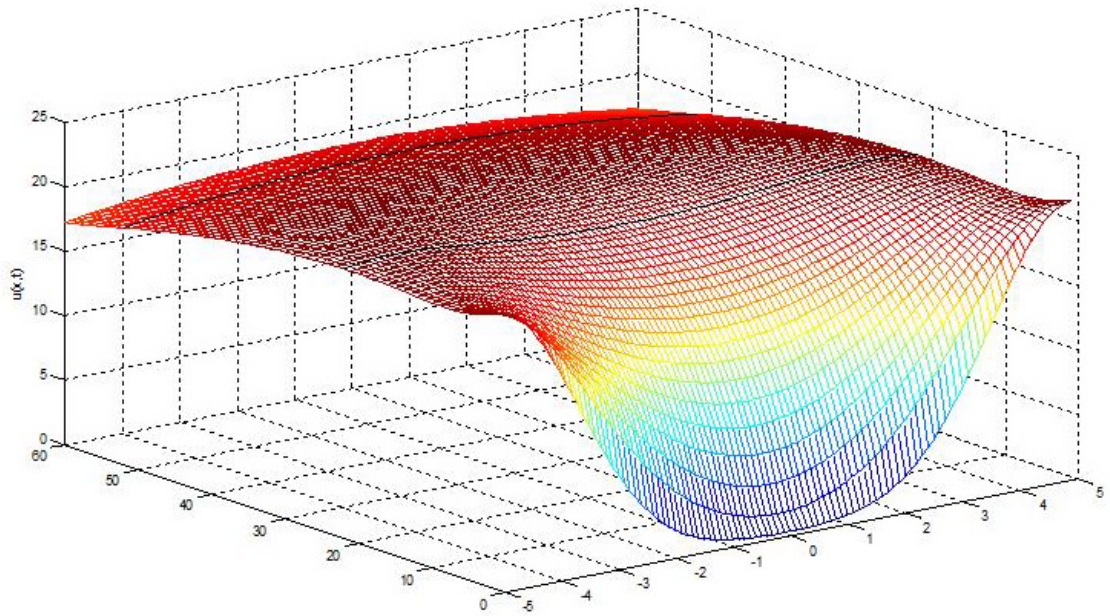

Figure 6. Three dimensional graph representing drug concentration variation within the tumor, when drug is injected in a blood vessel and reaches the tumor from the periphery.

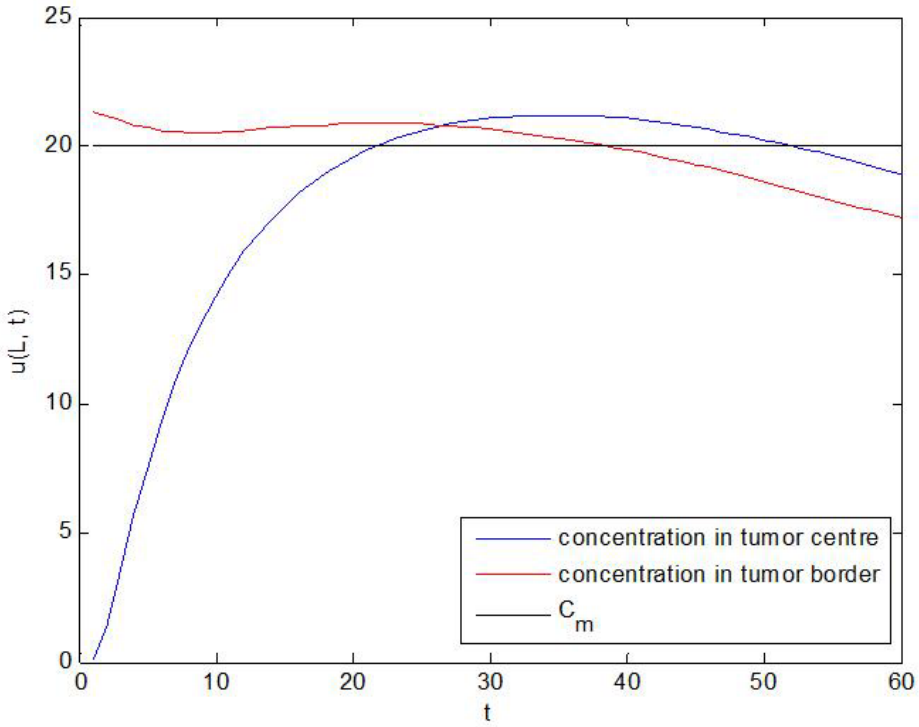

Figure 7. Drug concentration variation in the centre and border of the tumor, when drug is injected in a blood vessel and reaches the tumor from the periphery.

origin to more resistant clones that may be responsible for relapses, whilst the latter supports the existence of cancer stem cells with potential to be more tumorigenic, resistant and likely to give origin to relapses.

This tumor heterogeneity is certainly an important issue when we discuss chemotherapy response, because it is one of the characteristics that correlate better with tumor resistance. In order to supervene it, higher drug

concentrations (which would be lethal even to the most resistant cells) should be achieved or, otherwise, drug combinations should be administered. This can only be possible by a local administration technique like the one we present, since drug systemic toxicity is an important limitation in chemotherapy.

6. THERAPEUTIC APPLICATIONS

Although designed to accomplish other functions, microdialysis equipments may be used to deliver chemotherapy locally. Therefore, it may be applied to tumors within neoadjuvant approaches to downstage tumors and render them more accessible to surgery or radiotherapy. Its application is especially opportune in brain tumors (both primary and metastatic), since systemic chemotherapy hardly crosses blood-brain barrier and these tumors are frequently inaccessible to surgery. It may also be used to locally sensitize cells to radiotherapy, in a chemo and braquitherapy combined-regimen in cervix or prostate tumors.

In certain specified cases, this technique can be used with a curative intent. We already described the broadness of its applications in a previous article (7). It can be used not only in easily reached organs (breast...), but also in almost every internal organ with the development of endoscopic technical skills which can be adapted in order to insert catheters in the appropriate location (lung, prostate, stomach, colon...).

Nevertheless, there is a major caveat in this chemotherapeutic approach. It cannot be used by itself in disseminated diseases (unless if used to treat well-limited secondary tumors) and, when applied within neoadjuvant or adjuvant strategies, it does not help prevent metastasis and may not be effective enough, since chemotherapy will not reach, maintaining therapeutic concentrations, lymphatic nodes or distant sites where possible micrometastatic foci may be located. This limitation may be overcome by combining local delivery of chemotherapy by the microdialysis probe with lower doses of conventional chemotherapy, monoclonal antibodies or radiotherapy.

7. SUMMARY AND PERSPECTIVE – SHORT WAY FROM THE ANIMAL MODEL TO THE CLINICAL USE

Microdialysis, as a chemotherapy delivery mechanism, is empirically feasible and also theoretically effective, as we demonstrate in this article by using a mathematical model. However, before translating this knowledge to clinical practice, more studies should be performed in animals and in human tumors cultured *ex vivo*, to determine further important parameters necessary to determine what drug concentrations and delivery times should be used in each case. After obtaining these parameters, results obtained by computer simulation should be compared with practical experimentation and other more complex models may be built to optimize therapies in cancer. For tumors which present central necrosis, we should consider the placement of different probes in the periphery and this model may be adapted in order to represent this setting.

Since microdialysis has already been applied to human tumors, within research studies performed with diagnostic purposes (*in vivo* measurement of tumor estradiol and vascular endothelial growth factor in breast tumors) (12), once its effectiveness in animal models has been demonstrated its application in cancer therapy within clinical trials should be a straightforward step.

This technique carries with it great potential since, apart from delivering drugs, it can also measure cell metabolites, detecting cellular processes and possibly the rate of cell death. Further studies should be performed in order to exploit all the applications of this new, easy and effective technique, which may transform chemotherapy into an increasingly successful, more specific and less aggressive resource for patients.

8. ACKNOWLEDGEMENTS

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