PROTON IRRADIATION OF STEM CELLS: RADIATION DAMAGE AND CHEMICAL RADIOPROTECTION[1]

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Although many systems for studying radiobiological effects of high energy protons are available, much work has utilized whole mammals as the test system. Cellular level studies in mammals have included characterization of hematopoietic sequelae of total body exposure. In this study the effects of high energy protons on erythropoietic stem cells (erythropoietin responsive cells) and radioprotection by chemical agents have been measured.

The facilities of the NASA Space Radiation Effects Laboratory, Newport News, Virginia, were used. Carworth Farms CF-1 mice were exposed (five in tandem) to a parallel beam of 600 MeV protons. The fluence distribution was determined by ^{11}C foil counting. The fluence, when converted to dose, was referenced to the synchrocyclotron beam monitors which were then used to administer radiation exposures. Mice were given graded doses to 300 rads to determine the dose-response curve. Other mice received saline, AET, or 5-hydroxytryptamine 10-15 minutes before irradiation.

All mice received six units human urinary erythropoietin on day 2 following exposure, 0.5 μCi ^{59}Fe on day 4, and were killed on day 7. Percent iron incorporation was then determined as a measure of the fraction of stem cells remaining. Preliminary experiments, carried out as described, show a dose-response curve similar to that obtained with 250 kV X-rays indicating an RBE of approximately one. Chemical radioprotection can be shown under these conditions for both AET and 5-HT with doses well below the LD_{50} dose. Under the conditions of these experiments AET and 5-HT are equally effective and a dose reduction factor of 2.5 is obtained.

The specification of radiation tolerance doses for manned space missions requires quantitative information from sub-lethal doses. These experiments indicate that the stem cell system can be further utilized to obtain such information.

INTRODUCTION

In recent years radiation damage and chemical radioprotection have been studied for many types of radiation using a wide variety of biological endpoints. (ref. 1,2). One of these, the stem cell system, offers the opportunity of studying cellular radiation damage *in vivo*. Interest in the use of the stem cell compartment of the polycythemic mouse to study radiation effects has arisen for two principal reasons. First, studies in a cellular system *in vivo* provide information on basic mechanisms of radiation damage which cannot be obtained from lethality studies in a population of animals. Second, since it is primarily damage to the hematopoietic system which is responsible for the acute radiation syndrome, elucidation of the kinetics of normal and irradiated hematopoietic stem cells is of critical importance in radiobiology.

The transfusion-induced polycythemic mouse

was first shown to be ideally suited for *in vivo* studies of erythropoiesis by Jacobson *et al* (ref. 3). Subsequently Gurney and his co-workers showed that hypoxia could induce polycythemia (ref. 4) and that these mice could be used to study the effect, on the stem cell compartment *in vivo*, of X-rays (ref. 5, 6), gamma rays and neutrons (ref. 7).

In these studies mice are induced to stop elaboration of red cells by first producing polycythemia. This is accomplished by confinement for three weeks in a chamber at one-half atmosphere. By the fourth day following return to atmospheric pressure, the consequence of differentiation of stem cells (maturation of erythroblasts to mature red cells) is complete and erythropoiesis is nil (ref. 4). The status of the "static" stem cell compartment can then be ascertained by measuring the erythropoietic response to a constant dose of erythropoietin. Incorporation of a tracer dose of ^{59}Fe in red cells is used as a measure of erythropoietic response which in turn reflects the status of the stem cell compartment. Thus, the effect of radiation on the stem cell compartment *in vivo* and the ability of chemical radioprotective agents to modify that effect can be measured in this system.

Our unpublished experiments have shown that 2-aminoethylisothiouronium bromide (AET) protects stem cells from X-radiation. Recently, Vittorio *et al* (ref. 8, 9), using the stem cell compartment in polycythemic mice, have reported increased iron incorporation in blood, spleen, and liver when irradiation is preceded by administration of AET or serotonin (5-hydroxytryptamine, 5-HT). Using lethality as an endpoint, Oldfield *et al* (ref. 10) demonstrated protection in mice from 440 MeV protons with mercaptoethylamine (MEA) and p-aminopropiophenone (PAPP).

Since man may be exposed to protons in a space environment at doses which, although sublethal, may still produce hematological damage, we decided to examine the response of the hematopoietic stem cell system to high energy protons. This paper records preliminary observations on the effect of 600 MeV protons on stem cells and the modifying effect of AET and 5-HT.

METHODS AND MATERIALS

Irradiation and dosimetry - Animals were irradiated with 600 MeV protons obtained from the synchrocyclotron at the NASA Space Radiation Effects Laboratory, Newport News, Virginia. Mice were exposed (five in tandem) in 2.5 cm ID plastic tubes to a parallel beam of protons. The mice were placed in a hole within a block of lucite made from four slabs 2" X 8" X 24" each (Fig. 1). Beam position was determined photographically with Polaroid film.

The fluence distribution was determined by activation of ^{12}C in a polyethylene foil by the ^{12}C (n,np) ^{11}C reaction. The ^{11}C thus formed was measured in a scintillation spectrometer calibrated with a ^{22}Na standard. Fluence was converted to dose using the data of Neufeld *et al* (ref. 11). This dose was referenced to syncrocyclotron beam monitors and thermoluminescent dosimeters. The beam monitors were then used to deliver the nominal dose and the TLD's were used to measure the dose actually delivered.

Animals - Carworth Farms CF-1 virgin female mice twelve weeks of age were placed in hypoxia chambers at one-half atmosphere for three weeks. Four days after removal from the chamber 10-12

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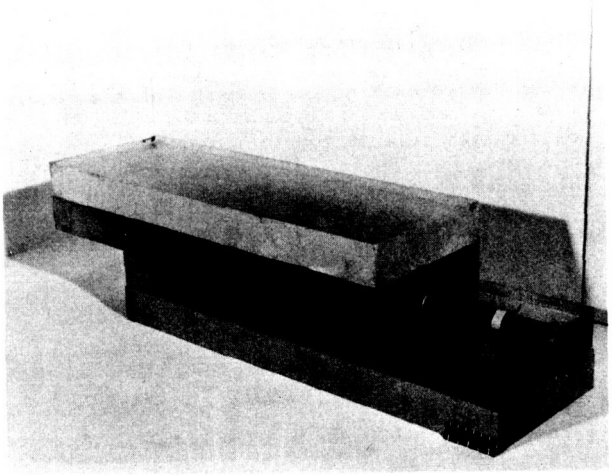

Figure 1. Proton exposure apparatus. The top half of the lucite block has been withdrawn to demonstrate the placement of the mice during proton irradiation. The beam was incident normally upon the 8 x 8 inch surface of the block. (Dark colored mice used for photography).

animals per group were exposed to specified doses of protons without injection or 10-15 minutes following intraperitoneal injection of 0.2 ml AET (360 mg/kg body weight), 5-HT (150 mg/kg body weight), or saline. The animals were housed ten per cage in shoe-box type cages and allowed Purina mouse chow and water ad libitum. On the second post-irradiation day each mouse received six units of human urinary erythropoietin subcutaneously. On day 4, they received intraperitonealy 0.5 μCi ^{59}Fe as ferric chloride. The mice were then killed on day 7 and the hematocrit, body weight, and percent iron incorporation in blood determined. Animals with hematocrits less than 55 were not included in the experiment because of the possibility of elevated radioiron incorporation due to endogenous erythropoietin production.

RESULTS

Radioiron incorporation in newly formed peripheral red cells of proton irradiated mice decreases with increasing radiation dose. Unirradiated control mice incorporate approximately 36% of the tracer dose of radioiron. Figure 2 shows the radioiron incorporation for mice irradiated with 600 MeV protons in two experiments. Values are normalized to 100% for controls. An ED_{50} of 65 rads is obtained from Figure 2. For comparison, the response of the stem cell compartment to 250 kV X-rays (ref. 12) is also shown.

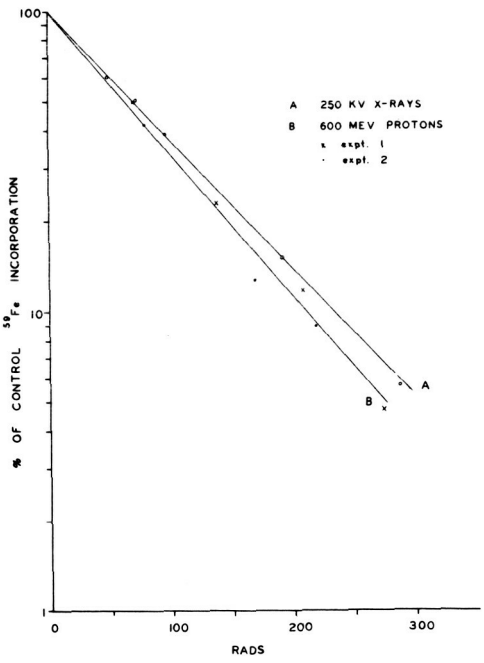

Figure 2. Iron incorporation in polycythemic mice following irradiation with protons or X-rays. Curve A: 250 kV X-rays (ref. 12). Curve B: 600 MeV protons.

Intraperitoneal injection of AET or 5-HT 10-15 minutes before proton irradiation yields higher iron incorporation than correspondingly unirradiated, untreated mice (Fig. 3). A slightly increased normalized iron incorporation in saline injected irradiated groups was observed. This was due to a slightly lower level of iron incorporation in the unirradiated, saline injected mice. Thus, the curve for saline injected mice does not differ significantly from uninjected controls.

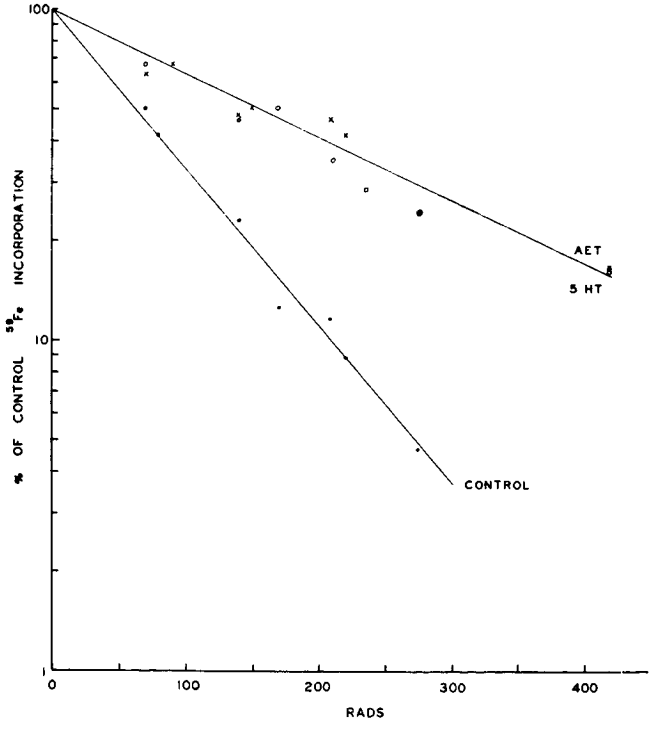

Figure 3. Modification of proton-induced radiation damage of stem cells by prior administration of AET or 5-HT. Solid circles, controls; open circles, AET; crosses, 5-HT.

DISCUSSION

From Figure 2 it is seen that 600 MeV protons are similar to 250 kV X-rays in their ability to damage the erythropoietin responsive or "stem" cell; therefore, an RBE of approximately unity can be assigned for protons of this energy in producing decreased iron uptake in hematopoietic stem cells. For this endpoint 600 MeV protons act as low LET radiation.

Both AET and 5-HT were effective in protecting the stem cell compartment from proton radiation damage. Oldfield et al (ref. 10) reported dose reduction factors (DRF) for mortality of approximately 1.5 for MEA and PAPP with both 440 MeV protons and X-rays. In our experiments with 600 MeV protons a DRF of 2.5 is obtained for iron incorporation in hematopoietic stem cells. This is comparable to the level of protection obtained in the same system by Vittorio et al (ref. 8) for ^{137}Cs gamma rays. Radioprotective chemicals are at best only slightly effective against exposure to high LET radiation. The high DRF of 2.5 obtained in these experiments further supports the observation from the dose-response curve that the major dose contribution from 600 MeV protons is low LET in nature.

Although considerable additional information is necessary to define radiation tolerance doses for space missions, this low LET behavior is significant. Low LET radiation shows a dose-rate dependence for most endpoints and part of

the damage it produces is reparable; in contrast, damage from high LET radiation is accumulated with little repair even at low dose-rates. In manned space missions the major portion of dose accumulated is expected to occur at low dose-rates. Since 600 MeV protons have been shown in these experiments to act at low LET radiation, a higher tolerance dose can be assigned for a given risk level than would be possible if the dose contribution from a high LET component were more preponderant. It appears to be possible, then, to consider the use of chemical radioprotective agents suitable for man for protection from high proton flux events.

The experiments reported here are being extended to include lower energy portions of the solar proton spectrum.

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