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The Influence of a Heat Pulse on the Thermally Induced Damage to Tumour Microcirculation

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Abstract—*The effect of an initial short period of higher-temperature heat application on the stoppage of the microcirculation in the experimental rhabdomyosarcoma BA1112 in 'sandwich' chambers was investigated. The treatment consisted of an initial heat pulse of* 45°C *for* 10 min *which was followed by a continuous exposure at* 42.5°C *for* 3 hr. *Using the* '$t_{\frac{1}{2}}$ *per* °C' *rule, the time equivalent of the heat pulse was* 94 min. *Taking this contribution into account, the derived* 50% *stoppage time of* 151 min *is essentially the same as the* 152 min *observed for* 42.5°C *only treatments. The data therefore indicate that the effect of a heat pulse in the treatment can be accounted for by the customary correction procedure of one time exposure doubling per* °C. *However, it appeared that the microcirculation in the surrounding tumour bed was impaired more than was expected by this treatment.*

INTRODUCTION

MODERATE hyperthermia of between 41 and 45°C not only kills tumour cells [1–4] but also appears to have a detrimental effect on tumour microcirculation [5–16]. At least part of the tumoricidal effect of a hyperthermic treatment is attributed to the presence of nutritively depleted hypoxic cells in an acidic environment, where cells have an increased thermal sensitivity [17, 20–22]. If hyperthermia is combined with irradiation, the net effect results in a decrease in the radiation dose required for cure [17–19]. The effect of hyperthermia depends, of course, on the intensity of the treatment. The latter is related to the treatment temperature as well as the treatment time in a well-established relationship, i.e. for every additional °C the exposure time can be halved [3, 18, 23, 24]. Regarding the use of different temperatures in the same treatment, relatively little information is available, and that only applies to *in vitro* systems [24, 25]. The present experiments were conducted to investigate to what extent a 10-min heat pulse of 45°C followed by a 3-hr treatment at 42.5°C would influence the treatment time, with the integrity of the tumour microcirculation as the endpoint.

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MATERIALS AND METHODS

The tumour used in these investigations was the undifferentiated isologous rhabdomyosarcoma BA1112 [26] growing in transparent 'sandwich' chambers [5, 27] in the subcutis of female WAG/Rij rats. The 'sandwich' chamber consists of a thin layer of subcutis in which the tumour grows and which is enclosed by two transparent surfaces. For the latter, a mica plate is used as a base on one side, while the other is covered with a small, round glass coverslip. The tumours in these preparations grow in a sheet-like fashion and their thickness is restricted to about 200 μm. This allows transillumination with observation of the microcirculation with, in these experiments, a stereomicroscope. Also, photographic records of the aspect of the tumour microcirculation was made before the treatment and every 60 min during the treatment as well as on the following day. The observations (every 15 min) as well as the photographic recordings reveal that after some time at elevated temperatures the microcirculatory flow in the tumour vessels may decrease and may eventually stop. This process appears to be irreversible under the conditions of these experiments, and the time required to achieve this effect is used as the parameter in these investigations. Regardless of observed microcirculatory stoppage, all tumours

were exposed for a full 180 min after the 42.5°C exposure temperature was reached.

The heating method has been described before [5, 13]. Briefly, only the skin flap carrying the 'sandwich' chamber is inserted into an isolated perspex box. Heating in this box is performed with air via a small electronically controlled heating device. Temperature control and temperature measurements are done via thermocouples which are attached to the coverslip with adhesive gum. To avoid overshoot when changing the treatment temperature, the temperature adjustment is done by hand in 0.1°C increments until the desired temperature is reached. With air-heating systems, it is obviously not possible to induce abrupt changes in the heated object. Therefore it required 9 min on average to increase the temperature of the 'sandwich' chamber from 42.5 to 45°C and 5 min to reverse this process.

RESULTS

The results of two types of heat treatment are shown in Fig. 1. In this figure, as in the following, the percentage of tumours showing intact circulation is plotted against exposure time. Through the pooled 42.5°C data obtained with continuous heating a curve representing a least-squares best fit to the Weibull distribution function is drawn [28]. The 50% inactivation time is 152 min, with 95% confidence intervals of 148–157. The step curve on the left side of Fig. 1 represents the data obtained when the treatment consists of an initial heat pulse of 10 min duration (consisting of 9 min rise time from 42.5 to 45°C, the 10 min at 45°C and 5 min cooling down time) followed by continuous treatment at 42.5°C. Obviously, the time required to induce micro-circulatory stoppage has become much shorter. No best-fitting inactivation curve has been drawn through these points because the treatment intensity was not constant over the entire exposure time, due to the heat pulse. Methods for correction of the effect of differences in exposure temperature have been reported by Henle and Roti-Roti [29]. In their method the integral is obtained for the biological effect of exposure to different temperatures on the basis of the '$t_{\frac{1}{2}}$ per °C' rule and this is expressed as an equivalent time at a reference temperature. This method was used to convert the 'heat pulse' data of Fig. 1a into points representing the equivalent exposure at 42.5°C. This is shown in Fig. 2. The Weibull probability curve is also shown in this diagram. The 50% inactivation time is 151 min, with 95% confidence intervals between 145 and 156 min.

In experiments with continuous exposure at 42.5°C, the microcirculation in the tissue surrounding the chamber never becomes impaired by the treatment. In the present experiments with the heat pulse, however, it appeared that the microcirculation in the surrounding tumour bed almost inevitably ceased. This is shown in Fig. 3. The 50% inactivation time for the equivalent exposure at 42.5°C was 190 min (95% confidence for intervals of 186–194 min). The difference between the two curves at the 50% effect level is significant ($2P < 0.01$).

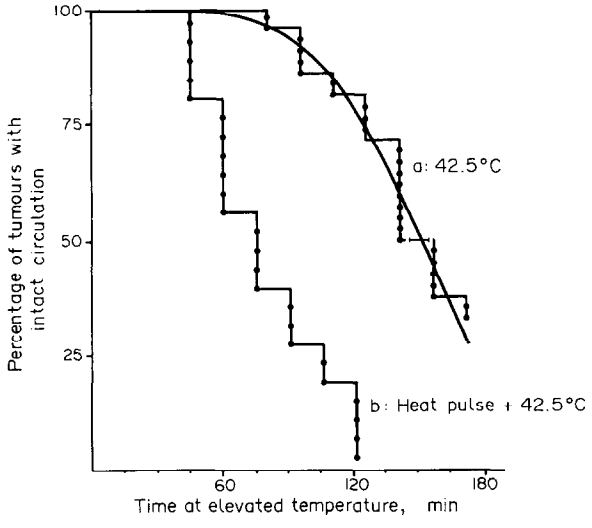

Fig. 1. The effect of heat at 42.5°C (curve a; n = 41) and 42.5°C with a heat pulse (curve b; n = 24) on the microcirculation of tumours. The heat pulse consisted of an initial rise (starting at zero time) from 42.5 to 45°C in 9 min, followed by a 10-min exposure at 45°C and a 5-min cooling to the further continuous exposure temperature of 42.5°C. Ordinate: percentage of tumours with intact microcirculation. Abscissa: exposure time. The curve (through the 42.5°C only data) was calculated according to [28].

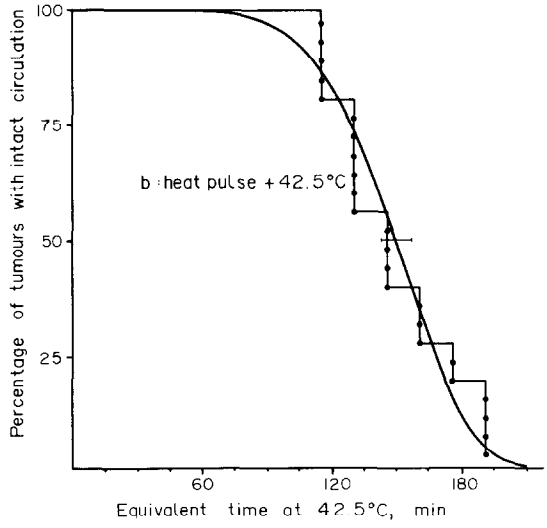

Fig. 2. Same data as Fig. 1b, but now the influence of the heat pulse is corrected via [29] to the equivalent exposure time at 42.5°C. Parameters as in Fig. 1.

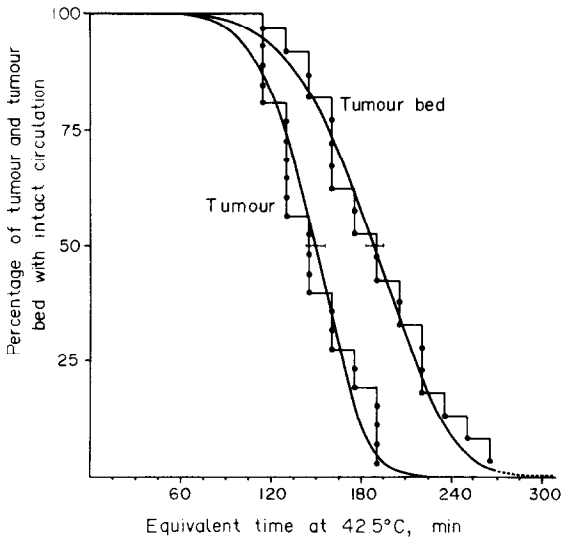

Fig. 3. The effect of the heat pulse as expressed as equivalent time at 42.5°C [29] on the tumour microcirculation and the microcirculation of the tumour bed. Parameters as in Fig. 1. The 50% effect times are 151 min and 190 min.

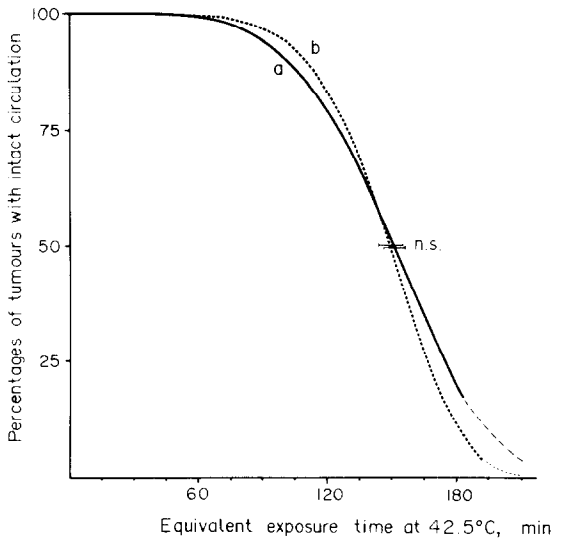

Fig. 4. The curves of Fig. 1a and Fig. 2 plotted in one diagram. Parameters as in Fig. 1. The 50% effect times are 152 and 151 min respectively.

DISCUSSION

The deteriorating effect of hyperthermia on tumour tissue and in relation on tumour microcirculation has been very well established, not only for this rhabdomyosarcoma [5, 9, 10, 13] but also for many other experimental tumours [7, 11, 12, 14, 15]. The exact cause of the damage to tumour microcirculation by hyperthermia is not known. It has been suggested to be related to metabolic factors [20] or a decrease in pH [30] resulting in a decreased flexibility of erythrocytes [6, 31]. According to recent investigations by Vaupel, a temperature higher than 42°C is required for the induction of a hyperthermia-induced decrease in tumour pH [30].

When one employs the '$t_{\frac{1}{2}}$ per °C' rule as suggested by many authors and incorporates the integration method introduced by Henle and Roti-Roti [29] for correction of the contribution of the heat pulse to the effect of hyperthermia, the results obtained by the two different treatments are surprisingly similar (Fig. 4). This indicates that, at least for temperatures of 42.5°C and higher, the effect of the heat pulse on the microcirculation of these 'sandwich' tumours can be adequately accounted for by correction in exposure time. The '$t_{\frac{1}{2}}$ per °C' rule applies to many systems. Initially introduced into this field by Westra and Dewey [32], it was recognized as early as 1974 by Suit and Shwayder [23] that this rule also applied to Crile's tumour data. Overgaard and Suit later demonstrated that this relationship also applied for tumour cure in an experimental fibrosarcoma [18]. More recently, Kang *et al.* demonstrated in the SCK tumour a similar relationship, providing that the treatment temperature exceeded 42.5°C [12]. These data, together with the ones provided in this report, support the suggestion of Henle [24] that for tumour treatment the application of a heat pulse in a hyperthermic treatment regimen may be an efficient way of shortening hyperthermic treatment time.

The reason for the apparent increased sensitivity of the microcirculation in the tissue surrounding the tumour, i.e. the tumour bed, is not known. Taking into consideration the contribution of the heat pulse, the total *equivalent* treatment time of this group is 94 min longer than the 42.5°C only group. Nevertheless, if the 39-min difference between the 50% stoppage time of the tumour circulation and tumour bed were also to occur in the 42.5°C only group, some incidence of damage should be expected to occur within the 180 min treatment time. That this was not observed may point to some specific sensitizing factor applying to the tumour bed in the 'heat pulse' situation. Whether or not this bears any relationship to the tumour bed or to other factors remains open for investigation.

REFERENCES

1. FIELD SB, BLEEHAN NM. Hyperthermia in the treatment of cancer. *Cancer Treat Rev* 1979, **6**, 63–94.

Methods in Tumor Biology: Tissue Culture and Animal Tumor Models for Basic and Clinical Oncology. New York, Marcel Dekker, in press.

27. REINHOLD HS, BLACHIEWICZ B, VAN DEN BERG-BLOK A. Reoxygenation of tumours in 'sandwich' chambers. *Eur J Cancer* 1979, **15**, 481–489.
28. CAMPOS JL. Application of the Weibull distribution to some instances of cell survival; of neoplastic survival, and of ageing. *Br J Radiol* 1975, **48**, 913–917.
29. HENLE KJ, ROTI-ROTI JL. Time-temperature conversions in biological applications of hyperthermia. *Radiat Res* 1980, **82**, 138–145.
30. VAUPEL P. Einfluss einer lokalisierten Mikrowellen-Hyperthermie auf die pH-Verteilung in bösartigen Tumoren. *Strahlentherapie* 1982, **158**, 168–173.
31. VAUPEL P, OSTHEIMER K, MÜLLER-KLIESER W. Circulatory and metabolic responses of malignant tumors during localized hyperthermia. *J Cancer Res Clin Oncol* 1980, **98**, 15–29.
32. WESTRA A, DEWEY WC. Variation in sensitivity to heat shock during the cell-cycle of Chinese hamster cells *in vitro*. *Int J Radiat Biol* 1971, **19**, 467–477.