# Radioprotection by Phenylhydrazine: Evaluation of Stem Cell Radiosensitivity and Potential Stimulation of the Reticuloendothelial System[1] (37541)

L. H. SMITH AND T. W. MCKINLEY, JR.

*Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830*

Phenylhydrazine is radioprotective when administered to mice about 1 wk before X-irradiation. There are indications that protection is associated with changes in the number and distribution of hemopoietic stem cells (1, 2), but the possibility that other mechanisms are involved has not been ruled out. In the present study, we examined two factors which conceivably could enhance survival of irradiated mice given phenylhydrazine ($PhNHNH_2$) before exposure: (a) increased *in vivo* radioresistance of hemopoietic stem cells, and (b) stimulation of the reticuloendothelial system (RES) by drug-damaged red blood cells (RBC). The first factor would result in survival of a larger number of cells, and the second factor might improve defenses against bacterial infection, thereby providing sufficient time for surviving hemopoietic cells to regenerate a blood-forming system.

*Materials and Methods.* The mice were (C3H/Anf Cum ♀ × C57BL/Cum ♂)$F_1$ males 12–16 wk old. Irradiation procedures and administration of $PhNHNH_2$ (phenylhydrazine hydrochloride) have been described (1). Hemopoietic stem cells were assayed as endogenous spleen colony-forming units (CFU) on the parietal surface of the spleen (3). In some experiments mice were maintained in an environmentally controlled chamber (1 atm) at an $O_2$ concentration of 8%. This hypoxic environment was achieved by pumping into the chamber a mixture of room air with an appropriate amount of $N_2$ gas generated from liquid $N_2$.

*Results.* In vivo *CFU radiosensitivity.* Radiation inactivation curves for endogenous CFU in mice given saline (0.15 *M* NaCl) or 3 mg of $PhNHNH_2$ 7 days before whole-body X-irradiation are shown in Fig. 1. Statistical analysis of the least-squares calculations shows that the respective slopes and therefore the $D_{37}$'s are not significantly different ($p > 0.4$). Thus, radioprotection by $PhNHNH_2$ is not the result of increased radioresistance of hemopoietic stem cells assayed as endogenous CFU.

Data in Fig. 1 also show that the survival curve for endogenous CFU of $PhNHNH_2$-treated mice was displaced to the right of the control curve. Thus, enhanced hemopoietic recovery occurred in the drug-treated mice, and over the 600–900 R exposure range, mice given $PhNHNH_2$ had $\sim$ 7 times more endogenous CFU than controls.

*Indirect evaluation of RES stimulation.* Two approaches were used to test the possibility that RES stimulation is involved in radioprotection by $PhNHNH_2$. This drug evokes marked proliferation of the RES (4) as a result of drug-damaged RBC. We reasoned that injection of $PhNHNH_2$-damaged RBC would also be expected to stimulate the RES in a manner similar to that evoked by injection of heat-damaged RBC (4). Accordingly, 3 mg of $PhNHNH_2$ was injected intraperitoneally into mice, and 1, 6, 18, 24, 48, or 72 hr later blood was collected by cardiac puncture. One milliliter of this blood was injected intravenously into mice which were exposed to 850 R 7 days thereafter. None of the 120 mice (20 mice per interval) injected with blood survived 30 days, so any RES stimulation that occurred was not sufficient to increase radioresistance 7 days after injection of drug-damaged RBC.

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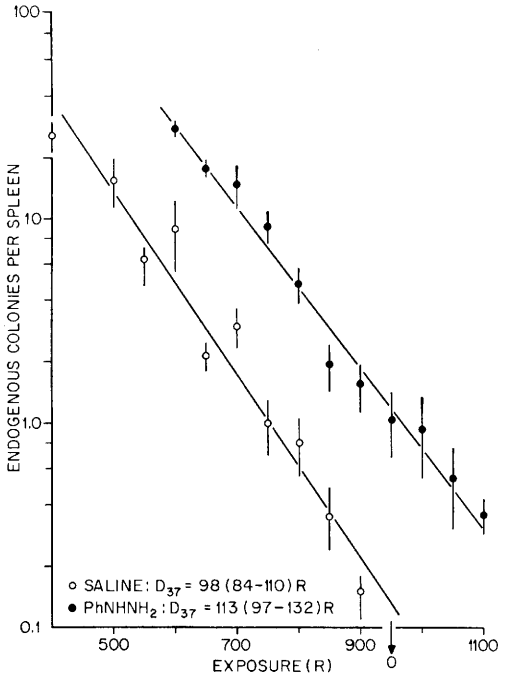


FIG. 1. Endogenous CFU as a function of whole-body X-ray exposure in mice given either 3.0 mg of $PhNHNH_2$ (●) or 0.5 ml of saline (○) 7 days before irradiation. Ten spleens per point.

The second approach involved suppression of $PhNHNH_2$-induced hypererythropoiesis. This approach was predicated on results which suggest a relationship between erythropoiesis and radioprotection by $PhNHNH_2$ (1, 2). If a factor other than induced hypererythropoiesis, *e.g.*, RES stimulation, were involved in radioprotection by $PhNHNH_2$, offsetting hypererythropoiesis would not be expected to diminish radioprotection by the drug. We attempted to offset erythropoietic stimulation by two different procedures. In one type of experiment, mice were injected intravenously with twice-washed isogenic RBC from normal mice at intervals after injection of $PhNHNH_2$. In the second type of experiment polycythemia was achieved by keeping mice in a hypoxic chamber for 11 days, and 3 days after removal from hypoxia (hematocrits 60–65) these mice were injected with $PhNHNH_2$. For both types of experiments, the mice were irradiated 7 days after drug injection. Results showed that protection was reduced by RBC transfusion and that survival was inversely related to the size of the RBC mass injected (Table I). Hematocrit data indicate that RBC injection increased the circulating RBC mass in proportion to the size of the RBC injections. However, even two injections of 0.7 ml of RBC did not completely restore hematocrits to normal, and therefore some erythropoietic stimulation could still have occurred in this group as suggested by the fact that slight (12% survival) protection was observed. In mice made polycythemic by hypoxia, the radioprotective effect of $PhNHNH_2$ was also markedly reduced. Of 20 hypoxia polycythemic mice given 3.0 mg of the drug, only 3 survived 30 days after exposure to 850 R, whereas 17 out of 20 normoxic drug-injected controls survived, 0 out of 20 hypoxic vehicle-injected mice survived, and none of the 20 irradiated controls survived.

*Discussion.* The present results show that radioprotection by $PhNHNH_2$ cannot be explained by increased *in vivo* radioresistance of hemopoietic stem cells, because the $D_{37}$ for endogenous CFU in drug-treated mice was almost the same as that for vehicle-treated animals. These results corroborate our previous observations on the *in vitro* radiosensitivity of exogenous CFU from PhNH $NH_2$-treated mice (2).

We considered stimulation of the RES to be a possible contributor to radioprotection by $PhNHNH_2$ solely because altered RBC are known to activate this system (4). The observation that 30-day survival was not enhanced in irradiated mice given blood from $PhNHNH_2$-treated donors implies that the RES is not involved in radioprotection by the drug. This implication is strengthened further by the fact that the degree to which erythropoiesis is spoiled by injection of normal RBC correlates inversely with radioprotection (Table I), and there is no reason to believe that transfusion of normal RBC would prevent $PhNHNH_2$-induced RES stimulation. The data, however, do not discount the possibility that radioprotection by $PhNHNH_2$ involves synergism between RES stimulation and hypererythropoiesis.

The present results do not explain the basis for radioprotection by $PhNHNH_2$, al-

TABLE I. Effect of Graded Doses of Isogenic RBC on Radioprotection by $PhNHNH_2$.

| Treatment[a] | | | | | |
|---|---|---|---|---|---|
| Initial injection | Subsequent iv injections[b] | | 30-Day survival (%) | No. mice treated | Hematocrit[c] (± SE) |
| | Day 1 | Day 2 | | | |
| V | V | V | 0 | 58 | 47.4 ± 0.9 |
| $PhNHNH_2$ | V | V | 93 | 45 | 29.1 ± 0.7 |
| $PhNHNH_2$ | RBC (0.5) | None | 75 | 20 | 33.8 ± 0.6 |
| $PhNHNH_2$ | RBC (0.5) | RBC (0.5) | 50 | 24 | 38.9 ± 1.2 |
| $PhNHNH_2$ | RBC (0.7) | RBC (0.7) | 12 | 25 | 44.4 ± 1.1 |
| V | RBC (0.5) | None | 0 | 20 | 52.8 ± 1.4 |
| V | RBC (0.5) | RBC (0.5) | 8 | 24 | 58.7 ± 1.4 |
| V | RBC (0.7) | RBC (0.7) | 0 | 15 | 68.9 ± 1.1 |

[a] Mice exposed to 850 R 7 days after initial intraperitoneal injection of 0.5 ml of saline vehicle (V) or 3.0 mg of $PhNHNH_2$.

[b] Numbers in parentheses indicate volume (ml) of washed packed RBC from normal mice.

[c] Determined on Day 4 after $PhNHNH_2$ or saline vehicle (V) injection for about one-half of the mice in each group.

though from past (2) and present data, it appears that the major if not the only factor associated with protection by this drug is a consequence of hypererythropoiesis. This conclusion is supported by the fact that other methods for inducing hypererythropoiesis, *i.e.*, hypoxia or bleeding, are also radioprotective (6–9). How induction of a hypererythropoietic state is translated to radioprotection is questionable, although we have favored the explanation that translation involves an increased number of hemopoietic stem cells at risk at the time of irradiation (2). An alternative explanation is that the growth of hemopoietic cells can be augmented after irradiation as a result of a preirradiation stimulus or condition. Hanks and Ainsworth (9), working with the biostimulant radioprotector endotoxin, discussed several possibilities that could explain augmentation. These possibilities are also applicable to radioprotection by $PhNHNH_2$ and include the following factors related to hemopoietic stem cells: redistribution, proliferation rate, selective differentiation, division delay, repair, and activation of dormant cells. Any one of these could be involved in an earlier onset of cell proliferation after irradiation. Also, it is conceivable that in mice subjected to a hemopoietic stimulus before irradiation, a residual heightened sensitivity of hemopoietic cells would permit a quicker response to stimuli resulting from radiation-induced hemopoietic depression. The earlier response would lead to faster hemopoietic regeneration, thus giving an animal a better change for survival.

*Summary.* The evidence presented indicates that radioprotection by phenylhydrazine is not associated with an increase in the *in vivo* radioresistance of hemopoietic stem cells (measured as endogenous spleen colony-forming units) nor with stimulation of the reticuloendothelial system. When the erythropoietic-stimulating effect of phenylhydrazine was quantitatively reduced, radioprotection was also reduced. This finding indicates that protection depends on induction of a hypererythropoietic state by the drug.

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