COO-4800-5

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Division of Radiation Oncology

950 9712

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TECHNICAL REPORT

EFFECT OF CHEMICAL CARCINOGENS ON HEMATOPOIETIC
STEM CELLS: TEMPORAL CORRELATION BETWEEN METHYL
METHANE SULFONATE INDUCED DEPRESSION OF CFU-S
AND POTENTIATION OF FRIEND VIRAL LEUKEMOGENESIS IN VIVO

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May 1979

Prepared for

THE U.S. DEPARTMENT OF ENERGY
UNDER CONTRACT

EP-78-S-02-4800.A000

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ABSTRACT

Methyl methane sulfonate (MMS) was shown to reduce the number of transplantable pluripotent hematopoietic stem cells (CFU-S) in the mouse spleen. A mild depression in CFU-S numbers was seen as early as 1 hour after injection of the chemical carcinogen. Maximal depression was found to be at 5 hours, followed by a recovery of up to 75% of normal levels by 24 hours. Comparison of the data with earlier results which demonstrated a potentiating effect of MMS on viral leukemogenesis in vivo revealed a good temporal correlation, suggesting a possible relationship between the two effects.

INTRODUCTION

Methyl methane sulfonate (MMS) causes single strand breaks in DNA, which are similar to those caused by x-rays in that they are quickly repaired by "short-patch" repair (1). Two hours after the administration of 100 mg/kg of MMS i.p. into rats, Petzold and Swenberg (2) could demonstrate breaks in the DNA of all organs tested. By four hours partial repair of this damage was evident. MMS has been shown to directly alkylate DNA, particularly at the N-7 position of guanine (3). Such alkylation can cause base mispairings during subsequent DNA replication, resulting in point mutation (4). When administered alone MMS is weakly carcinogenic, but enhances the in vitro neoplastic transformation of Syrian hamster embryo cells induced by N-acetoxy-2-acetylaminofluorene or by benzo(a)pyrene (5). MMS can also potentiate virally-induced carcinogenesis. This has been demonstrated by Casto et al. (6) in vitro using adenovirus and by us (7,8) in vivo using Friend murine leukemia virus (FLV). In both cases involving a virus the carcinogenic potentiation was observed only when the intervals between MMS and virus administration were relatively short (less than 24 hours). Such timing suggests a direct effect of MMS on the virus target cells, which in the case of FLV includes the hematopoietic stem cells (9) found in the bone marrow and spleen.

The present studies were undertaken to determine if exposure to MMS in the doses and manner which induce potentiation of viral leukemogenesis might also result in an effect on the CFU-S population of the exposed animal, and whether any correlation

might exist between such effects on the stem cells and the incidence of leukemogenic potentiation observed when similarly MMS exposed animals are also exposed to FLV.

MATERIALS AND METHODS

Animals - Female SJL/J mice were purchased from Jackson Laboratories when 6-8 weeks of age. These were then housed in autoclaved plastic filter-topped cages and given autoclaved Purina mouse chow and autoclaved, acidified water ad libitum. The mice were maintained in a controlled environment room, and the experiments were initiated when they reached 10-12 weeks of age.

Methyl methane sulfonate - For administration MMS (Sigma) was diluted with sterile isotonic saline and injected i.p. in the dose of 100 mg/kg of body weight as a single dose. As reported previously (7), this dose given to mice 5 hours before FLV resulted in a significant potentiation of Friend viral leukemogenesis, but at the same time had no chemically lethal toxic effects on the recipient animal.

Virus - Our Friend leukemia virus stock was originally obtained from NCI in 1969 and has been stored at -60°C . For the present studies it was passaged once in SJL/J mice and a plasma preparation obtained. This was titered by the spleen enlargement dose ($\text{SED}_{50/14}$) assay (10). The cell-free preparation was then diluted with phosphate buffered saline to give the appropriate dose to cause leukemia in approximately 20% of the animals exposed to virus alone.

CFU-S assay - For assay of pluripotent hematopoietic stem cell (11) levels donor spleen cell preparations were obtained by mashing the spleens of the control and MMS exposed mice in Ca^{++} and

Mg⁺⁺ free Hank's solution (GIBCO): Cell clumps were broken into a single cell suspension by gently passing them through hypodermic needles, using first a 21 gauge and then a 25 gauge needle. Concentrations of 1.25, 2.0, and 4.0 x 10⁵ cells were then injected via the tail vein into 7-8 SJL/J mice for each concentration. These recipient mice had been previously prepared for use as assay animals by exposing them two hours previously to the hematopoietic ablating dose of 950R total body irradiation. Eight days later the recipient mice were sacrificed and their spleens fixed in Bouin's solution. The spleen colonies formed were counted using a dissecting microscope.

Leukemogenic potentiation - Determination of leukemogenesis was done as previously described (7,8).

Plaque forming cell (PFC) assay - Effect of MMS on antibody producing PFC number was determined using the Kennedy-Axelrad (12) technique as previously employed by us (13,14).

RESULTS

The number of transplantable spleen colony forming units (CFU-S) per 10⁶ spleen cells was significantly depressed by injection of MMS (Fig. 1). This depression was evident as a slight reduction in CFU-S numbers as early as 1 hour after exposure of the mice to MMS. At 5 hours the level was reduced to 44% of the control. Recovery had started by 12 hours after MMS, and by 24 hours the CFU-S number was 75% of control.

The upper curve of Figure 1 represents MMS potentiation of Friend viral leukemia as described by us in earlier studies (7,8).

EFFECT OF MMS ON CFU-S AND FLV POTENTIATION IN SJL/J MICE

Figure 1. The upper panel shows the effect of MMS on FLV potentiation in SJL/J mice. Percent dead of leukemia by day 150 after virus inoculation are plotted. Each point represents one experiment involving 10-15 mice. The control animals received FLV only and are plotted at 0 time. For the other points the abscissa indicates the hours allowed to elapse after MMS injection before FLV was inoculated.

The lower panel shows the effect of MMS on CFU-S in SJL/J mice. To obtain the data of each time point 5 SJL/J mice were injected with MMS, sacrificed at the times indicated and their spleens were assayed for CFU-S as described in the Materials and Methods section. 24 lethally-irradiated SJL/J assay mice were used for each time point and the means of their colony counts ± 1 S.E. are plotted.

A control group, plotted at 0 time, received 0.1 SED Friend leukemia virus (FLV) only. Other groups received FLV at 4, 5, 18 or 24 hours after MMS. The virus dose given was less than that which would cause leukemia in 100% of the mice. Leukemia incidence among the control mice given FLV only was 43% at 150 days after virus infection. Injecting MMS 5 hours before FLV significantly increased leukemia incidence, while injection of MMS at 18 or 24 hours had little effect. MMS given alone had no effect on leukemia incidence during the 150 day observation period. Incidence of leukemia was evaluated using the three principal symptoms of Friend disease as described in the literature (15), increased white counts, hepatosplenomegaly at autopsy, and death (7,8). All mice had received the same virus preparation at the dose of 0.1 SED_{50/14} units, and there were at least 15 per group.

Figure 2 shows the effect of MMS on antibody producing plaque-forming cells (PFC) as a function of time, determined from our previous studies (7). Although MMS exposure also depressed the ability of PFCs to respond to sheep red blood cell antigen in vitro, this effect was not evident until at least 24 hours after administration of MMS. Decreased PFC response was then maintained for about twelve days before the onset of return to normal levels. Hence, although MMS depressed both the CFU-S and PFC response, of the two only the CFU-S response showed a temporal correlation with the leukemogenic potentiating effect of MMS.

DISCUSSION

CFU-S assays are a measurement of the number of reproductively capable pluripotent hematopoietic stem cells in the spleen or marrow of the mice being assayed. We have chosen the CFU-S for evaluation

Figure 2. The effect of MMS on the plaque forming response to sheep red blood cells in SJL/J mice. The time intervals on the abscissa are those allowed to elapse between MMS injection and the initiation of the PFC assay (12-14). This initiation is the i.p. injection of sensitizing sheep red blood cells. Each point represents the mean of values obtained from 6 mice $\pm$ 1 S.E.

in the present study for two reasons. First, stem cell populations represent the most critical cell populations in terms of carcinogenesis in that transformation of these cells is more likely to lead to establishment of a reservoir of reproductively potent malignant cells than would be expected from transformations occurring among differentiated cell populations. Second, the pluripotent stem cell has been identified as one of the target cells for Friend leukemia virus (9). The rapidity with which the potentiating effect of MMS on Friend viral leukemia is both gained and lost suggests a direct effect of the agent on the target cell level. It also suggests that the effect is quickly reversed. This can be seen from the fact that virtually all of the potentiating action of MMS is lost when there is a twenty-four hour interval between the chemical and the virus administration. Our previous data also showed that the potentiating effect could only be demonstrated if MMS were given *before* FLV (7). Thus whatever disruption of normal cell function is caused by MMS that allows it to be a potentiating agent must be existent at the time the cell is exposed to the virus.

Several previous studies have shown that as a part of the transformation process in viral leukemia a portion of the DNA encoded for by the virus is incorporated into the DNA of the cellular genome (16-18). It has also been suggested that active DNA replication is a requirement for incorporation of the viral information (19,20). In the mammal *in vivo* the vast majority of pluripotent hematopoietic stem cells are in a resting state (21). These can be called into active cell cycle by a partial destruction of the stem cell pool, such as occurs with radiation, which is known to be a potentiating agent for murine viral leukemia (22,23). Regan

and Setlow (1) have shown that MMS produces a type of DNA damage similar to ionizing radiation, resulting in short strand breaks that are easily repaired by repair enzyme. Based on their findings and our present observations we can suggest at least two mechanisms by which MMS may be facilitating viral leukemogenesis. The first of these would be by calling a greater number of the surviving stem cells into cycle than normal, thus increasing the number of possible available targets for the virus. The second would be increasing the number of possible points of insertion into the cellular genome by the breaks caused by the agent and facilitating their entry with the action of the repair enzyme. Moreover, these mechanisms are not mutually exclusive, and both may be operative at the same time.

Additional mechanisms could possibly be also proposed, but one of these, that of immunosuppression by MMS thereby reducing the animal's ability to destroy leukemogenic virus by antibody action (24,25), does not seem highly probable in view of the fact that the present data indicates that the immunosuppressive effect of MMS on antibody producing PFC does not coincide with the timing for the potentiating effect of the chemical.

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