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Letter to the Editor

A Possible Bias in Growth-Kinetic Estimates of the Thickness of the Growing Layer in Multicellular Tumor Spheroids

C. D. Thron

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03755

ABSTRACT

Yuhas and Li (Cancer Res., *38:* 1528–1532, 1978) have proposed a method for estimating the thickness of the growing layer in multicellular tumor spheroids. Their method assumes, however, that the thickness of the growing layer is independent of spheroid radius; this assumption seems implausible in view of the fact that, for purely geometrical reasons, oxygen diffusion distance is greater in small than in large spheroids. In this communication, theoretical growth rates are calculated for a growth kinetic model based on Burton's (Growth, *30:* 157–176, 1966) model of oxygen diffusion, and it is shown that for this model the Yuhas-Li estimates of thickness of the growing layer are 10 to 30% below the true thickness of this layer in the larger of the two spheroids used for the estimation. To generalize beyond this particular model, it is shown that, for any model where the growing layer is thicker in small than in large spheroids, the Yuhas-Li method underestimates the thickness of the growing layer. However, for our particular model at least, the bias in the Yuhas-Li estimates is fairly constant and relatively small, so that these estimates may be quite serviceable, especially for purely comparative studies.

Yuhas and Li (11) have suggested that multicellular tumor spheroids consist of an outer uniformly growing layer of constant thickness and an inner mass of nondividing cells. These authors have derived an equation for estimating the thickness of the growing layer from the relative growth rates, as measured by [^{125}I]IdUrd[1] incorporation, of spheroids of differing diameter. Curphey (4) has offered refinements and further discussion. Conger and Ziskin (3) have also made the assumption of constant thickness of the growing layer. This assumption, however, while plausible for large spheroids, seems unlikely to be true for small spheroids, in view of the fact that for purely geometrical reasons the oxygen diffusion distance is greater in small than in large spheroids (5).

This communication reports calculations done to determine how the Yuhas-Li analysis will be affected if in fact the thickness of the growing layer, like the oxygen diffusion distance, decreases with increasing spheroid radius. We begin by calculating the results of the Yuhas-Li analysis for a specific theoretical example, a model of spheroid growth kinetics based on the oxygen-diffusion model of Burton (1). Burton's model assumes that local tissue oxygen consumption is a step function of local tissue oxygen concentration; *i.e.*, oxygen consumption is either zero or a fixed value, according to whether oxygen concentration is less or greater than some critical value. Such a step function is undoubtedly somewhat unrealistic, but it makes the diffusion equations mathematically tractable, and it seems a reasonable approximation to reality in systems where oxygen consumption rises from near zero to near the maximum over a rather narrow range of oxygen concentrations.[2]

From Burton's equation 11 (see Ref. 1), we easily derive

$$\left[\frac{\theta}{R}\right]^3 - 3/2\left[\frac{\theta}{R}\right]^2 + 1/2\left[\frac{R_0}{R}\right]^2 = 0 \qquad \text{(A)}$$

where R is the spheroid radius, θ is the thickness of the oxygen-consuming layer, and R_0 is the radius of a just-fully oxygenated sphere, *i.e.*, the largest sphere in which all of the tissue has an oxygen concentration at or above the threshold for oxygen consumption.

For the sake of simplicity, we measure all distances (R, θ, X) relative to R_0; so $R_0 = 1$. Trigonometric Solution (9) of Equation A gives 3 real roots, of which the only one lying between 0 and 1 is

$$\frac{\theta}{R} = 1/2 + \cos\{1/3\cos^{-1}[1 - 2/R^2] - 2\pi/3\} \qquad \text{(B)}$$

For computation in Dartmouth BASIC, which lacks an inverse cosine function, I substituted $\cos^{-1} u = \pi/2 - \tan^{-1}(u/\sqrt{1-u^2})$ (the inverse tangent is taken to lie between $\pm\pi/2$). With a little algebra, we arrive at

$$\frac{\theta}{R} = 1/2 - \sin\left\{1/3\tan^{-1}\left[\frac{(R^2-1)^{1/2} - (R^2-1)^{-1/2}}{2}\right]\right\} \qquad \text{(C)}$$

The growing layer is certainly no thicker than the oxygen-consuming layer. It may well be thinner, but to avoid additional arbitrary variables we will assume for these illustrative calculations that the whole oxygen-consuming layer is growing uniformly, *i.e.*, that the oxygen-consuming layer is the same as the growing layer. With this assumption, a plot of Equation C (Chart 1) shows that the thickness of the growing layer is not constant in spheroids of radius less than about $2R_0$. This size range includes part of the size range best suited to the Yuhas-Li equation (4). Although the thickness of the oxygenated layer approaches constancy in larger spheroids, these cannot be used for the Yuhas-Li equation, because the equation would require impossibly small experimental error (4).

By Equation E of Yuhas and Li, the amount of [^{125}I]IdUrd incorporated is given by

$$C = 4/3\pi K[R^3 - (R-\theta)^3] \qquad \text{(D)}$$

[1] The abbreviation used is: IdUrd, iododeoxyuridine.

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[2] The Burton assumption of a step function for oxygen consumption is in fact quite analogous to the Yuhas-Li assumption for cell division: that the growth rate rises abruptly from zero to the maximum on passing from the inner nondividing core to the outer growing layer of the spheroid.

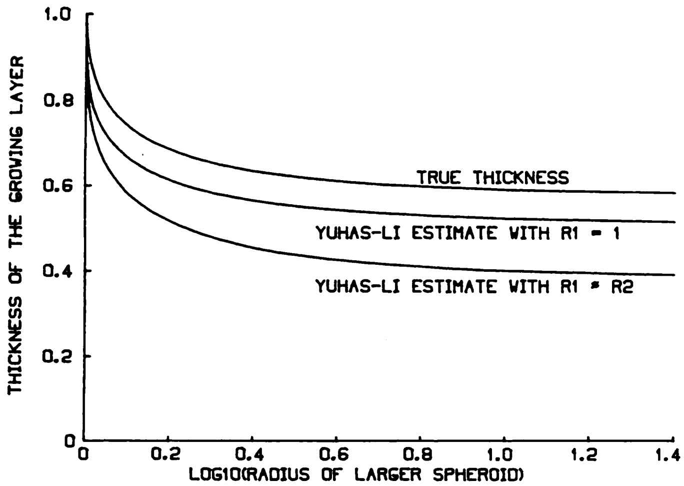

Chart 1. Calculated results for a theoretical model of spheroid growth kinetics based on Burton's (1) model of oxygen diffusion: true thickness of the growing layer [Equation C] as a function of the $\log_{10}$ of the spheroid radius, and estimates from the Yuhas-Li equation [Equations E (with $R_1 = 1$) and F] as functions of the $\log_{10}$ of the radius of the larger spheroid. Spheroid radius and growing layer thickness are measured relative to the radius of the largest spheroid that is fully oxygenated and hence, according to our model, is growing uniformly throughout.

where K is a proportionality constant. The Yuhas-Li equation for the estimated thickness X of the growing layer is

$$(Z - 1)X^2 - 3(ZR_1 - R_2)X + 3(ZR_1^2 - R_2^2) = 0 \quad \text{(E)}$$

where Z (= C_2/C_1) is the ratio of [^{125}I]IdUrd incorporations in spheroids of radii R_2 and R_1, respectively. For any choice of R_2 and R_1, we can calculate the ratio of the corresponding [^{125}I]-IdUrd incorporations for our theoretical model by means of Equations D and C. Taking this ratio as Z, we can then compute X from Equation E and compare this estimated thickness with the true thickness θ.

The results depend, of course, upon the choice of R_1 relative to R_2. The choices fall between 2 extremes: choose R_1 as distant from R_2 as possible (*i.e.*, let $R_1 = 1$); or choose R_1 as close to R_2 as possible. For the latter limiting case, note that $Z = C_2/C_1 = 1 + (R_2 - R_1)G$, where $G = (C_2 - C_1)/[(R_2 - R_1)C_1]$. Replacing Z in Equation E by $1 + (R_2 - R_1)G$, canceling out a common factor $R_2 - R_1$, and taking the limit as $R_1 \rightarrow R_2$, we have

$$gX^2 - 3(R_2g - 1)X + 3(R_2g - 2)R_2 = 0 \quad \text{(F)}$$

where

$$g = \lim_{R_1 \to R_2} G = \left[\frac{\partial \log C}{\partial R}\right]_{R = R_2} \quad \text{(G)}$$

The evaluation of g from Equations D and C is tedious but straightforward, and X can then be calculated from Equation F.

Chart 1 shows the estimated thickness X of the growing layer calculated by Equations E (with $R_1 = 1$) and F. Other curves (not shown) calculated by Equation E with constant differences $R_2 - R_1$ fell between these 2 curves, starting on the upper one and approaching the lower one asymptotically with increasing radius. The 2 limiting curves show that the Yuhas-Li method consistently underestimates the thickness of the growing layer in the larger spheroid by 10 to 30%. Note that X does not approach the true thickness θ as R becomes large, even though the assumption of constant thickness is approximately true at large R. This is because as R becomes large the Yuhas-Li calculation of X becomes increasingly sensitive to small deviations from constant thickness.

Since θ approaches constancy as R becomes large, it follows that the radial growth rate in our theoretical model approaches constancy, just as it does in a constant-thickness model (3). In fact, the increase in θ as R becomes small acts to extend the lower end of the range of linear radial growth, in comparison to a constant-thickness model.

This theoretical growth-kinetic model has been presented as an illustration, but it might prove to be approximately valid for some real spheroids. In such cases, the thickness of the growing layer should be calculated from the equations for this model and not from the Yuhas-Li equation. Unfortunately, the present model offers no easy way to make this calculation, but presumably a curve-fitting procedure can be followed with Equations C and D. Since in real measurements R would not be measured relative to R_0 (R_0 being unknown), R and θ in Equations C and D must be replaced by R/R_0 and θ/R_0, respectively. One can then adjust the unknown parameters R_0 and K to obtain the best fit of the modified Equations C and D to the experimental values of C as a function of R. The statistical properties of these parameter estimates would of course have to be investigated.

As to whether the present theoretical growth-kinetic model is valid, I have tried fitting the Burton model to published data on oxygen concentration within spheroids, and I have found a reasonably good fit to the data of Mueller-Klieser and Sutherland (8) but not to the data of Carlsson *et al.* (2). Of course, even if the Burton model holds for oxygen consumption, the growing layer may not be the same as the oxygen-consuming layer; *i.e.*, it may comprise only the outer part. A fuller analysis of spheroid growth kinetics would require consideration of other models of oxygen consumption [*e.g.*, Tosaka and Miyake (10), Hiltman and Lory (6)], oxygen depletion in the unstirred layer surrounding the spheroid (12), and other factors such as diffusible inhibitory substances, changes in cell size, and cell shedding [see Landry *et al.* (7), and references therein]. At the present time, the validity of any growth-kinetic model, including the Yuhas-Li constant-thickness model, would seem to be a matter requiring experimental verification for each case under consideration.

We turn now from our particular model to a more general model of diffusion-limited growth kinetics. It seems likely that the geometrical factor in spherical diffusion, not only of oxygen but also of other nutrients and products of metabolism, must almost always result in a thicker-growing layer in small than in large spheroids. Consider, therefore, spheroids of radii R_1 and R_2, in which the thickness of the growing layer is θ_1 and θ_2, respectively. Assume that $R_2 > R_1 > \theta_1 > \theta_2 > 0$. The ratio of [^{125}I]IdUrd incorporations is obtained from Equation D; and Yuhas and Li's Equation F is then

$$\frac{R_2^3 - (R_2 - \theta_2)^3}{R_1^3 - (R_1 - \theta_1)^3} = \frac{R_2^3 - (R_2 - X)^3}{R_1^3 - (R_1 - X)^3} \quad \text{(H)}$$

It is easy to see that substituting θ_2 for θ_1 on the left-hand side of Equation H makes the left-hand side larger, since $\theta_2 < \theta_1$; and after multiplying out the numerators and denominators we arrive at

$$\frac{3R_2^2 - 3R_2\theta_2 + \theta_2^2}{3R_1^2 - 3R_1\theta_2 + \theta_2^2} > \frac{3R_2^2 - 3R_2X + X^2}{3R_1^2 - 3R_1X + X^2} \quad \text{(I)}$$

This is an inequality between 2 values of the same function with different arguments θ_2 and X. To show that this inequality implies $X < \theta_2$, we need only show that the derivative of this function is positive. The derivative of the right-hand side of the inequality (I) with respect to X is a positive multiple of the factor

$$\begin{aligned} N &= (3R_1^2 - 3R_1X + X^2)(2X - 3R_2) \\ &\quad - (3R_2^2 - 3R_2X + X^2)(2X - 3R_1) \qquad \text{(J)} \\ &= 3[(2R_1 - X)(2R_2 - X) - R_1R_2](R_2 - R_1) \end{aligned}$$

We consider only the case where $0 < X < R_1$, since in any case where this is not so the estimate X will obviously be rejected.[3] From $R_2 > R_1 > X > 0$, it follows that N is positive; therefore, the derivative of the right-hand side of the inequality (I) is positive, and the Yuhas-Li estimate X is less than the true thickness θ_2.

Since spheroid growth rates are almost certainly diffusion limited and since the spherical geometry of diffusion will very probably make $\theta_1 > \theta_2$, it seems very likely that the Yuhas-Li method underestimates the thickness of the growing layer whenever it gives a physically possible result ($0 < X < R_1$). For the model of Chart 1, this bias in the Yuhas-Li estimates is fairly constant and relatively small. If this is the case generally, then Yuhas-Li estimates may be quite serviceable, especially for purely comparative studies. However, in more critical work, the bias in these estimates might prove to be an important source of error.

[3] Equation H reduces to a quadratic equation in X, and under present assumptions it can be shown that there is at most one root X between 0 and R_1. However, both roots may lie outside this range, or the roots may be complex.

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