Enhancement of 5-Fluorouracil Action on Normothermic Tumors by Generalized Hypothermia

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Summary
In experiments reported earlier it has been shown that subsequent to cooling of the whole body of golden hamsters their normothermically kept tumors disappear (11-13). However, in order to induce tumor disappearance the differential hypothermia (tumor-body) has to last at least 10 hr. Since extreme hypothermia lasting several hours is not well tolerated by non-hibernators, experiments were now undertaken in which the differential hypothermia was combined with 5-fluorouracil (FU), an anticancer drug. It was reasoned that the anticancer drug might become preferentially effective in the tumor tissue because of higher volume blood flow as well as of higher metabolic rate of normothermic tumors when compared with the rest of the hypothermically kept body. For this purpose the bodies of 75 hamsters were cooled to a temperature of 4°C while their tumors (Toolan H. Ad. No. 1U) remained uncooled at 37°C. The FU (50 mg/kg) was administered i.v. in a single injection. One hr later the animals were rewarmed. Twenty days later all tumors regressed and disappeared completely without resuming their growth afterward. When the same amount of FU was administered into normothermic tumor-bearing animals or into hypothermic animals with hypothermic tumors, neither tumor size nor body weight of the animals was affected.

Introduction
It has been shown that normothermically kept tumors disappear when the whole body of animals is cooled to 4°C for 10 hr or to 30°C for 24 hr (11-13). Subsequently, the authors found in preliminary (unpublished) experiments that the rate of volume blood flow is considerably higher to a normothermic tumor in a hypothermic animal than it is to a tumor cooled together with the animal. Based on this observation, and the well-established fact that normothermic cells have a higher metabolism than hypothermic ones, the question arose whether anticancer drugs administered to hypothermic animals with normothermic tumors might become preferentially effective in the tumor tissues without reaching toxic levels in the rest of the body. If this should be the case, the normothermic tumors in hypothermic animals might disappear even after a single administration of anticancer compounds. Thereby, it might be possible to avoid the detrimental consequences of repeated treatments, such as adaptation of cancer cells to drugs or selective development of resistant cancer cells. The purpose of the present investigation was to test this hypothesis.

Materials and Methods
In this investigation, 114 male hamsters (Mesocricetus auratus), 95 ± 9 gm of weight, were kept in separate cages on Rockland rat diet and water ad libitum. Toolan adenocarcinomas were transplanted in their cheek pouches (11-13). Ten days thereafter the right jugular vein was permanently cannulated (14) to permit subsequent injection of anticancer drugs without use of anesthesia or restraint. When the tumors reached suitable size for the experiments, the animals had fully recovered from the cannulation and their body weights had already surpassed the preoperational values.

In a typical experiment a hamster was slightly anesthetized and cooled to a body temperature of 4°C by immersion in ice water up to the neck. The deep colonic temperature was monitored continuously. The tumor was kept normothermic by a thermostated heating device and a servomechanism (11-13). The device was made out of a piece of 30 gauge (Brown and Sharpe) nickel-chrome wire 20 cm long, insulated in a Teflon-fiberglass sheath and molded into a pad with adhesive tape. The heat was delivered by a 6-volt battery connected to the heating pad by a temperature controller (Honeywell). The temperature sensor was an iron-constantan thermocouple inserted in the center of the tumor. As soon as the body temperature of the animal decreased to 4°C and the temperature of the tumor was stabilized at 36°C, a single dose of 250, 200, or 50 mg/kg of 5-fluorouracil (FU) was administered through the jugular cannula. The FU was dissolved in 0.85% sodium chloride, making a 1.3% solution, and was injected slowly, 5 mg/kg/min. The injections were given i.v. because they were found to be more effective than i.m. or i.p. ones (8). FU was chosen because of its high uptake by the tumors (9). One hr after the injection the animal was dried and returned to the cage to rewarmin and to recover from hypothermia. After re-warming, the animal's body weight was measured twice weekly for 100 days. During the same period of time as well as before the treatment, the size of tumor was measured every other day by a caliper and expressed in cm.

Results
Forty normothermic animals with transplanted cheek pouch Toolan tumors served as a control for all subsequent experiments.
No hypothermia or medications were used in the control experiments. All tumors increased in size (Chart 1).

**SERIES A, SINGLE DOSE OF 250 mg/kg FU.** In this series of experiments 21 animals were divided into 3 equal groups for the administration of a single dose of 250 mg/kg of FU per hamster. The 1st group consisted of 7 tumor-bearing animals. They were given FU while both the tumor and the animal body remained at normal temperature. The tumors regressed in size (upper part of Chart 2a, crosses). Concomitantly the animals lost weight and died after 8 days (lower part of Chart 2a, crosses). In the 2nd group, FU was administered to 7 tumor-bearing hamsters at a body temperature of 4°C while their tumors were kept normothermic. The tumors regressed at essentially the same rate (upper part of Chart 2a, circles) as those in Group 1. The loss of body weight was practically the same as in Group 1. The animals died after 7 days (lower part of Chart 2a, circles). In the 3rd group, FU was injected in 7 tumor-free animals at a normal body temperature. These hamsters lost weight at the same rate as the tumor-bearing animals and died after 10 days (lower part of Chart 2a, full dots).

**SERIES B, SINGLE DOSE OF 200 mg/kg FU.** The groups in this series were identical with those in Series A, except that the dose of FU was reduced from 250 mg/kg to 200 mg/kg. The results were similar to those in Series A. However, the tumor regression as well as the body weight loss of the animals in Series B was less pronounced and the survival was prolonged (Chart 2b).

Both series of experiments demonstrated that whenever a single dose of FU was large enough to reduce the size of the tumors, it always resulted in death of the animals, irrespective of their body temperature at the time of injection. Therefore, the dose was greatly reduced (Series C).

**SERIES C, SINGLE DOSE OF 50 mg/kg FU.** The same grouping was made as in Series A and B, except that 9 animals instead of 7 were used in each group. In Group 1 the tumor-bearing normothermic hamsters did not die and continued to gain weight after the administration of FU (lower part of Chart 3, crosses). Their tumors continued to grow (upper part of Chart 3, crosses). In Group 2 the tumor-bearing hypothermic animals survived and continued to gain weight, although there was a temporary weight loss after the exposure to hypothermia (lower part of Chart 3, open circles). However, the tumors, which had been kept normothermic in these animals, disappeared within 14–20 days after FU had been injected (upper part of Chart 3, open circles). In Group 3 the tumor-free animals gained weight and survived the administration of FU (lower part of Chart 3, full dots).

**SERIES D.** After this demonstration of tumor disappearance, an
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**Chart 3.** Upper part, Recession of normothermic tumors in hypothermic animals (open circles) after single injection of 50 mg/kg of 5-fluorouracil (FU). The same amount of FU when injected in the normothermic animals with normothermic tumors did not affect the growth of tumors (crosses). Lower part, After a single injection of 50 mg/kg of FU the body weight of normothermic tumor-free (full dots) or normothermic tumor-bearing animals (crosses) continued to grow normally. The body weight of hypothermic hamsters with normothermic tumors (open circles) regressed somewhat after injection of FU, a fact observed in all control animals after deep body cooling.

An experiment was devised to test the effect of the temperature difference between tumor and body upon the tumor regression in one and the same animal. Five hypothermic animals, bearing tumors in both cheek pouches, were given 50 mg/kg of FU. One tumor was kept at 37°C at the time of FU injection, but the other was lowered to the same degree of hypothermia as the rest of the hamster's body (4°C). All normothermically kept tumors disappeared completely, but those which were hypothermic at the time of FU injection continued to grow (Chart 4).

**Discussion**

In these experiments it has been shown that after a single injection of anticancer drug (FU) normothermic tumors of hypothermic animals regress and disappear eventually. The amount of FU was so small that neither body weight nor tumor size was affected when the drug was injected into normothermic animals with normothermic tumors or into hypothermic animals with hypothermic tumors. It appears that the most promising results may be obtained by further experiments combining chemotherapy and differential hypothermia.

However, since the mechanisms responsible for obliteration of normothermic tumors in hypothermic animals are unknown, the findings of these experiments should be considered together with the findings of other investigators on the effects of temperature and anticancer drugs on tumors in order to arrive at a working hypothesis or some speculations that might suggest future experimental approaches.

The idea of changing the temperature of 1 part of the body or of the body as a whole in order to influence the development of tumors is about 100 years old. Hyperthermia, general body warming induced in a Turkish bath, was first suggested in 1874 as a treatment of cheek tumors (3). Later developments concerned destruction of spontaneous mammary cancers in mice by microwave heating (4) and regression of sarcomas and adenocarcinomas incubated on chorioallantois of chicken embryos at 39°C (5). Cancer decrease in young patients warmed to a body temperature of 44°C for 30-45 min was also reported. The heat treatment was repeated several times, but the tumors continued to grow as before (1). Apparently 1 single exposure to hyperthermia was not sufficient to produce complete regression to tumors, while several heat treatments led to adaptation of cancer cells to hyperthermia and the treatment became ineffective.

Attempts to combine hyperthermia with regional perfusion of anticancer agents were not successful either (19). Shingleton com-
combined regional hyperthermic chemotherapy with a slight cooling of the rest of the body in order to protect the bone marrow from the harmful effects of the anticancer drugs (16, 17). He was able to extend the survival of most of his patients for several months, though he found that “the outstanding limiting factor, so far, has been inadequate drug effect on the tumors.” Shingleton and Parker say also that patients responded unpredictably to cancer chemotherapy irrespective of the dose and that “only 50% of the patients show an objective response” (17). It remains an open question whether this is caused by an “adaptation” of cancer cells to repetitive drug application, by a decreased action of anticancer agents induced by hyperthermia, or by an insufficient cooling of the body to only 32°C, which instead of producing a decrease of the metabolic rate could have left it unchanged or even increased (10). It seems that, when chemotherapeutic perfusions are used alone, or in combination with hyperthermia, the treatment has to be repeated often and always leads to the “tragic inevitability of the emergence of resistant cell population in tumors” (6).

Hypothermic cooling alone has also been attempted in order to diminish tumor growth. The results were negative (2, 7, 15, 18) except with “differential hypothermia” (11–13). In the present work successful combination of a single dose of anticancer medication with “differential hypothermia” appears to eliminate the adaptation of tumor cells not only to repeated drug injections but also to the temperature alterations alone. Both aspects, the nontoxic single dose and the short cooling period, may point toward the possibility of an application in patients. However, before such steps are considered, an understanding of the mechanism of action should be obtained and the animal experiments must be extended to other types of tumors, especially to those spontaneously developed or chemically induced.

At the moment one can merely speculate about the mechanism of tumor disappearance in our experiments. Since the volume flow of blood to the normothermic tumors during body hypothermia is very high, the anticancer drug may be preferentially embedded in the tumorous tissue. It needs to be elucidated whether the suspected concentration of FU is the sole cause of tumor regression or whether it is aided in its anticancer effects by the probably profound thermogenic changes in the tumor metabolism. The fact that 10 hr of differential hyperthermia at 4°C alone deletes normothermic tumors makes it likely that a combination of several factors is involved.

References


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