The dynamics of tumour–vasculature interaction suggests low-dose, time-dense anti-angiogenic schedulings

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Abstract

Objectives: The administration schedule appears to be a particularly relevant factor in determining the effectiveness of an antiangiogenic drug. A better quantitative knowledge of the interactions between tumour growth and the development of its vasculature could help to design effective therapies.

Material and Methods: Biological and clinical inferences were derived from the analysis of a mathematical model proposed by Hahnfeldt et al. (1999), and some of its variants. In particular, we compared the effect of constant continuous infusion of an anti-angiogenic drug that induces vascular loss, to the effect of periodic, bolus-based therapy.

Results and Conclusions: The role of drug elimination rate and of dose fractionation was investigated, and we show that different schedulings, guaranteeing the same mean value of drug concentration, may exhibit very different long-term responses according to their concentration vs. time profile. For a large class of tumour growth laws, the profiles that approach the constant one are the most effective. This behaviour appears to depend on the ‘cooperativity’ of the tumour–vasculature interaction and on the functional form of the relationship between tumour growth and vasculature extent. Moreover, we suggest that a therapy approaching constant drug infusion might be advantageous also in the case of cytostatic anti-angiogenic drugs.

Introduction

Primary tumours and metastases require formation of new blood vessels in order to grow beyond 1 to 2 mm³. This process is sustained by different mechanisms (1–3). Tumours may co-opt existing vessels, may induce formation of new vessels from pre-existing ones or may exploit endothelial precursor cells originating in the bone marrow. Vessel formation is regulated by a number of pro- and anti-angiogenic molecules, released by tumour cells, and inflammatory and stromal cells (4). There is compelling evidence from experimental work that inhibiting angiogenesis may induce tumour regression or may sometimes even provide a cure (5–7). Targeting tumour vasculature, composed of genetically stable endothelial cells, has been regarded as a means to overcome acquired drug resistance (8,9). Angiogenesis inhibitors are commonly classified as ‘direct inhibitors’, acting on endothelial cells and inhibiting their proliferation and migration or inducing their apoptosis; as ‘indirect inhibitors’, blocking production of angiogenic factors by malignant cells; as mixed agents, targeting both endothelial and malignant cells (10). Most angiogenesis inhibitors are cytostatic, inhibiting formation of new blood vessels. Some of the direct inhibitors may also have cytotoxic action, inducing rapid destruction of existing blood vessels. Various anti-angiogenic drugs have undergone clinical development in recent years, and some of them have led to improvement in overall survival or disease-free survival in various clinical scenarios.

Among the factors influencing clinical effectiveness of angiogenesis inhibitors, the administration schedule appears to be particularly relevant. Anti-angiogenic therapy has always been proposed as uninterrupted, long-term treatment to obtain effective tumour growth control (10). Despite the fact that this concept has pervaded clinical development of anti-angiogenic drugs, a deeper insight into relationships between drug pharmacokinetics and antivascular activity could be useful to improve clinical results.

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Modelling the interplay between tumour growth and development of its vascular network, as well as the action of angiogenesis inhibitors, could help to plan effective anti-angiogenic therapies. Some mathematical models have recently been proposed (11–20). Hahnfeldt *et al.* (12) formulated a simple mathematical model that describes the vascular phase of tumour growth assuming that tumour growth is strictly controlled by dynamics of the vascular network, and that vascular dynamics is the result of opposing influence of pro-angiogenic and anti-angiogenic factors produced by the tumour itself (12). This model provides a framework to portray effects of anti-angiogenic therapies, and it was successful in fitting experimental data on growth and response to different anti-angiogenic drugs, in Lewis lung carcinoma implanted in mice. Mathematical analysis of the model was presented by d'Onofrio & Gandolfi, focusing on tumour eradication under regimens of continuous or periodic anti-angiogenic therapy (16). On the basis of that model, the problem of determining an optimal continuous delivery of drug has also been studied (21).

In this paper, we illustrate some new biological and clinical inferences derived from analysis of the model by Hahnfeldt *et al.* (12) and of its variants. In particular, through numerical simulations, we compare the effect of a constant continuous infusion of a drug that induces vascular loss, to the effect of a periodic, bolus-based therapy. We investigate the role of drug elimination rate and dose fractionation, and show that different schedulings, guaranteeing the same mean value of drug concentration, may exhibit very different long-term responses according to their concentration vs. time profile. Profiles that approach constant infusion, result in being more effective. This behaviour appears to be related to cooperativity of tumour–vasculature interaction as well as to functional form of the relationship between tumour growth and vasculature extent, and it was found to apply to a large class of tumour growth-rate laws. Moreover, we suggest that a therapy approaching the constant drug infusion might be more effective also in the case of cytostatic antiangiogenic drug, extending to the clinical stage some results obtained by Tee & DiStefano (22) from a model of the early, preclinical, growth of vessels in the peritumoural region.

Methods

Tumour growth model by Hahnfeldt et al. (1999)

Growth of a tumour has been described by different empirical growth curves, such as the Gompertzian and generalized-logistic curves (23). According to Gompertzian kinetics, relative growth rate declines linearly with the

logarithm of tumour size, and tumour size tends to a limiting value. The limiting size is meant to reflect maximal available nutritive support, and it may be viewed as the 'carrying capacity' of tumour environment. According to the hypothesis that tumour growth be strictly dependent on the development of its vasculature, Hahnfeldt *et al.* (12) replaced the fixed carrying capacity of the Gompertzian law with a variable carrying capacity $K(t)$, representing the maximal tumour size potentially sustainable by the vasculature at time t (12). Tumour growth is thus described by:

$$V'(t) = -\alpha V(t) \ln \frac{V(t)}{K(t)}, \quad (1)$$

where $V(t)$ is tumour volume (superscript ' denotes time derivative). Tumour growth described by eqn (1) will be bounded only if tumour vasculature will tend to a limited maximum extent. Development of the vascular network depends on balance among stimulatory and inhibitory factors, as well as on spontaneous loss of functional vessels and on possible action of angiogenesis inhibitors administered as drugs. The differential equation for $K(t)$ proposed by Hahnfeldt *et al.* (12) includes the above four factors and is given by

$$K'(t) = bV(t) - dV^{2/3}(t)K(t) - \mu K(t) - \eta g(t)K(t), \quad (2)$$

where $bV(t)$ represents the pro-angiogenic effect exerted by the tumour; $dV^{2/3}(t)K(t)$ represents the effect of endogenous anti-angiogenic factors (this term has been derived under the assumption that rate constant of disappearance of inhibitory molecules is much smaller than δ/R^2 , δ being their diffusion coefficient and R the tumour radius); $\mu K(t)$ is the rate of spontaneous vasculature loss; and $\eta g(t)K(t)$ is the rate of therapy-induced vasculature loss. $g(t)$ denotes concentration of the drug. Therefore, eqn (2) embodies the following key assumptions: (i) the amount of functional vasculature produced in unit time is proportional to tumour size; (ii) the relative rate of vasculature loss related to endogenous causes grows unboundedly with tumour size; and (iii) the anti-angiogenic drug acts by destroying vessels at a rate proportional to drug concentration (and to the extent of existing vasculature).

We note here that system (1), (2) is *cooperative* (i.e. $\partial V'/\partial K > 0$, $\partial K'/\partial V > 0$) in a realistic set of V , K values. In other words, according to this model, if K (if V) increases, there is an increment of growth rate of V (of K). In the absence of therapy, eqns (1) and (2) predict the tendency for carrying capacity and for tumour size to reach the following plateau value:

$$V_e = K_e = \left(\frac{b - \mu}{d} \right)^{3/2}. \quad (3)$$

This equilibrium results in being globally attractive (16): tumour size and carrying capacity tend to it whatever be their initial values. However, the limiting volume V_e will be normally incompatible with the host's life. It must be noted that the tumour growth predicted in the absence of therapy by the full system (eqns 1 and 2) is actually not Gompertzian; that is, it is not of the form $V(t) = V(0)\exp[\beta(1 - e^{-\gamma t})]$ because in eqn (1) the carrying capacity is not constant, but evolves according to eqn (2).

The model given by eqns (1) and (2) has been successfully fitted to experimental volume data of Lewis lung carcinomas, implanted subcutaneously in mice, either untreated or treated with angiogenesis inhibitors endostatin, angiostatin and TNP-470, obtaining estimates of parameters (12).

Variants of the model

A. As pointed out by Sachs *et al.* (24) + not in ref list, the functional form of eqn (1) is not mandatory to describe tumour growth as driven by vasculature growth. We may therefore consider for V' , as an alternative to eqn (1), the following generalized-logistic functional form:

$$V'(t) = \alpha V(t) \left[1 - \left(\frac{V(t)}{K(t)} \right)^\nu \right], \quad (4)$$

where $\nu > 0$ (see discussion on the generalized-logistic growth in Bodnar & Forsys (25)). A difference between eqn (4) and the Gompertzian-like eqn (1) is that, according to eqn (4), when tumour volume becomes very small with respect to carrying capacity, the relative growth rate V'/V has an upper bound given by α , while according to eqn (1) V'/V is unbounded. Since the doubling time of tumour size will be limited by the cell cycle time of tumour cells, eqn (4) appears, in this regard, to be more accurate (26).

B. Inhibition of formation of new vessels is an important effect, if not the prevalent one, of some drugs. For instance, drugs that inhibit production, or antagonize the action, of pro-angiogenic factors would act ultimately on the vasculature as inhibitors of endothelial cell proliferation and migration. Inhibition of endothelial cell proliferation was included in the mathematical models proposed by Tee & DiStefano (22) for early spatial growth of new vessels in the peritumoural region, and by d'Onofrio & Gandolfi (16) for the case in which birth rate of new vessels is independent of tumour size.

The effect of cytostatic drugs can be incorporated in eqn (2) by introducing a drug-dependent inhibition function $U(t)$ that modulates the vasculature growth term

$$K'(t) = bU(t)V(t) - dV^{2/3}(t)K(t) - \mu K(t) - \eta g(t)K(t), \quad (5)$$

and choosing for $U(t)$ the following form:

$$U(t) = \frac{g_{50}}{g_{50} + g(t)}, \quad (6)$$

where g_{50} is the 50% inhibition concentration.

Results

Constant infusion therapy

In order to study the conditions for tumour eradication under (arbitrarily long) anti-angiogenic therapy, the simplest case to analyse is that of constant continuous infusion of drug, which implies $g(t) = G$. When G is insufficient for tumour eradication, analysis of eqns (1) and (2) shows that tumour volume under therapy will tend to a new, smaller equilibrium value (16):

$$K^*(G) = V^*(G) = \left(\frac{b - \mu - \eta G}{d} \right)^{3/2} < V_e. \quad (7)$$

Note that this equilibrium is approached as long as therapy is continued: if therapy is interrupted, the tumour starts to regrow towards the plateau value V_e . When $G = (b - \mu)/\eta$, it is $V^* = 0$, so that to have tumour eradication, G must be greater than (or equal to) the critical value $(b - \mu)/\eta$, that is,

$$G \geq G_{cr}, \quad G_{cr} = (b - \mu)/\eta. \quad (8)$$

Thus, this threshold is proportional to a parameter, b , related to the pro-angiogenic factors concentration within the tumour. As it is intuitively expected, tumours characterized by greater release of pro-angiogenic factors should thus be treated with higher drug dosage. Note that G_{cr} is independent of the proliferative kinetics of tumour cells, according to the hypothesis that fate of the tumour is ultimately dictated by fate of its vasculature.

Periodic therapy

Different considerations apply to the case of periodic anti-angiogenic therapy, which is more frequently used in clinical trials and can be continued, in principle, for very long times. In this case, denoted by T the constant interval between two successive administrations, $g(t)$ becomes a periodic function of period T , i.e. $g(t + T) = g(t)$. In the following, we will assume that the drug is administered as a sequence of boluses, supposing, for simplicity, a first-order linear pharmacokinetics for the drug. This implies

mono-exponential drug decay. After a sufficiently large number of administrations, drug concentration in blood stabilizes to a periodic function of time given by:

$$g(t) = D \frac{P}{1 - e^{-cT}} e^{-c \text{Mod}(t, T)}, \quad (9)$$

where D is the dose of each bolus, P is the inverse of drug distribution volume, c is the rate constant of drug elimination, and $\text{Mod}(t, T) = t - nT$, n being the largest integer so that $t \geq nT$ (see Appendix). Drugs with different pharmacokinetic parameters give rise to quite different concentration–time curves, and, as we will see, the shape of $g(t)$ has a major impact on possibility of tumour eradication.

The mean value of drug concentration, $\bar{g}$, is expected to be related to the eventual efficacy of treatment. It can be shown (16), indeed, that the condition $\bar{g} \geq G_{\text{cr}}$ is necessary but not sufficient for tumour eradication under periodic therapy, according to the model of eqns (1) and (2). In the case of a drug with mono-exponential pharmacokinetics, when the periodic regimen described by eqn (9) is reached, we have for any t

$$\bar{g} = \frac{1}{T} \int_t^{t+T} g(t) dt = \frac{DP}{cT}. \quad (10)$$

Thus, a critical dose level $D_{\text{cr}}(c) = cT(b - \mu)/(\eta P)$, yielding $\bar{g} = G_{\text{cr}}$, must necessarily be exceeded to allow tumour eradication without, though, guaranteeing it. Note that constant infusion with infusion rate equal to D_{cr}/T would give $\bar{g} = G_{\text{cr}}$ and would guarantee eradication. According to this theoretical analysis, constant infusion is the optimal means of administration (under linear pharmacokinetics).

The nature of interaction between tumour cells and vasculature indeed implies that depending on the shape of the concentration profile $g(t)$, eradication may not occur for values of $\bar{g}$ that would be successful in the case of continuous therapy. Indicating with g_{min} the minimal value of $g(t)$, if $g_{\text{min}} \geq G_{\text{cr}}$, there is eradication (this intuitive property can be easily proven). Otherwise, if $g_{\text{min}} < G_{\text{cr}}$, numerical simulations of eqns (1) and (2) performed keeping $\bar{g}$ fixed and greater than G_{cr} , suggest, as a general rule, that when amplitude of the oscillation of $g(t)$ around the mean value is too large, $V(t)$ will not tend to 0 even if $\bar{g} > G_{\text{cr}}$. $V(t)$ and $K(t)$ will oscillate with period T around a positive value that is smaller than equilibrium in absence of therapy and might be clinically tolerable. Simulations show that this periodic solution of the system is unique and is reached whatever be the initial tumour volume (see Fig. 1). This implies that if tumour volume is small when treatment starts, there may be an initial growth before reaching the steady state.

Figure 1. Simulation of evolution of tumour volume over time according to eqns (1) and (2), in the case of periodic administration of an anti-angiogenic drug, with dose not sufficient for eradication of the disease. Independently of the initial conditions, there is convergence towards a unique periodic solution. Tumour volume V (mm^3); time t (days). $T = 2$ days, $D = 20$ mg/kg. Parameters values: $a = 0.192/\text{day}$, $b = 5.85/\text{day}$, $d = 8.73 \times 10^{-3}/\text{day}/\text{mm}^2$, $\mu = 0.0$, $\eta P = 0.66/\text{day} * \text{kg}/\text{mg}$, $c = 1.7/\text{day}$. Parameter values estimated in Hahnfeldt *et al.* (12) from tumour volume data of Lewis lung carcinoma implanted in mice (animals untreated and treated with endostatin).

Figure 2. Dependence of V_m on c , when dose is changed with c to keep $\bar{g}$ constant: $\bar{g} = G_{\text{cr}}$ (upper curve), $\bar{g} = 1.1 G_{\text{cr}}$ (middle curve) and $\bar{g} = 1.5 G_{\text{cr}}$ (lower curve). $T = 1$ day; c , $1/\text{day}$. Other parameters as in Fig. 1, except $\eta P = 1.3/\text{day} * \text{kg}/\text{mg}$.

Impact of rate constant of drug elimination

In the case of mono-exponential drug decay, the rate constant of drug elimination is critical, as well as the value of T , in determining how long $g(t)$ remains above G_{cr} during each period T , even if dose D is changed with c according to eqn (10) to maintain constant $\bar{g}$. Thus, it may be expected that tumour eradication or tumour mean volume during the periodic steady state, V_m , will depend on c . Figure 2 shows mean tumour volume V_m , obtained by means of simulations of the model, as a function of rate constant of drug elimination for a given period and three values of $\bar{g}$ (with $\bar{g} \geq G_{\text{cr}}$). Only if c is lower than a threshold value which increases with $\bar{g}$ do we obtain

Figure 3. (a) Mean tumour volume at steady state as a function of c and D , $V_m(c, D)$. (b) For $D > D_{cr}(c)$, the surface gives the value $V_m(c, D)$; for $D \leq D_{cr}(c)$, the surface gives the value $V_m(c, D_{cr}(c))$ for each c . The curve D_{cr} vs. c is given by the projection of the edge of the surface on the plane (c, D) . Note that as c increases, the minimal D needed for tumour eradication increases more quickly than D_{cr} . $T = 1$ day; c , 1/day, D , mg/kg. Other parameters as in Fig. 1, except $\eta P = 1.3/\text{day} \cdot \text{kg}/\text{mg}$.

Figure 4. Dependence of V_m on T , when $c = 10/\text{day}$ and $\bar{g}/P = 5 \text{ mg}/\text{kg}$. Other parameters as in Fig. 1, except $\eta P = 1.3/\text{day} \cdot \text{kg}/\text{mg}$.

eradication of the tumour; for larger c , eradication does not occur and V_m increases as c increases.

Impact of drug dose and period: drug fractionation

In order to optimize anti-angiogenic therapy, we can intervene in dose and schedule of administration. As it is intuitive, the value of V_m decreases as the dose of a given drug, administered within a given period, increases. Figure 3a summarizes the above results showing tumour size at the periodic steady state as a function of c and D . Figure 3b reports the quantity $V_m(c, D)$ for $D > D_{cr}(c)$, whereas for $D \leq D_{cr}(c)$ the surface yields for each c the value $V_m(c, D_{cr}(c))$. As c increases, the difference between

D_{cr} (which is proportional to c) and the effective minimal dose needed to eradicate the disease with periodic therapy also increases.

A relevant issue emerging from the model is the impact of the administration period. This is exemplified in Fig. 4 that reports V_m as a function of T , for a given value of c and $\bar{g}$. The plot shows that a considerable gain in efficacy, while keeping mean drug concentration constant, can be obtained by reducing the interval between administrations. This property can be further illustrated by the following comparison. Let us consider two different schedulings: scheduling 1, characterized by a given bolus dose D and a given period T , and scheduling 2, characterized by dose $D/2$ and period $T/2$. Note that they deliver the same amount of drug in time intervals of duration T . Figure 5, reports simulated tumour responses for two values of c , showing that scheduling 2 is preferable. If the drug were targeting tumour cells with log-kill activity, according to a linear model of tumour growth, the two schedulings would instead have the same effect. Scheduling 2 remains more effective also if $d = 0$ in eqn (2) (not shown: in this case, however, either eradication is achieved, or the tumour eventually grows boundlessly). The numerical study of models (1) and (2), summarized by Figs 4 and 5, thus supports the hypothesis that time-dense fractionated schedulings are more effective. We note that this modality of scheduling approaches, a constant continuous infusion, as D and T become small together.

Figure 5. Tumour volume vs. time for different schedulings. The left panel refers to the administration of a drug having $c = 10/\text{day}$: in the upper curve the scheduling is characterized by $T = 2$ days and $D = 100 \text{ mg/kg}$, whereas for the lower curve $T = 1$ day and $D = 50 \text{ mg/kg}$. The right panel refers to a drug with $c = 1.7/\text{day}$: in the upper curve the scheduling is characterized by $T = 2$ days and $D = 16 \text{ mg/kg}$, whereas for the lower curve, corresponding to eradication, $T = 1$ day and $D = 8 \text{ mg/kg}$.

Bi-exponential drug elimination

A more realistic description of drug pharmacokinetics should include the possibility of multimodal decay of drug concentration after bolus administration. In such a case, analysis of the effect of periodic therapy is rather complex, and we provide here some results only in the case of bi-exponential drug elimination. Let us consider periodic bolus administration, and suppose that the periodic regimen for drug concentration is reached. We can write (see, e.g. Gibaldi & Perrier (27), and the Appendix):

$$g(t) = D(a_1 e^{-c_1 \text{Mod}(t,T)} + a_2 e^{-c_2 \text{Mod}(t,T)}). \quad (11)$$

Parameters c_1 and c_2 , with $c_2 \geq c_1$, are the rate constants of the slow and, respectively, the fast mode of elimination. Keeping the mean drug concentration fixed at a given value, different shapes of drug concentration time-course are possible, according to the values of c_1 , c_2 and of the ratio a_2/a_1 . To measure the distance between the profile of $g(t)$ and the constant value $\bar{g}$, we may compute the following 'dispersion' index:

$$I_g = \left(\frac{1}{T} \int_t^{t+T} |g(t) - \bar{g}| dt \right) \frac{1}{\bar{g}}. \quad (12)$$

Through numerical simulations, we have investigated the relationship between mean value of tumour volume during the periodic steady state (according to eqns (1) and (2)), and dispersion of $g(t)$ around its mean value. Figure 6

Figure 6. Mean tumour volume V_m (open symbols) and dispersion index I_g (closed symbols) vs. c_2/c_1 , when the drug decay is bi-exponential ($g(t)$ given by eqn 11). Ordinates normalized to maximal values. Upper panel: $c_1 = 1/\text{day}$; lower panel: $c_1 = 4/\text{day}$. In both panels: $a_2/a_1 = 5$, $T = 1$ day. Other parameters as in Figure 1, except $\eta P = 1.3/\text{day} * \text{kg/mg}$. The dose is changed with c_2/c_1 in order to keep $\bar{g}$ constant and equal to $1.1 G_{cr}$.

reports an example of these results, showing V_m and index I_g as functions of the ratio c_2/c_1 , for two different values of c_1 and a fixed value of a_2/a_2 . In these simulations, drug dose was varied for each combination of the parameters to keep $\bar{g}$ fixed at a value greater than G_{cr} . We can observe that when the dispersion index decreases, the mean tumour volume also decreases (the maximum of the two curves is indeed attained for the same combination of parameters). This result confirms the suggestion that therapies producing drug concentration profiles close to a constant profile have greater efficacy.

Response of the logistic-like model

The above-described behaviour of tumour response appears related to vasculature regrowth during the portion of period in which drug concentration attains lower values, and it is also influenced by tumour dynamics because of the pro-angiogenic cooperative role of the tumour (see eqn 2). The question, thus, arises of how much this behaviour is dependent on functional choices made in building the model.

If dependence of tumour growth on vasculature is expressed by eqn (4) with $\nu \geq 1$, it can be proved that the condition $\bar{g} \geq G_{cr}$ is necessary and sufficient for tumour eradication under periodic therapy (16). This sufficiency is lost if $\nu < 1$. Simulations of steady-state response according to the model of eqns (4) and (2), with different values of $\nu < 1$ and given $\bar{g}$ greater than G_{cr} , are shown in Fig. 7 (in these simulations, mono-exponential drug decay was assumed). In all the simulated therapies, eradication is no more guaranteed and mean tumour size at the steady state increases with c as in Fig. 2. Moreover, therapy appears less efficient as ν decreases from unity.

Figure 7. Mean tumour volume at steady state vs. c , according to the model of eqns (4) and (2). $T = 1$ day, $\bar{g}/P = 5$ mg/kg; $\nu = 0.25$ (upper curve), 0.50 (middle curve), 0.75 (lower curve). Dose varied with c to keep $\bar{g}$ constant and equal to $1.1 G_{cr}$. Other parameters as in Fig. 1, except $\eta P = 1.3/\text{day} * \text{kg}/\text{mg}$.

Inhibition of endothelial cell proliferation

We start illustrating the case of a drug that inhibits growth of new vasculature without inducing vessel loss (i.e. $\eta = 0$ in eqn 5). In the case of constant infusion therapy, i.e. when $g(t) = G$, the therapy effect corresponds in the model to reducing the actual value of parameter b to the value $bg_{50}/(g_{50} + G)$. As a consequence, the equilibrium point is smaller than the one in absence of therapy (see eqn 3):

$$\tilde{K}(G) = \tilde{V}(G) = \left(\frac{1}{d} \left(b \frac{g_{50}}{g_{50} + G} - \mu \right) \right)^{3/2}.$$

Thus, if $\mu > 0$, by solving $\tilde{V}(G) = 0$, it is easy to see that for

$$G = \tilde{G} = g_{50} \left(\frac{b}{\mu} - 1 \right),$$

the equilibrium value of V is zero, and for $G \geq \tilde{G}$ there is eradication. If spontaneous loss of vasculature were absent ($\mu = 0$), disease elimination would not be possible.

In the case of periodic therapy, outcome will depend on the shape of the drug concentration–time curve, firstly because the inhibition function (eqn 6) is not linear with respect to g . In the case of mono-exponential drug pharmacokinetics, if $c \gg 1/T$, it can be easily seen that $U(t) \approx 1$ for most part of the time interval between successive administrations. Thus, a blocking drug, acting through the inhibition function of eqn (6) and having a very high rate of elimination, is expected to be ineffective even if administered at very high doses. Figure 8 illustrates the dependence of the mean tumour size at the steady state on c , predicted by model of eqns (1), (5) and (6), in the case of a bolus-based therapy in which doses are changed with c to keep mean drug concentration constant.

Figure 8. Drugs inhibiting vascular proliferation but not increasing vessel loss. Mean tumour size at steady state as function of c , according to the model of eqns (1) and (5). $D/g_{50} = 2cT$ (upper curve), $3cT$ (middle curve), $4cT$ (lower curve). $T = 1$ day. Other parameters as in Fig. 1, except $\eta = 0$. Eradication is never reached.

In the case of drugs having both cytostatic and cytotoxic effects, for low drug doses under constant infusion therapy, the following plateau value is reached:

$$\tilde{K}^*(G) = \tilde{V}^*(G) = \left(\frac{1}{d} \left(b \frac{g_{50}}{g_{50} + G} - \mu - \eta G \right) \right)^{3/2} < \tilde{V}(G).$$

Of course, that $\tilde{V}^*(G) < \tilde{V}(G)$ is due to synergy between the two effects of the drug. This synergy has the effect of reducing drug concentration needed to eradicate the tumour. In fact, the eradication threshold $\hat{G}$ is the solution of

$$b \frac{g_{50}}{g_{50} + \hat{G}} = \mu + \eta \hat{G},$$

and we have that $\hat{G} < G_{cr}$. In the case of periodic therapy, if c is large, from $U(t) \approx 1$ it follows an equivalent behaviour to that illustrated in the previous sections.

Discussion

Theoretical remarks

The model of Hahnfeldt *et al.* (eqns 1, 2) predicts the tendency for tumour size to reach a plateau that is lower under anti-angiogenic treatment and potentially clinically tolerable (12). This behaviour also applies to the models of eqns (4)–(2), which use the generalized-logistic law. What indeed is actually required to reach a stable steady state, is that (i) the trade-off between pro-angiogenic and anti-angiogenic signals shifts towards inhibition as tumour size increases, and (ii) tumour growth rate is positive when tumour size is lower than the carrying capacity, and is negative otherwise. To simulate responses to drugs that do not induce apoptosis of endothelial cells or have mixed effects, we have extended the model of eqns (1) and (2) to include inhibition of endothelial cell proliferation (eqns 1, 5, 6). All these models predict that the tumour size reached at the plateau is the same regardless of tumour volume at the initial time of treatment, implying in some cases an initial tumour growth even under treatment eventually effective.

The above-discussed mathematical models suggest that pharmacokinetic properties of the drug and administration schedule are particularly relevant to the anti-angiogenic treatment efficacy. In the case of constant infusion of drug, models of eqns (1)–(2) and eqns (4)–(2) provide a critical serum level of drug sufficient for tumour eradication (or for obtaining a certain tumour control). The minimal dose of drug required to control tumour growth appears to depend on tumour pro-angiogenic activity rather than on tumour cell kinetics. It could therefore be important in clinics to evaluate this activity. In the case of periodic administration, the models of eqns (1)–

(2) (or the model of eqns (4)–(2) with $\nu < 1$) predict that keeping mean concentration of drug above the value that is eradicated with constant infusion may not exert the same effect. Not only the area under the curve but also the shape of the concentration–time curve influences activity, even when the vascular loss rate depends on the drug concentration in a linear way as assumed in Eqn 2. High peaks followed by rapid decay of serum concentration are less effective than lower, but more prolonged peaks, even if the area under the curve is the same. Constant concentration profile is found to be the most effective. Furthermore, the model predicts that giving the same total amount of drug in frequent divided doses (time-dense schedule) is more efficacious than giving more spaced administrations. Frequent dosing is indeed the best way of mimicking a constant infusion.

According to Eqn. 4 with $\nu \geq 1$, any mean drug concentration above G_{cr} causes instead eradication, independently of the shape of the periodic concentration–time curve. Since in both the cases of eqns (1), and (4) with $\nu < 1$, the relative tumour growth rate V'/V is a convex function of V/K (whereas the opposite is true if $\nu \geq 1$), we make the conjecture that this property, together with the cooperativity of the tumour–vasculature interaction, is required for optimality of the constant drug infusion. Simulations of other model variants (not shown) supported this conjecture.

The question arises whether the best model for the growth rate of a given tumour is Gompertzian-like or logistic-like with $\nu < 1$ (say, type I models), or logistic-like with $\nu \geq 1$ (type II models). To obtain some speculative insight, we can forecast the tumour behaviour by thinking the supplying vasculature as if it were ‘frozen’ at a fixed extent. We note that when the carrying capacity in eqn (4) is kept constant at a value larger than the initial tumour size, the predicted tumour growth for $\nu > 1$ is almost exponential for a prolonged time interval, after which it saturates rather sharply. By lowering ν under 1, instead, the exponential portion of the growth curve becomes shorter with slower approach to saturation. Since it is likely that tumour cells are pushed increasingly away from blood vessels because of their proliferation (28), it is reasonable to hypothesize that tumour cells are exposed during their lifespan to worsening microenvironment (29). Then, we can guess that parameter ν in eqn (4) reflects sensitivity of tumour cell kinetics to extracellular level of oxygen and nutrients. Low value of ν would be associated with a population of tumour cells that gradually slow down their proliferation before the point at which nutrient deprivation impairs viability (see also (25)). High value of ν would be predictive, instead, for tumour cells that do not change their proliferation rate even in poor environmental conditions. Therefore, following the above argumentation (and recognizing that the

Gompertzian growth law can be approximated by eqn (4) with $v \ll 1$, type I models appear to be more appropriate in most real cases. Moreover, according to d'Onofrio (2008), decreasing and convex relative growth rates characterize proliferating cell populations under the effect of long-range inhibitory cell–cell interactions that decrease with intercell distance (30).

The advantage of low-dose, time-dense schedules for drugs inducing vessel loss has been previously suggested by Hahnfeldt *et al.* (31), using a model that hypothesizes subdivision of the endothelial cell pool in two subpopulations with different sensitivities to the drug, and able to return rapidly to a mutual equilibrium after perturbations. Moreover, Tee & DiStefano found, simulating initiation of vascular development in the peritumoural region, a greater efficacy of the constant infusion of drugs that inhibit the proliferation of the endothelial cells (22). Such a finding is paralleled by the results discussed in 'Inhibition of endothelial cell proliferation' section. However, it must be noted that both the above studies did not model the tumour growth, whereas the interplay between the vascular growth and the tumour growth is instead at the basis of the predictions of our analysis.

In the present work, 'a vast array of spatial ... details of tumour cell expression' (12) has been disregarded, although the Hahnfeldt *et al.* model, which we have studied and extended here, was derived starting from a simple partial differential equation model describing diffusion of pro-angiogenic and anti-angiogenic factors within the tumour mass. We recognize that lack of an explicit spatial description is a limitation, and it implies that our predictions can be more qualitative than strictly quantitative. It would be actually important to describe phenomena such as the spatial heterogeneity of the production of stimulatory and/or inhibitory factors, the variability in blood flow and perfusion within the tumour volume, and the co-optation of host vessels in the peritumoural region. However, from a qualitative point of view, modelling all these phenomena should not lead to results significantly different from those illustrated here, as long as the overall functional relationships between the tumour mass and the effective vasculature extent are in agreement with the functional assumptions of the present models.

Preclinical and clinical evidences

Preclinical studies of anti-angiogenic drugs have shown an advantage in continuous vs. periodic drug administration. Continuous administration of TNP-470 at 5 mg/kg/week produced effects comparable to those of bolus administration of 20 mg/kg three times per week in rats with osteosarcoma (32). Angiostatin was significantly more effective when administered *via* continuous infusion, than

when the same daily dose was given as twice-daily bolus administration (33). A dose of 2 mg/kg/day of endostatin in continuous infusion was as effective as a dose of 20 mg/kg/day given as single bolus injections (34). Although the above results cannot be taken as a direct confirmation of our predictions because the mean serum concentrations have not been reported, such findings can be explained by our theory under the assumption of linear pharmacokinetics. Moreover, a frequent administration of cyclophosphamide in mice inhibited the growth of tumours resistant against the same drug given with a conventional schedule. This has been attributed to anti-angiogenic effect, because apoptosis of vascular endothelial cells precedes that of tumour cells (35). Continuous low-dose oral administration of cyclophosphamide and methotrexate produced tumour control in 32% of patients pretreated for metastatic breast cancer (36). However, interpretation of these results would require more complex modelling, because of the direct antitumour activity of the above drugs.

Phase I clinical trials of recombinant human endostatin were conducted mainly with daily intravenous administration (37–39). No objective response has been reported, but only minor signs of activity. Pharmacokinetics is linear with a terminal half-life of 10–13 h, and an initial half-life of 40 min. This short value could prevent maintenance of adequate serum concentrations for most of the period in bolus administration, explaining the reported lack of significant activity. Anyway, a further phase I trial involving a continuous intravenous administration of endostatin for 4 weeks followed by twice-daily subcutaneous injections showed only a few cases of disease stabilization (40), and a phase II trial with twice-daily subcutaneous injections of endostatin in patients with advanced neuroendocrine tumours did not show significant antitumour activity (41). Possible reasons for lack of activity have been proposed (40); we further suggest that serum concentration could have been lower than G_{cr} . Intravenous administration of recombinant human angiostatin produced serum half-life of 20 min (42), while subcutaneous injection yielded a half-life of about 3 h (43). Twice-daily subcutaneous injections produced long-term (> 6 months) stable disease in 6 of 24 patients (43). Moreover, twice-daily subcutaneous angiostatin, continued until disease progression, in combination with paclitaxel and carboplatin for up to six cycles, yielded overall disease control rate and time to progression exceeding that of historical controls treated with the same chemotherapy alone (44). These pieces of evidence of activity could be related to longer half-life of subcutaneous angiostatin and to the use of a fractionated administration schedule. TNP-470 has an extremely short-terminal half-life of about 2 min for the parent compound and 6 min for its main active metabolite AGM-1883 (45,46).

Plasma concentrations higher than those needed for *in vitro* inhibition were obtained during a 4-h infusion, but plasma levels decreased abruptly becoming undetectable after 1 h from the end of infusion. Thus, prolonged infusion has been advocated as a better schedule (45). Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has a long-terminal half-life in the range of 13–21 days (47), which implies plasma concentration profile close to constant one even in the case of spaced administrations. This might be one reason for the relevant efficacy of this drug, which improved overall survival when added to chemotherapy in patients with metastatic colorectal cancer (48) and lung cancer (49), and disease-free survival in patients with metastatic breast cancer (50).

Various inhibitors of receptor tyrosine kinases are under clinical development. Many are anti-angiogenic, whereas others also have effects on tumour cells (51). SU5416 (semaxinib) is an inhibitor of VEGF receptor-2 (VEGFR-2). Its terminal half-life is short (21–87 min) and after a brief intravenous infusion plasma concentration remained only for 2 h greater than that required for activity *in vitro* (52). This might be a reason for the disappointing results of clinical trials (52–54). Drugs with longer half-lives showed more promising results. PTK787/ZK 222584 (vatalanib) is an oral inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, and c-kit, with terminal half-life of 3–6 h (55). In a phase I trial, twice-daily administration of the drug yielded a high rate of disease stabilization and one partial remission (56), while early results from two phase III trials in advanced colorectal cancer showed an improvement in disease-free survival (57,58). Among multitargeted agents inhibiting both endothelial and cancer cells, SU11248 (sunitinib), which inhibits VEGFR-2, PDGFR, c-kit and FLT-3, and BAY43-9006 (sorafenib), which inhibits Raf kinase as well as VEGFR1, VEGFR2, VEGFR3, PDGFR, FLT-3, and c-Kit, have terminal half-lives up to 40 h (59,60), and both these drugs shown efficacy in phase III clinical trials (61,62).

Although multiple important factors, such as the number of targets and pathways inhibited and their relevance for the growth of specific tumour types, may explain the variable results of anti-angiogenic agents, these examples of clinical studies underline the importance of the strategies of administration in relation to the pharmacokinetic parameters of each drug. As our analysis suggests, the crucial role of scheduling may also originate from the nature of the interplay between the tumour growth and the growth of the vascular network. An effective use of angiogenesis inhibitors, as well as of any targeted therapy, will require the consideration of the tumour and its host as an interacting system. Mathematical modelling may help to identify hidden relationships and unexpected

responses of a highly complex system and could thus be valuable in assisting the clinical development of new drugs.

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Appendix

Let us consider the dynamics of the concentration x of a drug characterized by a first-order linear pharmacokinetics with elimination rate constant c , and suppose that a single dose D is delivered as a bolus at time $t = 0$. For $t < 0$ it is

$$x(t) = 0,$$

whereas for $t > 0$ the drug concentration will obey the following linear differential equation:

$$x' = -cx, \quad (13)$$

with the initial condition

$$x(0^+) = DP, \quad (14)$$

where P denotes the inverse of the distribution volume. Eqns (13) and (14) have the exponential solution:

$$x(t) = DP e^{-ct}.$$

If the drug is delivered each T time units, $x(t)$ is governed by the following impulsive differential equation:

$$x' = -cx \quad (15)$$

$$x(nT^+) = DP + x(nT^-), \quad n = 0, 1, \dots \quad (16)$$

In other words, for $nT < t < nT + T$ the decay of x follows the exponential law, but every T time units the concentration must be increased as consequence of drug delivery. By defining

$$x_n = x(nT^+),$$

we have that for $t \in (nT, nT + T)$ it is

$$x(t) = x_n e^{-c(t-nT)}, \quad (17)$$

so that eqn (16) implies:

$$x_{n+1} = DP + x_n e^{-cT}. \quad (18)$$

Eqn (18) defines a discrete dynamical system having the unique equilibrium

$$x_{eq} = \frac{DP}{1 - e^{-cT}},$$

which is globally asymptotically stable since $e^{-cT} < 1$. Thus, for sufficiently large n , eqn (17) is well approximated for $t \in (nT, nT + T)$ by the equation

$$x(t) = \frac{DP}{1 - e^{-cT}} e^{-c(t-nT)},$$

and thanks to the equality $\text{Mod}(t, T) = t - nT$ for $t \in (nT,$

$nT + T$), we get the steady-state periodic function given by eqn (9):

$$x(t) = \frac{DP}{1 - e^{-cT}} e^{-c \text{Mod}(t, T)}. \quad (19)$$

More formally:

$$\lim_{n \rightarrow +\infty} x_n e^{-c(t-nT)} = \frac{DP}{1 - e^{-cT}} e^{-c \text{Mod}(t, T)}.$$

In the framework of a multi-compartmental model with k compartments, let us denote by x_i the drug concentration in the i th compartment. Let $x = (x_1, \dots, x_k)^{Tr}$ be the vector of concentrations, Tr denoting transposition. If the pharmacokinetics is linear and the drug bolus is delivered in the first (blood) compartment, we can write the following vector differential equation

$$x' = Mx \quad (20)$$

$$x(nT^+) = DPu + x(nT^-), \quad (21)$$

where M is a $k \times k$ matrix whose eigenvalues are $-c_1, \dots, -c_k$, u is the vector $(1, 0, \dots, 0)^{Tr}$, and P is the inverse of the distribution volume of the first compartment. By using the matrix exponential notation and following the same steps we have used for the mono-exponential case, we obtain that $x(t)$ asymptotically becomes:

$$x(t) = DP(I - e^{MT}) e^{M \text{Mod}(t, T)} u.$$

The drug concentration in blood, $g(t) = x_1(t)$, is therefore asymptotically given by the periodic function:

$$g(t) = DP \sum_{s=1}^k b_s e^{-c_s \text{Mod}(t, T)} = D \sum_{s=1}^k a_s e^{-c_s \text{Mod}(t, T)}. \quad (22)$$