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# Experimental Hyperthermic Treatment of a Human Colon Carcinoma Xenograft. The Thermal Sensitivity of the Tumour Microcirculation

A.E. VAN DEN BERG-BLOK and H.S. REINHOLD
*Radiobiological Institute TNO, Rijswijk and Erasmus University, Rotterdam, The Netherlands*

**Abstract**—*The effect of hyperthermia on the microcirculation of a human colon tumour growing in a 'sandwich' observation chamber in immune-suppressed rats was investigated. Evaluation of the effect was based on microscopic observation, measurement of the relative flow rate of the blood in the capillaries of the tumour and photographic recording. The results indicated that moderate hyperthermia* (3 h *at* 42.5°C) *has a destructive effect on the microcirculation of the tumour, followed the next day by severe necrosis. These results indicate that this human colon carcinoma xenograft has—for the endpoints that were investigated—a heat sensitivity that is comparable with rodent tumours.*

## INTRODUCTION

HYPERTHERMIA is a rapidly developing treatment modality for the control of malignant tumours. Besides being intrinsically cytotoxic it also potentiates the effects of radiation and chemotherapy [1–6]. The effect of hyperthermia on cells *in vitro* under standard conditions seems to be less pronounced than in the *in vivo* situation in tumours. This is generally explained by environmental factors such as low pH, poor supply of nutrients and low oxygen tension in the tumour tissue [7–11]. This knowledge is based on numerous studies involving animal and human tumours, both *in vivo* and *in vitro*.

One of the proposed future applications of hyperthermia is the treatment of human gastrointestinal tumours, which are fairly resistant to both radiotherapy or chemotherapy. The question then arises whether human gastrointestinal tumours will be sufficiently sensitive to hyperthermia. The appearance of genetically immune-deficient hosts (both nude mice and nude rats) had made it possible to investigate the effects of a given treatment on human tumours under more or less comparable biological conditions as xenografts. However, the nude species are rather expensive, not always readily available and difficult to handle because of their susceptibility to infection. These animal models have been employed as hosts for tumour xenografts [12–15].

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Mailing address: A.E. van den Berg-Blok, Radiobiological Institute TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands.

The purpose of the present work was to obtain information on the effect of hyperthermia on the microcirculation aspect of a human colon carcinoma growing in a sandwich observation chamber in immune suppressed rats. The results indicate a satisfactory thermal sensitivity.

## MATERIALS AND METHODS

Fourteen-week-old female WAG/Rij rats were used. The tumour used in these experiments was a human colon carcinoma designated XCo1F. This tumour is histologically characterized as a well to moderately differentiated mucinous adenocarcinoma. The 3rd to the 5th passage of this tumour growing in male BALB/c nude mice was used.

Small fragments of the XCo1F tumour were implanted in 'sandwich' chambers. The latter consist of a skin flap of the rat in which a part of the subcutis is enclosed between a mica base plate and a glass coverslip. This yields an observation chamber with a clear, intact microcirculatory bed. The development of the tumour and its typical vascular bed can be followed during its growth. In the present investigations this is an advantage, as the tumour microcirculation can be evaluated continuously during a hyperthermia experiment. The details of the chamber design and surgical technique have been published elsewhere [16]. In this system its thickness is limited to about 200 μm. In order to keep the tumour close to the body temperature, after tumour transplantation the rats were individually housed in a warm cabinet

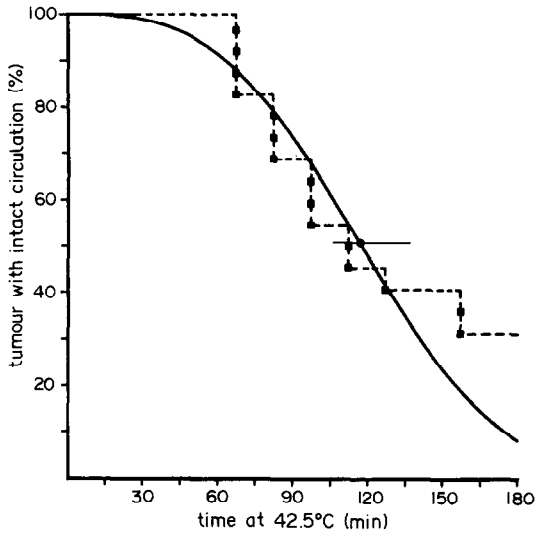


*Fig. 1. The effect of heat on the microcirculation stoppage in the main tumour mass of a human colon carcinoma xenograft (XCo1F) in 'sandwich' observation chambers* (n = 21).

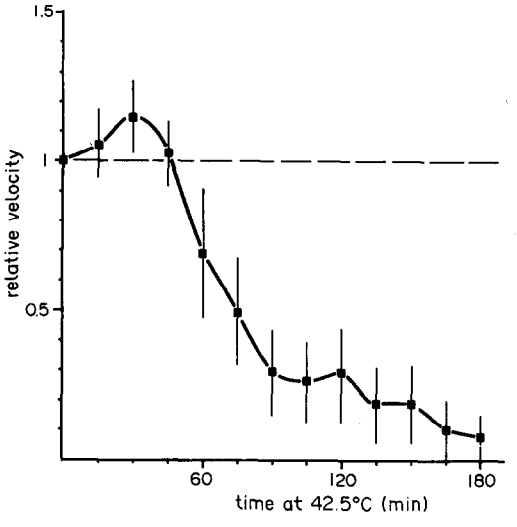


*Fig. 2. The effect of heat on the relative erythrocyte velocity in selected capillaries in a human colon carcinoma xenograft (XCo1F). The average velocity at time 0 was* 428 μm/sec, (n = 10). *Bars indicate S.E.M.*

(33.5°C, 40% humidity) and received acidified water and processed food *ad libitum*.

The rats were treated with Sandimmune® (cyclosporin A) to induce a state of immune suppression permitting the growth of human xenografts. Beginning on the day of tumour transplantation the rats were injected daily with a dose of 10 mg/kg i.m. (1 ml/kg). The last injection was given on the day of hyperthermic treatment.

Before treatment the animals were anaesthetized with Hypnorm® (Philips Duphar) at 1 ml/kg i.p. and the tumour bearing skin flap was inserted into an isolated perspex box in which heating was performed using warm air [2]. Twenty-one tumour-bearing rats were treated with a constant temperature of 42.5 ± 0.1°C and an exposure time of 180 min.

In 10 animals a single, centrally located capillary in the tumour was selected for erythrocytic velocity measurements during treatment [17]. Because of the limited thickness of the tumour it was possible to (a) measure the velocity of the blood microcirculation in the tumour by microscopic means during heat treatment; (b) to record the time to produce stoppage of the microcirculation flow (ST) in the entire tumour vascular system as another parameter. The recording of such changes in the tumour, including the development of necrosis, was made by visual observation (every 15 min) and photography (every hour).

## RESULTS

The effects of heat treatment at 42.5°C for 3 h on the microcirculation are depicted in Fig. 1. The dotted line shows the numbers of tumours with intact microcirculation observed at 15 min intervals during treatment. A time/effect curve (solid line)

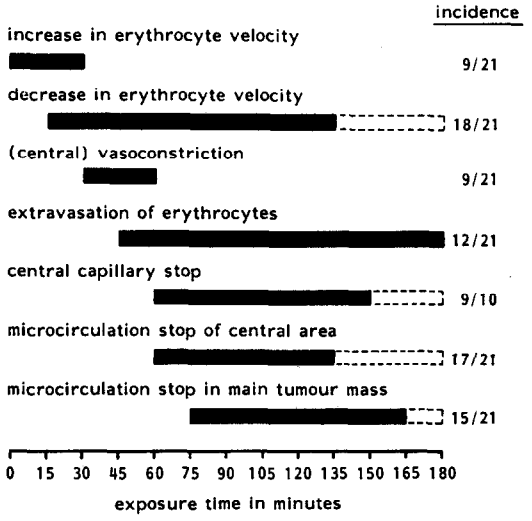


*Fig. 3. Schematic diagram of the observed time ranges for several events taking place in the microcirculation of the xenografted human colon carcinoma XCo1F in 'sandwich' chambers during a hyperthermic treatment at* 42.5°C *for* 3 h.

was fitted to the data points using the Weibull distribution model [18]. The $ST_{50}$, i.e. the time required to induce microcirculation stoppage in 50% of these XCo1F tumours, was 118 min (95% confidence limits 107–137 min).

Figure 2 demonstrates the effect of heat exposure on the average erythrocyte velocity in a number of centrally located capillaries. After a temporary increase, the erythrocyte velocity decreased and approached zero values after 3 h. This is in accordance with the observations demonstrated in Fig. 1.

The effects of hyperthermia on several circulation parameters during 3 h treatment are summarized in Fig. 3. First an improvement of the circulation was observed followed after 30 min by vasoconstriction and a slowdown of the circulation. This resulted

in a circulation stop in the centre of the tumour after 75–90 min. After 120 min a complete stoppage was obtained throughout the tumour except for its outer rim.

## DISCUSSION

The growth curve of the XCo1F xenograft in 'sandwich' chambers of immune-suppressed WAG/Rij rats shows a proliferative phase up to 12 days after tumour transplantation (volume-doubling time from 6 days), followed by a plateau phase. The hyperthermic treatment was carried out between day 9 and day 12, when the tumour had reached a diameter of 3–4 mm.

The behaviour of the human XCo1F tumour during heat exposure was not much different from that we observed with the rhabdomyosarcoma BA1112 [2, 17, 19, 20]. After an initial increase the red blood cell velocity gradually slowed down. A circulation stop in the centre of the tumour was rapidly followed by a complete stop of the tumour microcirculation. A similar observation was made by Kallinowski *et al.* [21], who reported that hyperthermic treatment causes a distinct reduction of the tumour blood flow in human mammary carcinoma xenografts in nude rats. Although the mechanisms underlying the hyperthermia-induced vascular stasis have not yet been fully elucidated [22] it is likely that heat-induced pH changes play an important role, next to haemodynamic changes [22]. Olch *et al.* [23] observed a reduction of tumour blood flow in patients during hyperthermic treatment. The blood flow in some experimental tumours, like the Walker 256 carcinoma, however, did not change during a 1 h treatment at 43°C or 45°C as compared to untreated tumours, but 3 h after the 45°C treatment the blood flow appeared to be less than that in control tumours [24]. It is noteworthy that the damage to the human XCo1F tumour vasculature during a hyperthermic treatment of 3 h at 42.5°C was irreversible and that the next day a necrosis had developed in much the same way as seen in the rhabdomyosarcoma BA1112.

The human colon carcinoma XCo1F appears somewhat more thermosensitive than two different rat tumours treated in exactly the same way. The ST50 values (3 h at 42.5° C) are:

XCo1F (human colon carcinoma) : 118 min (this paper)
BA1112 (rat rhabdomyosarcoma) : 152 min [20]
RMA (rat mammary carcinoma) : 190 min (unpublished data).

This may indicate that there may be differences in thermal sensitivity, as measured with this endpoint, that are related to the tumour type, regardless of the fact that the vascular bed of all the tumours is of rat origin. But even within a group of human colon cancers the response to hyperthermia as measured by growth delay is not uniform [25] and the same applies to a group of human melanomas [5, 11].

From histological and microscopical investigations it has been reported that, in general, xenografts have very much the same properties as the patient tumour [11, 12, 13, 26, 27] and the question arises as to whether the results of experiments with human tumours as xenografts are relevant to the treatment of tumours in patients. Bailey *et al.* [28] concluded that xenografts are of not very much practical use for patient drug-sensitivity testing, since patients with rapidly growing tumours died before the results of the test were known and moreover sometimes the take rate of xenografts was very low. On the other hand Giovanella *et al.* [29] and Steel *et al.* [14] supported the use of xenografts as a model for treatment of human tumours especially with respect to chemotherapy, be it on a non-individual basis. With this in mind, one may infer from the present experiments that it may be worthwhile to further investigate clinically the potential therapeutic benefits of adding hyperthermia as a treatment modality for human colon carcinoma.

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## REFERENCES

1. Field SB, Bleehen NM, Hyperthermia in the treatment of cancer. *Cancer Treat Rev* 1979, **6**, 63–94.
2. Reinhold HS, van den Berg-Blok A. Enhancement of thermal damage to the microcirculation of 'sandwich' tumours by additional treatment. *Eur J Cancer Clin Oncol* 1981, **17**, 781–795.
3. Senapati N, Houchens D, Ovejera A, Beard R, Nines R. Ultrasonic hyperthermia and drugs as therapy for human tumor xenografts. *Cancer Treat Rep* 1982, **66**, 1635–1639.
4. Rofstad EK, Brustad T. Radiation and heat sensitivity of cells from human melanoma xenografts. Lack of correlation with tumour growth parameters. *Eur J Cancer Clin Oncol* 1983, **19**, 427–432.
5. Rofstad EK, Wahl A, Brustad T. Radiation and heat sensitivity of cells from two slowly growing human melanoma xenografts. *Br J Cancer* 1984, **49**, 745–752.
6. Suit HD, Shwayder M. Hyperthermia: potential as an anti-tumor agent. *Cancer* 1974, **34**, 122–129.

7. 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.
8. Vaupel P. Einfluss einer lokalisierten Mikrowellen-Hyperthermie auf die pH-Verteilung in bösartigen Tumoren. *Strahlentherapie* 1982, **158**, 168–173.
9. Emami B, Song CW. Physiological mechanisms in hyperthermia: a review. *Int J Radiat Oncol Biol Phys* 1984, **10**, 289–295.
10. Emami B, Nussbaum GH, Hahn N, Piro AJ, Dritschilo A, Quimby F. Histopathological study on the effects of hyperthermia on micro-vasculature. *Int J Radiat Oncol Biol Phys* 1981, **7**, 343–348.
11. Rofstad EK, Brustad T. Effect of hyperthermia on human melanoma cells heated either as solid tumors in athymic nude mice or *in vitro*. *Cancer* 1982, **50**, 1304–1308.
12. Cobb LM. The behaviour of carcinoma of the large bowel in man following transplantation into immune deprived mice. *Br J Cancer* 1973, **28**, 400–411.
13. Floersheim GL. Comparative growth of human tumors in pharmacologically immunosuppressed, immune-deprived, cyclosporin A-treated and nude mice. *Eur J Cancer Clin Oncol* 1982, **18**, 589–594.
14. Steel GG, Courtenay VD, Peckham MJ. The response to chemotherapy of a variety of human tumour xenografts. *Br J Cancer* 1983, **47**, 1–13.
15. Hoogenhout J, Kazem I, Jerusalem CR *et al.* Growth pattern of tumor xenografts in Wistar rats after treatment with cyclophosphamide, total lymphoid irradiation and/or cyclosporin A. *Int J Radiat Oncol Biol Phys* 1983, **9**, 871–879.
16. Reinhold HS, Blachiewicz B, van den Berg-Blok A. Reoxygenation of tumours in 'sandwich' chambers. *Eur J Cancer* 1979, **15**, 481–489.
17. Reinhold HS, van den Berg-Blok AE. Hyperthermia-induced alteration in erythrocyte velocity in tumors. *Int J Microcirc Clin Exp* 1983, **2**, 285–295.
18. 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.
19. Reinhold HS, Blachiewicz B, van den Berg-Blok A. Decrease in tumor microcirculation during hyperthermia. In: Streffer C, van Beuningen D, Dietzel F *et al.* eds. *Cancer Therapy by Hyperthermia and Radiation.* Baltimore, Urban and Schwarzenberg, 1978, 231–232.
20. van den Berg-Blok AE, Reinhold HS. Time–temperature relationship for hyperthermia induced stoppage of the microcirculation in tumors. *Int J Radiat Oncol Biol Phys* 1984, **10**, 737–740.
21. Kallinowski F, Vaupel P, Schaefer C, Benzing H, Mueller-Schauenburg W, Fortmeyer JP. Hyperthermia-induced blood flow changes in human mammary carcinomas transplanted into nude (rnu/rnu) rats. In: Overgaard J, ed. *Hyperthermic Oncology* 1984, Vol. 1, *Summary Papers.* London, Taylor and Francis, 1984, 133–136.
22. Reinhold HS, Endrich B. Tumour microcirculation as a target for hyperthermia. *Int J Hyperthermia* 1986, **2**, 111–137.
23. Olch AJ, Kaiser LR, Silberman AW, Storm FK, Graham LS, Morton DL. Blood flow in human tumors during hyperthermia therapy: demonstration of vasoregulation and an applicable physiological model. *J Surg Oncol* 1983, **23**, 125–132.
24. Song CW, Kang MS, Rhee JG, Levitt SH. The effect of hyperthermia on vascular function, pH, and cell survival. *Radiology* 1980, **137**, 795–803.
25. Osieka R, Magin RL, Atkinson ER. The effect of hyperthermia on human colon cancer xenografts in nude mice. In: Streffer C, van Beuningen D, Dietzel F *et al.* eds. *Cancer Therapy by Hyperthermia and Radiation.* Baltimore, Urban and Schwarzenberg, 1978, 287–290.
26. Davies G, Duke D, Grant AG, Kelly SA, Hermon-Taylor J. Growth of human digestive-tumour xenografts in athymic nude rats. *Br J Cancer* 1981, **43**, 53–58.
27. Giovanella BC, Stehlin JS, Williams LJ Jr. Heterotransplantation of human malignant tumors in "nude" thymusless mice. II. Malignant tumors induced by injection of cell cultures derived from human solid tumors. *J Natl Cancer Inst* 1974, **52**, 921–930.
28. Bailey M, Jones A, Raghavan D *et al.* Limitations of human tumour xenografts in individual patient drug-sensitivity testing. *Br J Cancer* 1981, **43**, 725.
29. Giovanella BC, Stehlin JS Jr, Shepard RC, Williams LJ Jr. Correlation between response to chemotherapy of human tumors in patients and in nude mice. *Cancer* 1983, **52**, 1146–1152.