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Effect of Chemotherapy on Tumor Temperature in Rats

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Abstract—*Two transplanted rat immunocytomas with different degrees of sensitivity to single injection of cyclophosphamide (CY) were used to assess the reliability of recording of the tumoral and rectal temperatures may be useful to evaluate the efficacy of cytostatic drugs against experimental rat tumors. With the highly sensitive tumor (ISIS* 130), 25 mg/m^2 *of CY resulted in a pronounced tumor inhibition (Treated/Control tumors* = 28%); *with the less sensitive (ISIS* 208), *a CY dose of* 320 mg/m^2 *was necessary to inhibit tumor growth to the same extent. The more important the decrease in tumor size was, after the administration of the drug, the larger the decrease was in tumoral temperature. Since the rectal temperature remained fairly stable, there was an increase of the difference between the tumoral and rectal temperatures. From the comparison between the results obtained for the two tumors with a wide range of CY doses, it appeared that the decrease in tumoral temperature did not correlate with the drug dose itself, but with the actual antitumor efficacy of the drug in each particular case.*

INTRODUCTION

THE HIGH growth rate and the increased metabolism of tumors in comparison to normal tissues may be associated with increased heat production and increased tumoral temperature [1–3]. Thus, careful tumoral temperature recording compared to basic temperature of an organism could be an index of malignancy, and an indirect measurement of tumoral metabolism. Conversely, the decrease in tumoral metabolism, or the necrosis induced by cytostatic agents could result in tumoral hypothermia. If this hypothesis is correct, then measurements of the tumoral temperature would be an early and additional method to evaluate the activity of cytostatic agents.

Our laboratory has studied the temperature variations of tumors and the response to antineoplastic agents. The present paper deals with the preliminary results obtained with cyclophosphamide (CY) in two subcutaneously transplanted rat immunocytomas, one (ISIS 130) being very sensitive to CY, the other (ISIS 208) less sensitive.

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MATERIALS AND METHODS

Animals and tumors

LOU rats from our own substrain (LOU/dec) were used. Groups of four animals were kept in plastic cages under conventional housing conditions and fed with a commercial pelleted diet (type A03; U.A.R., Villemoison-sur-Orge, France) and clean water *ad libitum*. Room temperature was maintained at 21–22°C, and a regular diurnal lighting cycle (12 hr/day) was provided.

Transplanted immunocytomas ISIS 130 and ISIS 208 were established in 1964 and 1970, respectively. In both cases, the primary tumor was an immunoglobulin-secreting, spontaneously occuring tumor of the ileocecal node in a LOU rat. The tumor lines have since been maintained by serial transfers of neoplastic ascites or by cryopreserved batches. At the time when this study was carried out, both tumors showed the same microscopic structure as initially described [4–6]. Furthermore, secretion of immunoglobulin (IgG) in serum and ascites samples from ISIS 208–bearing rats was confirmed; ISIS 130, on the other hand, has lost its monoclonal component secretion since 1975 (unpublished results). The survival time after i.p. inoculation of 5×10^6 tumor cells was 8–10 days for ISIS 208, and 13–15 days for ISIS 130. After s.c. implantation of 10^6 tumor cells, a tumor size of 1000 mm^2 was usually reached by day 9 with ISIS 208, and by day 15 with ISIS 130; for a 1000

mm^2 tumor, the doubling time was 3.3 and 6.1 days for ISIS 208 and ISIS 130, respectively. Subcutaneously-implanted tumors killed 100% of the animals in 25 (ISIS 208) or 30 (ISIS 130) days.

Tumor transplantation and tumor treatment

For the purpose of this study, after demonstration of their viability by trypan blue staining, cancer cells obtained from the peritoneal cavity of an ascitic tumor-bearing rat were suspended in physiological saline (2×10^6 cells/ml) and grafted subcutaneously in the right flank of young adult male rats (10^6 cells/rat). Nine days later (in the case of ISIS 208) or 15 days later (in the case of ISIS 130), the rats were anaesthetized with a 2% 2,2,2-tribromoethanol solution and the hair in the region of the tumor was removed with the aid of a lab-made depilating-cream. Arbitrarily, the depilation day was defined as day 1 of the experiment.

Three days later at 12 o'clock (day 4), a single dose of CY (Endoxan; Cilag-Chemie, Herentals, Belgium) was administered by i.v. injection (tail vein) under ether anaesthesia. Because of the variations in body weight among the rats to be treated, the dose was adjusted for each rat according to body surface area rather than body weight. Individual body surface areas were estimated according to the following formula:

$$S = 0.1 \times W^{2/3}$$

where S represents surface area expressed in m^2 and W is the body weight in kg [7]. Because ISIS 130 is much more sensitive to chemotherapy than ISIS 208 [6, 8], the ISIS 130-bearing rats received CY at a dose of 2.5, 5, 15, 25, 50, or 100 mg/m^2, whereas those rats with ISIS 208 tumors received higher doses (25, 50, 70, 100, 150, 220 or 320 mg/m^2). The highest dose used in this study corresponded to approximately one half of the dose inducing a lethality rate of 50% in non-cancerous LOU/dec rats (unpublished results). Depilated tumor-bearing rats receiving under ether anaesthesia an i.v. injection of physiological saline on day 4 constituted the control groups. Each treated or untreated group included 12 animals.

Beginning on day 1, the tumor size was estimated daily by measuring the longest and shortest tumor diameters with calipers. The tumor size data presented in this paper refer to the product (mm^2) of these two dimensions. Beginning on day 2, both the tumor surface temperature (*TT*) and the rectal temperature (*RT*) were measured at 7 a.m. and 6 p.m. daily. *TT* was measured by applying a stainless steel 'banjo' surface probe (YSI model 408; Yellow Springs Instrument Co., Ohio, U.S.A.) to the same place on the depilated tumor for 30 sec. Immediately after measuring *TT*, *RT* was measured on the same rat by inserting a small flexible vinyl probe (YSI model 402) 2 cm into the rectum for 20 sec. The two probes were used with a multi-channel electronic thermometer (YSI model 47 TA) which delivered direct temperature measurements in the range of 20–42°C with 0.1°C accuracy. The instrument permitted continuous recording of all temperature data on a BD 401 recorder (Kipp & Zonen, Holland). Due to the fact that the tumoral and rectal probes could be operated alternately without thermometer recalibration, it took under 1 min to measure *TT* and *RT* on one rat.

The difference between the tumoral and rectal temperatures ($Dt = TT\text{-}RT$, in °C) was used as a parameter for assessing the thermic response of the tumors to therapy.

RESULTS

Whatever the tumor or the CY dose we tested, negative or zero *Dt* were observed all through these experiments (in the range −4–0°C), indicating that *TT* was constantly inferior or equal to *RT*. It should be noted that any fall of the *Dt* lines (Figs. 1 and 2) resulted from a decrease in *TT* and not from an increase in *RT*; actually, no tendency for *RT* to increase significantly was observed in any rat group in these assays.

In control ISIS 130-bearing rats, there was a continuous increase in tumor size; no growth inhibition was observed at a dose of 2.5 mg/m^2 of CY but the tumor growth inhibition was evident in the 25 mg/m^2-group (Fig. 1). Moreover, the dose of 2.5 mg/m^2 did not induce any significant *Dt* difference between the treated and untreated rats, whereas at 25 mg/m^2, tumor temperature was significantly lower in the treated rats than in the control rats, resulting in a statistically significant ($P<0.01$) difference in *Dt* (Fig. 1). In the 25 mg/m^2 group, the tumors began to shrink rapidly from the second day after injection; at the same time, the *Dt* line of the treated group began to diverge significantly from that of the control group. In comparison, this "tumoral thermic response" was not observed in the group receiving 2.5 mg/m^2. In general, similar results were obtained with the CY-less sensitive ISIS 208 tumor. In this case, a CY dose of 25 mg/m^2 failed to affect either the tumor size of *Dt* significantly, whereas 220 mg/m^2 had a pronounced effect on both of these parameters (Fig. 2). With ISIS 208, the decrease in tumor size was only transitory; even after being given 150 or 220 mg/m^2, prompt tumor regrowth took place within 5 days after injection. It is worth noting that, as the tumor regrew, the *Dt* difference between the treated and untreated rats became less significant and then disappeared. This phenomenon is shown in Fig. 2 for the 220 mg/m^2 dose.

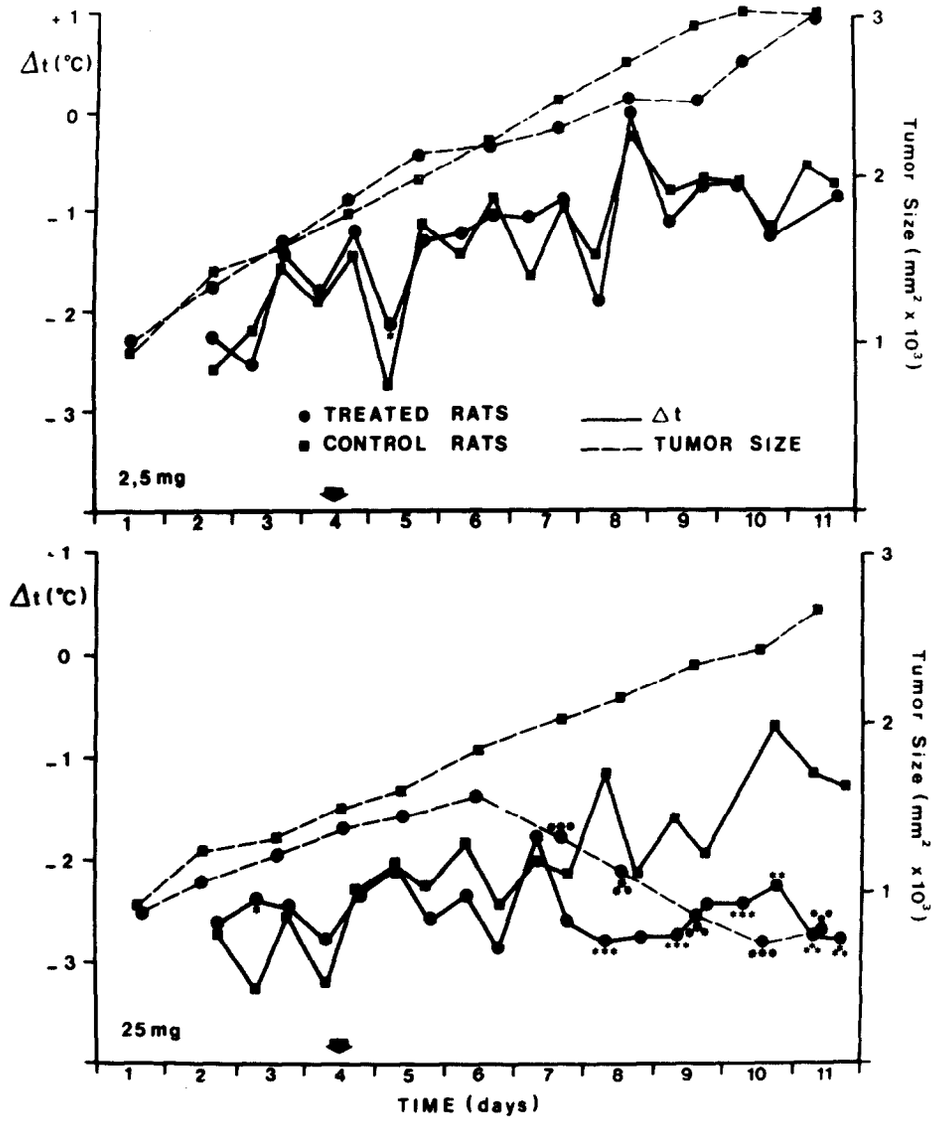

Fig. 1. Change in tumor size and in Dt *with respect to time in untreated and CY-treated ISIS* 130*-bearing rats; the results obtained at* 2.5 *and* 25 mg/m^2 *of CY (single dose on day* 4*) are depicted.* DT *is the difference between the tumoral and rectal temperatures (see text). Significant differences in tumor size or in* Dt *between the treated and untreated rats (Student's* t*-test) are indicated with one* (P<0.05)*, two* (P<0.02) *or three* (P<0.01) *stars.*

The 25 mg/m^2 dose, which was active against ISIS 130 but not active against ISIS 208, induced a thermic response of the former tumor but no response of the latter (Figs. 1 and 2).

For each CY dose tested, the thermic response was then estimated by computing the area between the *Dt* lines of the treated and untreated rats (from day 4 onward); a similar calculation was made for evaluating the antitumor effect (area between the tumor-size lines), and these parameters were plotted against the CY dose (Fig. 3). Although it was clearly dependent on the drug dose in both tumor systems, the tumoral thermic response proved to be proportional to the actual antitumor effect of the drug rather than to the drug dose itself. In the case of ISIS 130, the correlation between the tumoral thermic response and the antitumor effect of r was statistically significant ($P \leq 0.01$). In the case of ISIS 208, the correlation was also significant ($P \leq 0.02$).

DISCUSSION

Two subcutaneously transplanted immunocytomas of the rat were used as an experimental model to study the thermic response of tumors to chemotherapy. In the treated rats, any significant decrease in tumor size was accompanied with a decrease in *TT*. Conversely, *TT* rose as the tumor regrew.

In the untreated ISIS 130–bearing rats, *TT* did not vary significantly as time elapsed (from day 1 to day 11). Accordingly, we might have used *TT* instead of *Dt* as a basis for assessing the thermic response of ISIS 130 (by simply comparing *TT* of the treated and untreated rats). In the case of the untreated ISIS 208-bearing rats, on the other

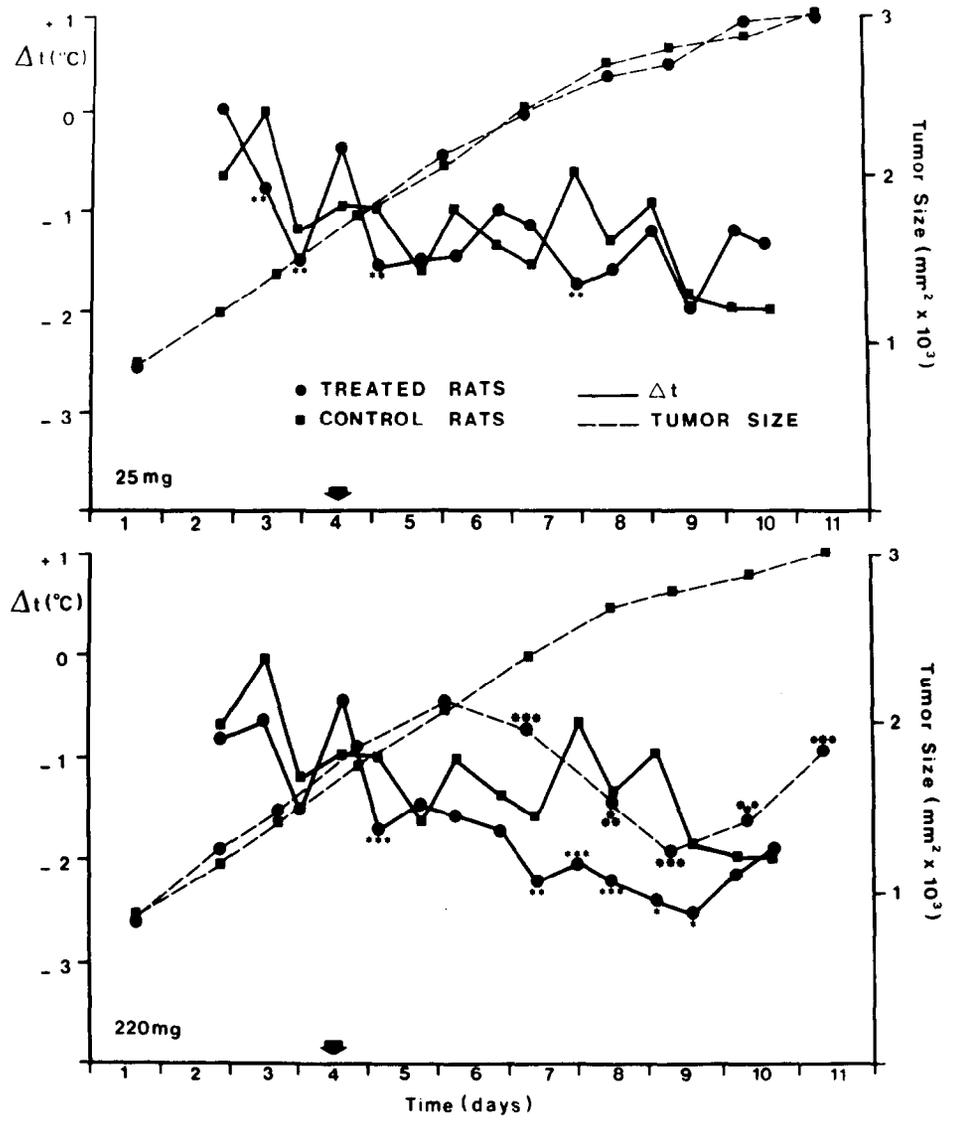

Fig. 2. Change in tumor size and in Dt *with respect to time in untreated and CY-treated* (25 *or* 220 mg/m^2 *in a single dose on day* 4) *ISIS* 208*-bearing rats. For other explanations, see legend of Fig. 1.*

hand, there was a tendency for *TT* to decrease as the tumor size increased, especially at the end of the experiment. This phenomenon was most likely to be due to the presence of more important hemorrhagic and necrotic components in this tumor, compared to ISIS 130 [8]. This decrease in control *TT*, however, was accompanied by a decrease in control *RT* most likely to result from the altered health status of the rats due to the higher malignancy of ISIS 208; as a result, the difference (*Dt*) between *TT* and *RT* was stable enough to serve for the comparison with the treated rats. Therefore, the tumoral thermic response of ISIS 208 was assessed by comparing the *Dt*s of the treated and untreated rats.

Based on these *Dt* measurements, consistent tumoral thermic responses to CY one-injection therapy were found for both the CY-sensitive ISIS 130 and CY-less sensitive ISIS 208 tumors. In both cases, a close correlation between the tumor growth inhibition and the tumoral thermic response was observed, within a wide range of CY doses. Moreover, from the comparison between the results obtained for the two tumors, it was clear that the tumoral thermic response did not correlate with the drug dose itself, but correlated with the actual antitumor efficacy the drug had in each particular case.

The results described suggest that *Dt* or *TT* may be useful as a new physiopathologic parameter to study the sensitivity of tumors to anticancer agents. It is hoped that continuous recording of tumor temperature with surface or intra-tumoral probes may permit us to assess the thermic behavior of various tumors, such as primary tumors, lymph nodes and metastases. Further studies with other experimental tumors and other drugs, as well as metabolic and histopathologic investigations, will contribute to a better understanding of the biological significance of these thermic phenomenons.

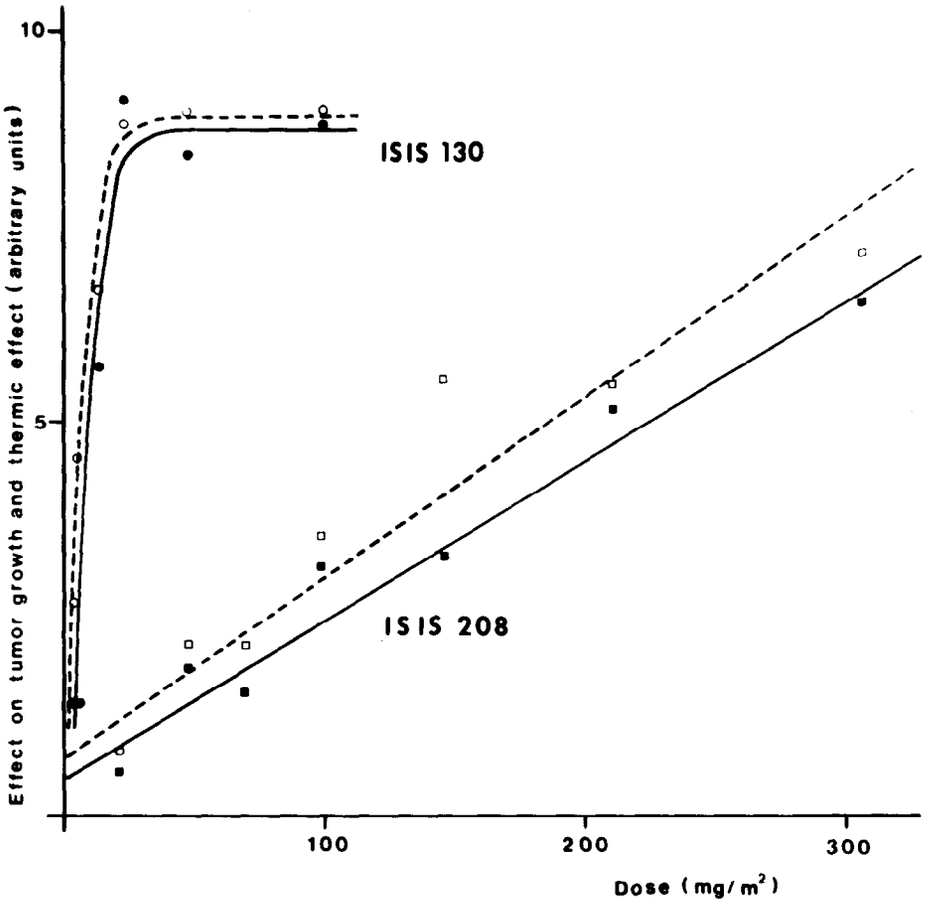

Fig. 3. Antitumor effect (open marks; dashed lines) and tumoral thermic response (filled marks; continuous lines) as a function of the CY dose in ISIS 130 *and* ISIS 208*-bearing rats.*

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