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Preferential Inhibition of DNA Synthesis in Mouse Hemopoietic Cells by Halothane

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SUMMARY

The effect of prolonged light halothane anesthesia (0.8%) on the proliferation rate of different mouse tissues was investigated, using [5-^{125}I]5-iodo-2-deoxyuridine uptake into DNA as the test parameter. It was found that DNA synthesis in spleen, femoral bone marrow, and, occasionally, the small intestine was significantly depressed after exposure for 24 hr to halothane *in vivo*. The time course of DNA synthesis inhibition was then investigated by utilizing a shorter (6-hr) exposure time. This period was found to be insufficient to cause DNA synthesis inhibition in any of the test tissues. Because anesthesia was found to be associated with hypothermia at normal room temperatures, it was established that the inhibition of DNA synthesis was not due to cooling of the mice under anesthesia by demonstrating that inhibition in sensitive tissues occurred at warmer temperatures as well. To examine the specificity of this finding, the DNA synthesis rate of cells in other normal tissues, *e.g.*, skin and muscle, and in s.c.-growing tumor cells of a mouse mammary carcinoma, L1210 leukemia, and a first transplant AKR lymphoma were examined. In none were responses noted with 24 hr of halothane exposure. However, halothane was found to inhibit DNA synthesis in regenerating marrow. Finally, it was found that after significant exposure to halothane, complete recovery was seen in the spleen after 24 hr, whereas femur DNA synthesis was still depressed by 20% at the same time.

INTRODUCTION

One of the major limiting factors in cancer chemotherapy is that the drugs used cause damage to critical normal tissues, such as in the hemopoietic and gastrointestinal systems. Since the lethal effect of several anticancer agents depends upon the proliferative state of the target cells, with cells that proliferate faster being more sensitive, a logical approach to chemotherapy would be to decrease preferentially the proliferative activity in the critical normal tissues during the administration of these cytostatic drugs.

Bruce *et al.* (3) showed that, after 24 hr of halothane administration to mice at a sedative concentration, the sensitivity of normal bone marrow stem cells to phase-specific drugs, *e.g.*, vinblastine and arabinosylcytosine, was markedly lowered. AKR lymphoma cells were not similarly affected. Further it was reported by Bruce and Koepke (4) that long-term light halothane administration prolonged the S phase (DNA synthesis) in rat intestinal cells. This paper reports the effects of similar light halothane anesthesia on the proliferative state of several normal and malignant mouse tissues.

MATERIALS AND METHODS

For studies on normal cell populations, CBA × C57BL F_1 hybrid male mice were used; the mammary carcinoma was grown in female mice of the same type. The AKR lymphoma and L1210 leukemia were grown in AKR male mice and DBA2 × C57BL F_1 hybrid male mice, respectively. Groups of 4 or 5 mice were exposed to either 0.8% halothane or to normal air, both in 7-liter Perspex boxes under controlled conditions. Air containing 0.8% halothane was produced by a Fluotec vaporizer at a flow rate of 1 liter/min, and analysis of the gas mixture was done by means of Fluotec analyzer at regular time intervals. This halothane concentration does not induce complete anesthesia, but allows the animals to ambulate during most of the experiment.

To measure the proliferation rate in the cells of the tissues under study, 0.5 μCi of [^{125}I]IUdR[2,3] (specific activity, 1 to 6 Ci/mmole) in a volume of 0.2 ml was injected i.p. into the experimental mice one-half hr before the end of halothane exposure. The mice were killed by cervical dislocation, and, immediately thereafter, tissues of interest were collected and weighed (except the bone marrow, of which a suspension was prepared from 2 femurs). The DNA from the tissue was extracted by means of a modified Schmidt-Tannhauser method. Briefly, the tissues were dissolved in 2 ml 1 N NaOH during 24 hr at room temperature. The tubes were then cooled to 0° and 10 ml of an ice-cold 15% trichloroacetic acid solution were added to precipitate the DNA. This precipitate was washed twice at 0° with a 5% trichloroacetic acid solution to achieve separation of DNA and RNA. Counting of the washed precipitates was done in a well-type NaI(T1) scintillation γ-counter for 20 min. The mean number of cpm/g of tissue was calculated and the number of cpm/g of halothane-exposed tissue was expressed as a percentage of the mean number of cpm/g of air-exposed tissue.

Whole spleen, bone marrow, skin (2 ears), small intestine (2-cm terminal ileum), and muscle (surrounding the femur) were used as normal tissues. Rapidly proliferating hemopoietic cells were produced by radiation depletion, followed

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[2] The abbreviation used is: [^{125}I]IUdR, [5-^{125}I]5-iodo-2-deoxyuridine.

[3] Like [^{3}H]thymidine, the [^{125}I]IUdR is exclusively incorporated into DNA (6, 7). At the dosages used, there is no measurable chemical toxicity.

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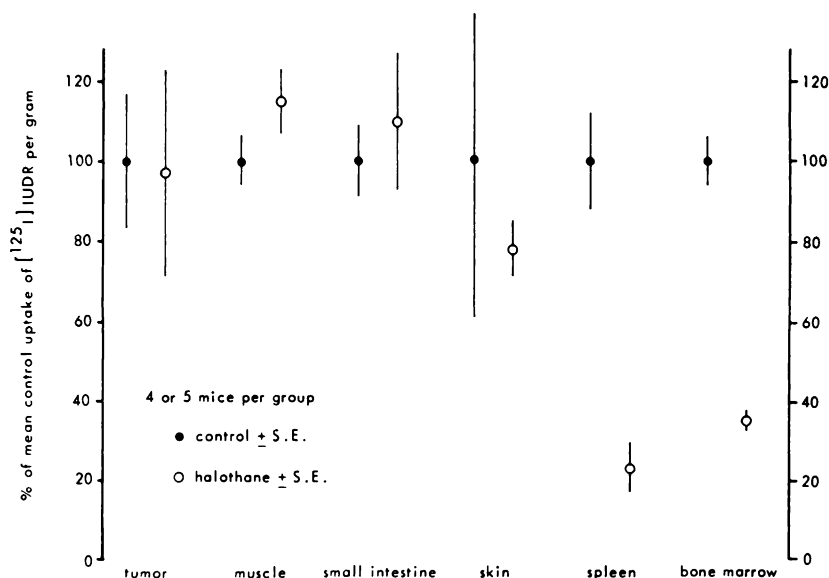

Chart 1. Diagrammatic representation of the effect of 24 hr of exposure to 0.8% halothane *in vivo* on the DNA synthesis rate in normal mouse tissues and in a transplantable mammary carcinoma. *Points*, mean ± S.E. for 4 to 5 mice. Mean values (cpm/g tissue) for the control mice were: tumor, 1707; muscle, 384; small intestine, 714; skin, 472; spleen, 6747; and marrow (2 femurs), 76.

by bone marrow transplantation. Mice received 950 rads γ-ray whole-body irradiation plus i.v. injections of a standard amount of bone marrow (one-quarter of that in 1 femur, *i.e.*, approximately 3 to 4 $\times$ 10^6 nucleated cells). Seven days later, their spleen and femurs contained large numbers of hemopoietic cells, engaged in rapid proliferation, and these mice were used then as experimental animals. The following malignant cell lines were used: a well-established transplantable mammary carcinoma (spontaneously induced in the CBA mouse; passage 60: undifferentiated carcinoma, tumor-doubling time in exponential phase, 2 days), the L1210 leukemia, and a 1st passage of an AKR lymphoma. All tumors were grown s.c. after transplantation of 10^6 cells and were used at Day 7 after transplantation.

RESULTS

The effect of 24 hr of light halothane anesthesia on the incorporation of [^{125}I]IUdR into DNA has been shown in Chart 1. In the halothane groups, a statistically significant depression of [^{125}I]IUdR uptake in spleen and bone marrow can be observed. However, no difference between the control and halothane group was observed for skin, small intestine, muscle, and a s.c.-growing mammary carcinoma. When the mice were exposed to halothane for a period of 6 hr instead of 24, no significant depression of [^{125}I]IUdR uptake in DNA could be observed in any of the tissues under investigation (Chart 2). The apparent inhibition in tumor and stimulatory effect in the other tissues measured are not statistically significant (Student's *t* test).

In an earlier phase of the study, the temperature in the animal cages during the experiment was maintained at 20°. Body temperature of the mice was observed to drop by 3°-5° after 24 hr of halothane exposure at this environmental temperature. To see whether cooling of the mice might have caused the depressed [^{125}I]IUdR uptake, the effects of 2 environmental temperatures, 20° and 30°, were compared. An environmental temperature of 30° protected the mice from this drop in body temperature. Chart 3 shows that an

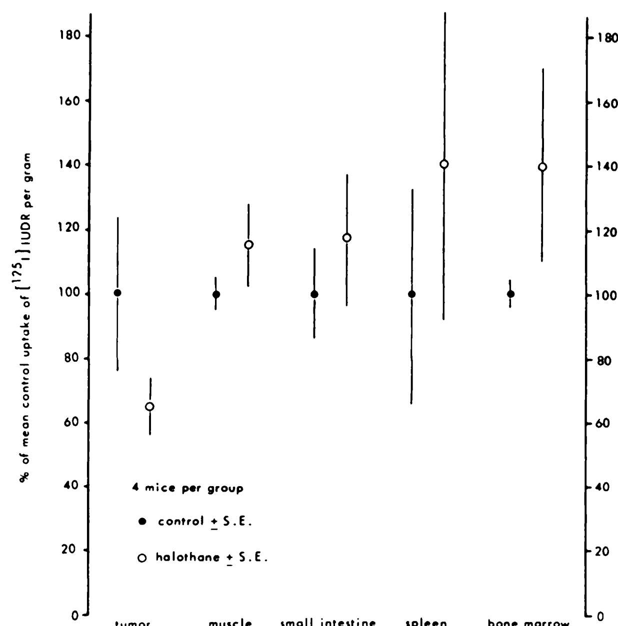

Chart 2. Diagrammatic representation of the effect of 6 hr of exposure to 0.8% halothane *in vivo* on DNA synthesis rate in normal and malignant mouse tissues. *Points*, mean ± S.E. for 4 to 5 mice. Mean values (cpm/g tissue) for the control mice were: tumor, 312; muscle, 45; small intestine, 1427; spleen, 3325; marrow (2 femurs), 49.

environmental temperature of 30° did not abolish the depressed DNA synthesis in spleen and bone marrow. In these experiments, halothane depressed DNA synthesis in small intestine. Here, the difference is statistically significant. Twenty-four hr after stopping the halothane anesthesia (Chart 4), complete restoration of DNA synthesis was seen in the spleen, whereas the femoral marrow was still 20% inhibited.

The hemopoietic stem cell population of normal mice is mainly in a resting state. However, the proliferation rate of the bone marrow may be increased after treatment with cytostatics or radiation. Therefore, we investigated the effect of halothane on DNA synthesis in the hemopoietic cells

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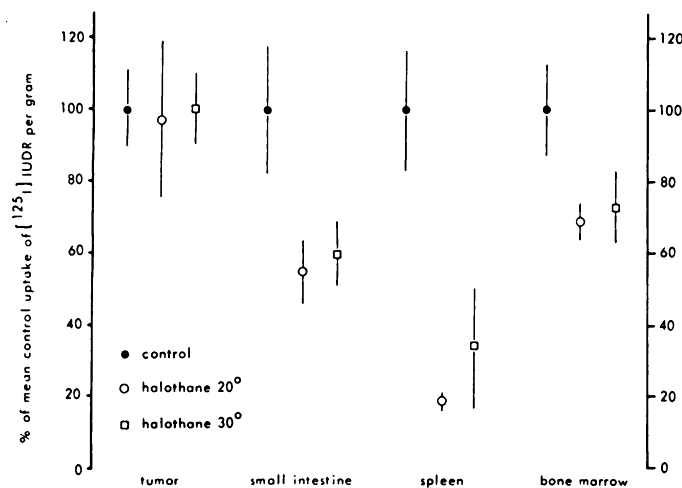

Chart 3. Diagrammatic representation of the effect of 24 hr of exposure to 0.8% halothane *in vivo* on DNA synthesis rate in normal and malignant mouse tissues; test for the effect of 2 temperature levels. *Points*, mean ± S.E. for 4 to 5 mice. Mean values (cpm/g tissue) for the control mice were: tumor, 487; small intestine, 1914; spleen, 3451; marrow (2 femurs), 69.

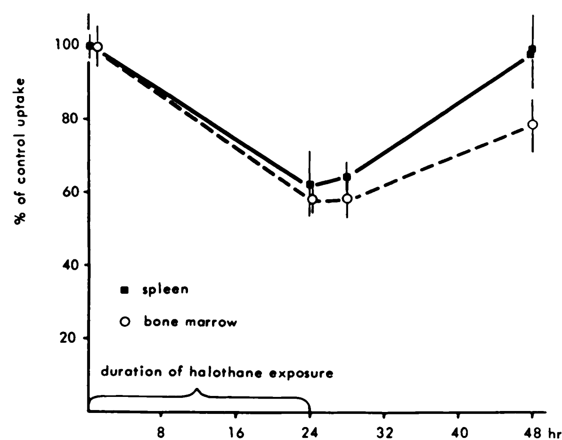

Chart 4. Diagram representing the time course of inhibition of the DNA synthesis rate by halothane in spleen and bone marrow cells. *Points*, mean ± S.E. for 4 to 5 mice. Mean values (cpm/g tissue) for the control mice were: spleen, 1875; marrow (2 femurs), 134.

of mice whose hemopoietic system was rapidly proliferating. Our results indicate that 24 hr of light halothane anesthesia also significantly depressed DNA synthesis in rapidly proliferating marrow and spleen cell populations (Chart 5). Twenty-four hr of halothane administration did not affect DNA synthesis in a s.c.-growing mammary carcinoma (Chart 1). Therefore, we extended our study to the L1210 leukemia grown s.c., but found that the DNA synthesis in this tumor was not inhibited either (Chart 5). It can be hypothesized that the lack of an inhibitory effect with these widely used transplantable tumors is a result of a loss of responsiveness to growth-regulating factors, which loss is related to the tumors having undergone many passages and not to their malignant state alone. Therefore, we also investigated a 1st transplant of an AKR lymphoma but found that, in this tumor also, DNA synthesis was not affected by halothane (Chart 5).

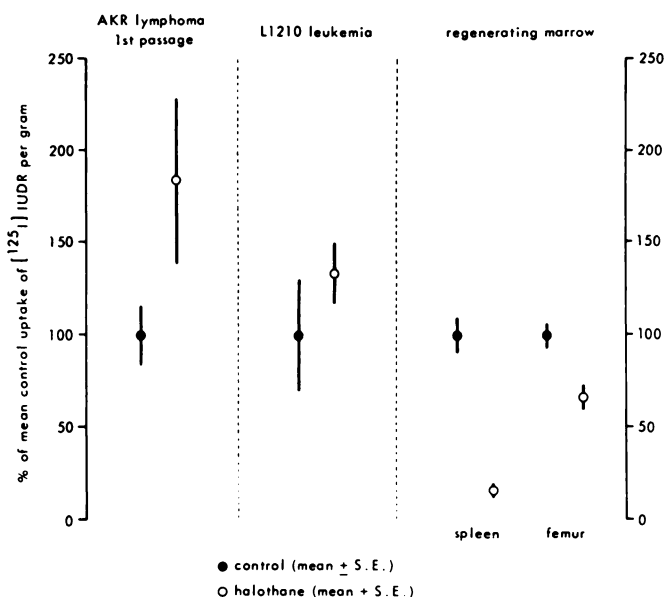

Chart 5. Diagrammatic representation of the effect of 24 hr of exposure to 0.8% halothane *in vivo* on DNA synthesis rate in cells from AKR lymphoma (1st passage, s.c.), L1210 leukemia (s.c.) and in regenerating marrow. *Points*, mean ± S.E. for 4 to 5 mice. Mean values (cpm/g tissue) for the control mice were: AKR, 3864; L1210, 2756; spleen, 11,400; marrow (2 femurs), 75.

DISCUSSION

The inhibitory effect on cell division by volatile anesthetics has been known since 1930. It was later reported that both halothane and nitrous oxide lowered the sensitivity of normal hemopoietic stem cells (colony-forming units) but not of AKR lymphoma cells to 1-β-D-arabinofuranosylcytosine and vincaleukoblastine (3). We have found that 24 hr of halothane exposure reversibly depressed DNA synthesis in normal hemopoietic cells in the femur and spleen and less consistently also in small intestine. In the femoral bone marrow, this depression is, although reproducible, rather variable. However, no inhibition of DNA synthesis was observed in other normal tissues, *e.g.*, skin and muscle, nor in several types of s.c.-growing transplantable tumors. Thus, the finding of a preferential effect of halothane on normal hemopoietic cells is in agreement with the finding of Bruce *et al*. (3). It is emphasized that the last authors showed the halothane effect for hemopoietic stem cells only. Our data indicate, however, that the total hemopoietic cell compartment, stem cells and more mature cells, must have been affected by the halothane treatment.

The inhibition of DNA synthesis also provides evidence that halothane renders normal hemopoietic cells less sensitive to phase-specific cytostatic drugs (3) by lowering the proliferation rate of these cells. The observation that several types of transplantable animal tumors are not affected by halothane might suggest a basic difference between tumor cells and murine hemopoietic cells concerning this effect. The suggestion that halothane might exert its inhibitory effect by preventing the entry of normal cells into cycle (3) is not supported by our findings, since DNA synthesis must also be blocked in the much larger non-stem cell compartment of the bone marrow cells. Indeed, even a rapidly proliferating bone marrow cell population is also affected by light halothane anesthesia. The results obtained with a

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1st passage of an AKR lymphoma, which is presumably still responsive to certain proliferation-regulating stimuli, suggest that the unresponsiveness to halothane of leukemia L1210 and of the mammary carcinoma is not due to the fact that these transplantable tumors are insensitive to growth-regulating factors after numerous passages of transplantation.

The mechanisms for these findings are not completely understood; some observations have suggested that anesthetic agents, for example, halothane, can reversibly interfere with fundamental mechanisms responsible for intracellular mobility and cellular translocation and can interact with at least some species of microtubular protein (1, 5, 11, 13). It is known that at least some of the injectable anesthetic drugs exert a slight radioprotective effect on mouse hemopoietic and intestinal cells (8, 10, 12). These drugs were also shown to have a slight protective effect on hemopoietic stem cells during fractionated chemotherapy (9). One of these drugs, pentobarbital, was shown to inhibit DNA synthesis in mouse hemopoietic cells *in vivo* (2, 10), an effect that is probably exerted directly at the DNA level (2).

In conclusion, our results show that reversible inhibition of DNA synthesis in both hemopoietic stem cells and maturing cells is possible. The use of halothane during chemotherapy of human cancers does not seem indicated in view of the occasional severe liver toxicity of this drug. Search for other less toxic drugs with similar effects on critical normal tissues might improve the therapeutic gain in cancer chemotherapy.

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