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Estimation of Liver Tumor Volume Using Different Formulas – An Experimental Study in Rats*

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Summary. Experimental solitary ellipsoid liver tumors in the rat can be induced by inoculation of a tumor-cell suspension of known potency into the liver parenchyma. During laparotomy, the largest (a) and the smallest (b) superficial diameters of the tumor were measured on the surface of the liver with vernier calipers. Four different formulas have been tested and compared with the actual volume from the extirpated tumor and tumor weight. Within the size range of 15–700 mm^3, based on the calculation of the difference between logarithmic tumor volume from the different formulas versus logarithmic volume of extirpated and dissected tumors and regression analyses, volume of the unremoved liver tumor can be best calculated according to the formula $V = a \times b^2/2$.

Key words: Liver tumors – Tumor measurements

Introduction

In malignant tissue, cells have escaped from the restrictions and controls of normal growth. The malignant growth process develops from a dynamic, frequently changing, and complex series of events. There is no single parameter better than tumor growth rate that can give information on cell population and the effect of different therapeutic maneuvers on tumor growth (Lala 1971).

A previously induced tumor transplanted into inbred animals is a convenient model for tumor experiments even if growth characteristics and histological findings change during repeated transplantation (Begg 1971; Moore and Dixon 1977). An experimental model for studying the changes in tumor volume of therapeutic manipulations on an unremoved liver tumor in rats would be most valuable. Inducing liver tumors with a hepatocarcinogenic substance or as a cell suspension of tumor cells injected into the portal vein in rats is accompanied by scattered tumor growth in the liver (Nilsson and Zettergren 1967; Fisher and Fisher 1959a). Factors affecting tumor take and growth rate are, for example, surgical trauma to the liver (Fisher and Fisher 1959b), repeated laparotomies (Fisher and Fisher 1959c), alteration of liver blood flow (Fisher et al. 1961; Fisher and Fisher 1963), nutrition (Fisher and Fisher 1961a), anticoagulants (Fisher and Fisher 1961b), and reticuloendothelial interference (Fisher and Fisher 1961c; Fisher and Fisher 1962). Scattered tumor growth in the liver is a disadvantage in many experiments as estimations of tumor size require examination of the entire liver after removal (Nilsson and Zettergren 1967; Fisher and Fisher 1959a). The changes in tumor size between subsequent repeated measurements express the effect of therapeutic manipulations. The most suitable experimental model is one that enables accurate repeated tumor size estimations in living animals.

Most solid tumors in animals and man grow as three-dimensional aggregates and, in most tissues, the tumors appear spheroid or ellipsoid in shape (Willis 1968). Estimation of tumor size can be done from measurements of the tumor in one (Mayneord 1932; Marsh 1933) or two (Brues et al. 1939; Mottram 1935) dimensions by vernier calipers. In a superficially growing tumor, repeated measurements of the product of the largest and smallest diameters give a good description of tumor growth, provided that the tumor does not change its average shape under the observation period (Steel et al. 1966). Calculation of tumor volume is, however, best done if the tumor is measured in three dimensions. Measurement of three diameters is easy to

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do if the tumor is situated superficially. This can be done with different formulas (Dethlefsen et al. 1968; Schrek 1935; McCredie et al. 1965; Hermens and Barendsen 1967), which gave a good agreement with in vitro measurements (Schrek 1935). With central growth in an organ such as the liver it is almost impossible to make accurate measurements in vivo in all three dimensions.

The aim of the present study was to design a reproducible method of developing a solitary tumor in the rat liver using a tumor-cell suspension as inoculum and to investigate which formula when measuring two perpendicular diameters of the visible outer surface of the unremoved tumor is the most accurate estimation of the tumor volume compared with the measurement in three dimensions of the dissected and extirpated tumor or tumor weight.

Material and Methods

Thirty-four inbred Wistar rats of both sexes with a weight of 180–240 g were used in the experiment. They were maintained on a standard pellet diet and water ad libitum. The tumor used in the study was a N-methyl-N-nitrosoguanidine-induced adenocarcinoma of the colon, transplanted into the kidney under sterile conditions every 10th day (Steele and Sjögren 1974). The cell suspension used in the experiment was made by dissecting the tumor from the kidney and then cutting it into small fragments. Trypsin-EDTA solution and normal rat serum were added and mixed. This procedure was repeated three times, then the test tube was centrifuged and the supernatant removed. The trypsinization was stopped by inactivated rat serum. After adding trypan blue, the vital cells were counted in a Büchner chamber (Boyse et al. 1962). The final cell solution was then diluted to a suspension containing 1.0×10^6 viable tumor cells per 0.1 ml.

Within 1 h after preparation, 0.1 ml tumor cell suspension was inoculated into the periphery of the central lobe of the liver just under the liver capsule. Access to the liver was achieved through a midline abdominal incision under ether anesthesia. To avoid leakage of tumor cells from the injection site after withdrawal of the injection needle, a Spongostan sponge was pressed against the opening until hemostasis was complete. Within varying intervals of up to 3 weeks the abdominal cavity was reopened and the tumors were measured with vernier calipers for the largest (a) and smallest (b) superficial visible diameters on the ventral side of the liver lobe. The tumors were then extirpated and freed from normal liver tissue by macroscopic dissection. Measurements with vernier calipers were then made of the same diameters as before, expressing the actual largest (c) and smallest (d) diameters and, finally, the third diameter (e) was measured. The tumor weight was recorded in mg.

Mathematical and Statistical Calculations

From the two superficial measurements in vivo the tumor size was calculated using four different formulas (Steel et al. 1966; Kopper and Steel 1975; Chambers and Scott 1930; Simpson-Herren and Lloyd 1970):

$$V_1 = a \times b$$

$$V_2 = \frac{\pi}{6} \times \left(\frac{a+b}{2}\right)^3$$

$$V_3 = a \times (b)^2/2$$

$$V_4 = (\sqrt{a \times b})^3$$

The three measurements from the extirpated tumors were used to calculate the tumor volume by the formula (Dethlefsen et al. 1968):

$$V_5 = \frac{\pi}{6} \times c \times d \times e.$$

The calculated tumor sizes were then compared with each other and with the tumor weight. The Student's t test and regression analyses were used as statistical methods.

Results

None of the 34 animals showed any signs of malnutrition during the experiments. No mortality was registered and at autopsy, no macroscopic signs of metastases were found. The extirpated tumors were in all animals, except one, ellipsoid in shape. The smallest actual tumor volume calculated according to V_5 was 15.4 mm^3 and the largest 710.3 mm^3. Calculations using the formulas V_1–V_5 for the 33 different ellipsoid tumors gave the results presented in Table 1. Logarithmic mean tumor size calculated from two superficial measurements according to V_2 and V_3 showed no statistically significant difference from V_5 ($p < 0.10$). The other formulas (V_1 and V_4) gave highly significant differences from formula V_5 ($p < 0.0001$). The standard deviation of the paired difference of logarithmic mean tumor volume between V_5 and V_3 was smallest (Table 2). Regression analyses on logarithmic values resulted in $V_5 = 0.572 + 0.900 \times V_3$, $R^2 = 0.938$ (Fig. 1, Table 3).

Calculation according to V_3 represented the best fit, the regression coefficient (0.900) being closest to 1 and the proportion of explained variation (R^2) being highest (Table 3). The correlation between logarithm of tumor volume according to V_3 and logarithm of

Table 1. Mean tumor size and geometric mean tumor size for different formulas used on 33 different tumors with an ellipsoid shape

Formula	Mean (±SD)	Logarithmic mean (±SD)
V_1	57 (± 37)	3.880 (±0.62)
V_2	263 (±241)	5.197 (±0.94)
V_3	219 (±175)	5.062 (±0.89)
V_4	483 (±425)	5.820 (±0.93)
V_5	223 (±163)	5.128 (±0.83)

Table 2. Standard deviation of paired difference between the formulas for 33 different tumors

Compared formulas	Standard deviation
V_1–V_5	0.282
V_2–V_5	0.265
V_3–V_5	0.226
V_4–V_5	0.247

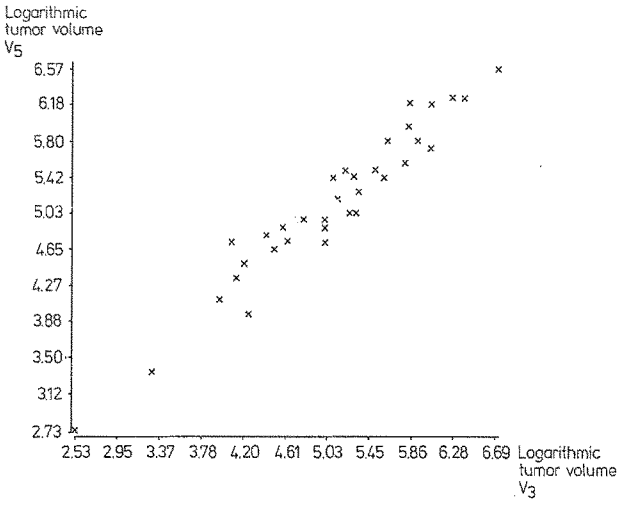

Fig. 1. Regression analysis for formulas V_3 and V_5 for 33 different tumors

Table 3. Regression analysis of logarithmic values for the different formulas

$V_5 = 0.092 + 1.298 \times V_1$	$R^2 = 0.935$
$V_5 = 0.713 + 0.849 \times V_2$	$R^2 = 0.927$
$V_5 = 0.572 + 0.900 \times V_3$	$R^2 = 0.938$
$V_5 = 0.092 + 0.865 \times V_4$	$R^2 = 0.935$

Table 4. Correlation between logarithmic tumor volume and logarithmic tumor weight for 33 different tumors

Formula	Correlation (r)
V_1	0.87
V_2	0.87
V_3	0.88
V_4	0.87
V_5	0.94

weight was the highest ($r = 0.88$) and the same correlation according to V_5 was 0.94 (Table 4). The optimal formula found in this investigation for calculation of tumor volume from two perpendicular superficial diameters was $V_6 = a^{1.05} \times b^{1.62} \times 0.999$.

Discussion

To gain better insight into the treatment of liver tumors in man it is necessary to have a suitable and reproducible in vivo animal model. By injecting a cell suspension with a known number of viable tumor cells in the periphery of the central lobe of the liver in the rat it was possible to produce solitary, ellipsoid liver tumors. There was no difference in the shape of the tumors whatever their size. Calculation of the tumor volume from superficial measurements of two visible tumor diameters in vivo gave different results depending on which formula was used. Based on the calculations of the difference between logarithmic tumor volume from the different formulas (V_1–V_4) versus logarithmic volume of extirpated and dissected tumors (V_5) and on regression analysis it can be stated that the most accurate formula seemed to be $V_3 = a \times b^2/2$.

The model may therefore be most suitable for use in studies following the influence of different therapeutic maneuvers on unremoved liver tumors having a size of 15–700 mm^3 in the rat. The method can thus be used to follow rat experiments of hepatic artery occlusion, local or general hyperthermia, cytostatic therapy, etc. Each measurement requires, however, a laparotomy which may influence tumor growth (Fisher and Fisher 1959c), and this makes the method unsuitable in clinical protocols. Which of the formulas is most appropriate for determining liver tumor volume in patients by computerized tomography or other roentgenological techniques needs to be investigated. Whether the same formula $V = a \times b^2/2$ is the best for tumors at other sites has not been investigated.

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