Direct and Abscopal Antitumor Action of Local Hyperthermia

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Growth-inhibition of GW-77 human colonic tumors growing in hamster cheek pouches was found to be proportional to the duration of heating the exposed tumors by 4 °C with short-wave diathermy. A maximal tumor growth-inhibition of 35 per cent resulted from administration of a daily 30 min. heating period repeated on 7 consecutive days. Further, hyperthermia to the left cheek pouch tumors resulted in growth-inhibition of the contralateral, presumably normothermic, cheek pouch tumors, thus causing us to question whether hyperthermia has abscopal antitumor effects.

Clinical and experimental observations on the antitumor effects of local and systemic hyperthermia are well documented. Recently, there has been a renewal of interest in the possible advantage of either total-body heating or heated perfusions. The availability of a relatively slow-growing human bowel tumor capable of continuous growth in the cheek pouch of unconditioned, adult golden hamsters which still retains human properties, the GW-77 tumor system, provides a convenient model for evaluating the effects of local heat to the exposed cheek pouch tumor. Also, use of tumors in both cheek pouches of the same animal affords an opportunity to evaluate any abscopal growth-inhibitory effects of heat radiation applied to one cheek pouch tumor on the other, contralateral one, as we have already exploited for X-irradiation.

Methods

GW-77 cheek pouch tumors were excised and minced in Ringer's solution containing penicillin (100 units/ml) and streptomycin (0.6 mg/ml) at a final suspension of 20% (w/v). Both cheek pouches of adult golden hamsters (Mesocricetus auratus) of both sexes, weighing 60 ± 10 g received 0.1 ml of this tumor suspension, and the animals thereafter randomized into 2 equal groups of 12 for each experiment. Inhibition of tumor growth was determined by comparing change in tumor size at 4 days post therapy. Using a small caliper, tumors were measured in three dimensions (length x width x depth) and the average products recorded. All successive measurements were expressed as simple multiples of the original one made prior to therapy, i.e., on the 7th day post transplantation. Each group's multiples were then averaged for each measurement day. The ratio of the mean increase in tumor size of treated vs. control tumors after completion of therapy, expressed as a percentage, was our criteria of growth-inhibitory effect.

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inhibition. The standard errors of these means were also determined. A more detailed description of our methods of transplantation and determining tumor growth-inhibition has been reported.

Heat was administered to the left cheek pouch tumor by means of a short-wave diathermy machine (Ultra-therm 525 of Siemens-Reiniger, Erlangen). The radiated animals were anesthetized with sodium hexobarbital (100 mg/kg i.p.) and then placed on a specially constructed plastic (polyvinylchloride) stage measuring 36 x 36 x 0.5 cm, and containing a vertical wall with an opening of 3 x 2 cm through which the hamster cheek pouch was drawn and immobilized for exposure to the radiation electrodes, while the rest of the animal was shielded (Fig. 1). The ideal distance between the diathermy machine’s two electrodes was empirically found to be 6 cm (2.5 below and 3.5 cm above the cheek pouch tumor). Various periods of heating the exposed cheek pouch tumors in single, in one case in 2 (six hrs. apart), therapy sessions daily, repeated for a total of 7 consecutive days (Days 9—15 post transplantation) were employed. The cheek pouch tumors of the control animals were likewise manipulated and fixed to the heating platform for similar periods of time.

Results

The normal temperature of the hamster's cheek pouch, measured by inserting a constantan-copper thermocouple through the anesthetized animal's mouth to the tip of its pouch, was found to be 35 °C, or about 1.6° below peritoneal temperature. During the first 2 min of diathermy, an increase of 1° temperature of the cheek pouch tumor resulted, followed by 1° per min up to 41°, after which the temperature rose by 2° per min. It thus required 3 min to increase the cheek pouch tumor temperature by 2°, 4 min for 3°, etc. After reaching a cheek pouch tumor temperature of 39°, the heating period commenced and was kept constant for the desired length of time by controlling the diathermy machine’s output.

Fig. 2 charts the effects of increasing the temperature of the exposed cheek pouch tumors by 4° for lengths of time ranging between 6 and 30 min. This figure represents the combined results of 4 different experiments, each with its own control group. Degree of tumor growth-inhibition is proportional to duration of heating, with a maximal effect of about 35 ± 5% growth-inhibition witnessed for a heating period of 30 min daily, repeated on 7 consecutive days. A divided dose period daily (2 x 15 min), administered 6 hrs. apart, appeared to yield poorer results than continuous irradiation for 30 min, though this difference certainly would not be significant from a statistical point of view. Of particular interest is the finding that even the presumably non-irradiated, contralateral cheek pouch tumors were affected with almost the same growth-inhibitory levels as the tumors heated intentionally. The apparently indirect action was especially marked at heating periods of 6 — 20 min.

Discussion

Our results indicate that the GW-77 tumor employed is only modestly responsive to a daily increase in temperature of 4 °C for periods ranging up to 30 min, repeated on 7 consecutive days. It may well be that this modest growth-inhibitory action of local hyperthermia in this in vivo cheek pouch tumor model is equivalent to the loss of trans-
Abb. 1. Querschnitt durch ein Ommatidium von *Formica polyctena* unmittelbar unterhalb des Kristallkegels (Zellkernregion der Nebenpigmentzellen). Eichmarke 1 μ. a) Dunkel adaptiertes Auge, b) 30 Min. lang hell adaptiertes Auge.
Abb. 2. Querschnitt durch ein Ommatidium von Formica polyctena in der Ebene der Zellkerne der 6 großen Retinula-
zenlen. Eichmarke 1 μ. a) Dunkel adaptiertes Auge, b) 30 Min. lang hell adaptiertes Auge.

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plantability found by Gericke\textsuperscript{10} when GW-77 cells were heated \textit{in vitro} at the same temperature and for comparable periods. However, we feel that thermal sensitivity \textit{in vitro} is, because of lack of temperature regulatory mechanisms, less predictable as a parameter of antitumor efficacy than alteration of tumor growth-rate \textit{in vivo}. Whereas a degree of tumor growth-inhibition has been verified for hyperthermia in our experiments, its relatively low level makes the reports disputing its value\textsuperscript{11} understandable.

The consistent finding of an abscopal, indirect, antitumor action of hyperthermia was certainly unexpected. Although every effort was made to shield the rest of the animal’s body, an early body-temperature rise of $1^\circ$ was in fact noted which, however, did not increase with increase in radiation time. If, therefore, this indeed be a true effect, then extreme local hyperthermia would merit further consideration in terms of possible plasma-borne factors, similar to those reported for X-rays\textsuperscript{12}, especially as an adjunct to other means of tumor therapy. Further, as in the case of the effects of certain levels of ionizing radiation\textsuperscript{13–15}, it may also be appropriate to question whether hyperthermia, local or systemic, might conceivably increase the immunological defenses of the host sufficiently as to account for possible abscopal antitumor effects.

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