Drug Toxicity in Mice Exposed to Mixed Gamma-Neutron Radiations (33656)

LAWRENCE W. DAVIS AND THOMAS A. STRIKE
(Introduced by E. Weiss)

Armed Forces Radiobiology Research Institute, Defense Atomic Support Agency, Bethesda, Maryland 20014

The use of medication in the treatment of the symptoms associated with radiation injury has generally been based on the assumption that the responses to the drugs are unaltered. Evidence from radiation accidents involving humans (1, 2) and from animal experiments (3–7) indicates that this assumption may not be valid for all drugs. Further study of drug response in irradiated animals is needed. The current study not only evaluated one aspect of drug response (drug toxicity) in irradiated mice but also evaluated the effect of radiation dose and postirradiation time on this response by determining the acute toxicity of 10 drugs (members of four drug classes) at selected times following 500, 1000, and 10,000-rad doses of mixed gamma-neutron radiations. The results of these studies are the subject of this report.

Material and Methods. A total of 6500 male CF_1 mice, 5–6 weeks old and weighing 18–28 g, was used. The animals were housed 3 or 4 per cage in environment-controlled rooms and were conditioned for a minimum of 1 week. Food and water were available *ad libitum*. The water, pH 2.8, was acidified with hydrochloric acid to control the post irradiation septicemia caused by *Pseudomonas aeruginosa* (8).

Mice were unilaterally exposed to mixed gamma-neutron radiations from the AFRRI-TRIGA reactor. Uniformity of the radiation field in air at the position occupied by the midline of the animals of each exposure group varied less than 4% from the mean. Depth dose measurements made in cylindrical phantoms constructed from Plexiglas rods indicated that the irradiations were Class A uniform (9). Dose rates were selected so that all exposure times were 10 min. Midline tissue doses of 500 (approximately the LD_{50} dose), 1000, and 10,000 rads were used. The exposure methods, dosimetric techniques, and reactor characteristics utilized in this investigation have been previously described.[1,2]

The following drug classes and drugs were used in this study: anticonvulsants—diphenylhydantoin, phenobarbital, and mephenytoin; hypnotics—barbital, pentobarbital, and hexobarbital; hypoglycemic-tolbutamide; psychopharmacologics—chlorpromazine, triflupromazine, and chlordiazepoxide. All drugs were administered by intraperitoneal injection. The volume of solution injected per gram of body weight was kept constant for each drug by adjusting the concentration of the solutions. (Mephenytoin was dissolved in an aqueous solution containing 80% propylene glycol by volume; all other drugs were dissolved in sterile water.) Drug doses were selected which would make calculation of the dose–response curve possible (LD_1 to LD_{99}). The end point for toxicity was death within 24 hr following drug injection. The mice used in each drug study were divided into 7 groups and tested as follows: unirradiated controls; 500-rad groups on day 0 (drug administered 2–3 hr post-irradiation), day 1, or day 6; 1000-rad groups on day 0 or day 1; 10,000-rad group on day 0. Each of the groups contained a minimum of 80 mice which were divided into drug dose subgroups. Each subgroup received one of a graded series of drug doses. A given mouse

[1] Strike, T. A., Seigneur, L. J., and Stanley, R. E. Acute mortality of mice and rats exposed to mixed gamma-neutron radiations or to X-rays, Bethesda, Maryland, Armed Forces Radiobiology Research Institute, Scientific Report SR 68-6 (1968).

[2] Sayeg, J., Compiler, Report on neutron and gamma dosimetry measurements at the AFRRI-DASA TRIGA reactor, Santa Barbara, California, Edgerton, Germeshausen and Grier, Inc., Report S-260 - R (1964).

TABLE I. Acute Toxicity of Anticonvulsants in Irradiated Mice.

Midline tissue dose (rads)	Postirradiation day tested	Diphenylhydantoin		Phenobarbital	Mephenytoin	
		LD_{50} (mg/kg)[a]	Relative potency[b]	LD_{50} (mg/kg)	LD_{50} (mg/kg)	Relative potency
None	—	264 (240–294)	—	223 (212–235)	258 (243–275)	—
500	0	191[c] (167–217)	0.7 (0.6–0.9)	214 (204–224)	225[c] (213–237)	0.9 (0.8–0.9)
500	1	212[c] (191–236)	0.8 (0.7–0.9)	248[c] (238–261)	256 (240–274)	1.0 (0.9–1.1)
500	6	232 (197–274)	0.9 (0.7–1.1)	180[c] (169–195)	215[c] (200–229)	0.8 (0.8–0.9)
1000	0	212[c] (190–237)	0.8 (0.7–0.9)	184[c] (176–189)	284 (265–307)	1.1 (1.0–1.2)
1000	1	241 (217–268)	0.9 (0.8–1.1)	211 (202–223)	264 (248–282)	1.0 (0.9–1.1)
10,000	0	150[c] (130–169)	0.6 (0.5–0.7)	166[c] (150–178)	272 (250–298)	1.1 (0.9–1.2)

[a] 95% confidence limits in parentheses.

[b] Ratio of equally effective doses of the irradiated group to the controls (95% confidence limits in parentheses).

[c] $p < .05$.

was used only once in the study. In addition to the mice used for the toxicity studies, a group of mice were simultaneously exposed to each radiation dose and then injected with saline. These latter mice totaled 360 and served as controls to characterize radiation-induced deaths.

Toxicity of the drugs was analyzed by the methods of Finney (10) using a digital computer. The regression line of log dose on the probit of the proportion of animals dying at a given drug dose was calculated by the maximum likelihood method. Each regression line was tested for significance of regression using an F test and each was judged significant at the .05 level. The median lethal dose, LD_{50}, and its 95% confidence limits were calculated for each fitted regression line. The toxicity data on each drug yielded 7 regression lines which were tested for parallelism. If the lines were parallel, the pooled data was used to calculate the appropriate slope for the 7 regression lines. The LD_{50} values and their 95% confidence limits were recalculated using this new slope then compared by calculating relative potency values (by dividing the LD_{50} for each irradiated group by the LD_{50} for unirradiated controls then calculating 95% confidence limits). Since the regression lines for a given drug were parallel, the relative potency gives the ratio for all levels of equally toxic doses. (A relative potency of less than 1.0 indicates a more toxic drug.) If the 95% confidence limits of the relative potency value did not include 1.0, the toxicity was different from the unirradiated controls at the 0.05 probability level.

The data for phenobarbital and chlordiazepozide did not yield parallel regression lines. The LD_{50} values for the 7 regression lines for each of these two drugs were compared by Scheffe's method of testing contrasts of means (11). Since these lines were not parallel, only the LD_{50} values of the regression lines were compared.

Results. The LD_{50} and relative potency values and their 95% confidence limits for the anticonvulsant, hypnotic, hypoglycemic, and psychopharmacologic agents are summarized in Tables I, II, III, and IV, respectively.

The anticonvulsants generally were more toxic in irradiated mice than in controls, and these changes were significant at a number of times tested. Several exceptions exist to this trend of increased toxicity, but the altered toxicity of phenobarbital when tested on day 1 in the mice receiving 500 rads was the only significant exception.

The toxicity of the hypnotics generally was unchanged in irradiated mice except on day 6 following 500 rads. At that testing time, all three drugs appeared more toxic, but this change was only significant with hexobarbital.

The toxicity of tolbutamide was unchanged

TABLE II. Acute Toxicity of Hypnotics in Irradiated Mice.

Midline tissue dose (rads)	Post-irradiation day tested	Barbital		Pentobarbital		Hexobarbital	
		LD_{50} (mg/kg)[a]	Relative potency[b]	LD_{50} (mg/kg)	Relative potency	LD_{50} (mg/kg)	Relative potency
None	—	498 (478–519)	—	117 (96–138)	—	254 (236–273)	—
500	0	501 (466–539)	1.0 (0.9–1.1)	114 (108–119)	0.9 (0.9–1.0)	256 (237–276)	1.0 (0.9–1.1)
500	1	540 (507–579)	1.1 (1.0–1.2)	136 (117–159)	1.2 (0.9–1.5)	234 (216–253)	0.9 (0.8–1.0)
500	6	441 (413–468)	0.9 (0.8–1.0)	90 (73–107)	0.8 (0.6–1.0)	197[c] (183–210)	0.8 (0.7–0.8)
1000	0	485 (456–516)	1.0 (0.9–1.0)	111 (95–129)	0.9 (0.8–1.2)	252 (234–272)	1.0 (0.9–1.1)
1000	1	515 (484–551)	1.0 (1.0–1.1)	116 (99–134)	1.0 (0.8–1.3)	232 (216–249)	0.9 (0.8–1.0)
10,000	0	457 (424–489)	0.9 (0.8–1.0)	128 (110–148)	1.1 (0.9–1.4)	263 (244–285)	1.0 (0.9–1.1)

[a] 95% confidence limits in parentheses.
[b] Ratio of equally effective doses of the irradiated group to the controls (95% confidence limits in parentheses).
[c] $p < .05$.

TABLE III. Acute Toxicity of the Hypoglycemic, Tolbutamide, in Irradiated Mice.

Midline tissue dose (rads)	Post-irradiation day tested	Tolbutamide	
		LD_{50} (mg/kg)[a]	Relative potency[b]
None	—	1138 (1096–1181)	—
500	0	1106 (1057–1156)	1.0 (0.9–1.0)
500	1	1185 (1132–1240)	1.0 (1.0–1.1)
500	6	1039 (988–1088)	0.9 (0.9–1.0)
1000	0	1213 (1173–1256)	1.1 (1.0–1.1)
1000	1	1040 (1006–1075)	0.9 (0.9–1.0)
10,000	0	995[c] (963–1027)	0.9 (0.8–0.9)

[a] 95% confidence limits in parentheses.
[b] Ratio of equally effective doses of the irradiated group to the controls (95% confidence limits in parentheses).
[c] $p < .05$.

in irradiated mice when compared to unirradiated controls with one exception. The drug was more toxic in the mice receiving 10,000 rads.

The psychopharmacologic drugs were less toxic on days 0 and 1 following 500 rads but had normal or increased (chlorpromazine) toxicity by day 6. These drugs also tended to be less toxic in the mice receiving 1000 rads but more toxic in those receiving 10,000 rads.

Discussion. Radiation-induced death is commonly ascribed to one of three mechanisms depending on the dose received. These are hematopoietic failure, gastrointestinal mucosal loss, and central nervous system changes. Each of the three doses selected for the current study results in death by one of the three mechanisms. The 500-rad dose causes hematopoietic depression, and radiation induced deaths do not occur in the first week following irradiation. The 1,000-rad dose causes gastrointestinal mucosal loss, and deaths usually begin on the third post-irradiation day. The 10,000-rad dose causes central nervous system changes and death occurs 2 or 3 days postirradiation. The post-irradiation times selected for drug toxicity testing should not have radiation induced deaths, and this was confirmed in the irradiated mice injected with saline. One might expect the toxicity of drugs to differ among

TABLE IV. Acute Toxicity of Psychopharmacologics in Irradiated Mice.

Midline tissue dose (rads)	Postirradiation day tested	Chlorpromazine		Triflupromazine		Chlordiazepoxide
		LD_{50} (mg/kg)[a]	Relative potency[b]	LD_{50} (mg/kg)	Relative potency	LD_{50} (mg/kg)
None	—	188 (171–206)	—	213 (190–241)	—	230 (223–238)
500	0	219 (201–240)	1.2 (1.0–1.3)	219 (197–244)	1.0 (0.9–1.2)	275[c] (258–302)
500	1	229[c] (210–251)	1.2 (1.1–1.4)	215 (194–241)	1.0 (0.9–1.2)	299[c] (282–327)
500	6	134[c] (119–150)	0.7 (0.6–0.8)	183 (164–203)	0.9 (0.7–1.0)	204 (178–236)
1000	0	217 (199–238)	1.2 (1.0–1.3)	193 (173–215)	0.9 (0.8–1.1)	257[c] (233–282)
1000	1	259[c] (235–286)	1.4 (1.2–1.6)	243 (218–272)	1.1 (1.0–1.3)	286[c] (263–323)
10,000	0	168 (153–184)	0.9 (0.8–1.0)	169[c] (148–189)	0.8 (0.7–0.9)	235 (211–254)

[a] 95% confidence limits in parentheses.

[b] Ratio of equally effective doses of the irradiated group to the controls (95% confidence limits in parentheses).

[c] $p < .05$.

the animals of the three radiation dose groups since the type of radiation injury differs in the three groups, and this was confirmed by the results.

All drugs were administered parenterally to avoid erratic drug absorption due to radiation induced gastrointestinal changes. Therefore, altered toxicity values should not be due to decreased drug absorption.

Other authors have investigated some aspects of drug toxicity. Frik (4) studied the toxicity of a number of drugs in mice, including four of those reported here, on day 4 following 500 rads of X-irradiation. He found an increased toxicity of phenobarbital and chlorpromazine, a decreased toxicity of chlordiazepoxide, and no change in the toxicity of hexobarbital. He concluded that, in general, drugs showing increased toxicity are those which act on the central nervous system. Frik did not study the mechanism of altered drug toxicity but thought that these changes were not due to altered membrane permeability alone since some drugs which act on the central nervous system had a decreased toxicity.

Danysz (12) studied the toxicity of several drugs which act on the autonomic nervous system. Mice receiving 200 rads or less of X-irradiation were used at the peak of radiation sickness. He reported that parasympathomimetics and sympatholytics tended to be less toxic, while parasympatholytics had increased toxicity. Danysz considered these changes in drug toxicity to be due to altered sensitivity of the receptor sites in the autonomic nervous system.

In the current study, except for the anticonvulsants, the drugs were less or equally toxic in irradiated mice on days 0 and 1 following 500 rads as in unirradiated controls. The anticonvulsants tended to be more toxic in this early period. However, by the sixth postirradiation day, there was a tendency toward increased toxicity of all drugs in the irradiated mice. This latter time is during the period of hematologic depression which may contribute to the toxicity of the drugs.

The anticonvulsants were generally more toxic in the mice receiving 1000 and 10,000-rad doses. This change supports the view that increased toxicity is likely in drugs which affect the central nervous system. However, the hypnotics showed no toxicity change, and the psychopharmacologics were less toxic in mice receiving 1000-rad doses. These changes contradict the theory.

Alteration of drug toxicity occurs in irradiated animals receiving sublethal to supralethal radiation doses. These radiation doses result in varying degrees of injury and death by different mechanisms. Undoubtedly the changes responsible for altered drug toxicity are complex and represent the end result of several mechanisms. The current study did not attempt to investigate these

mechanisms. The significance of these toxicity changes on drug safety can only be judged by comparing the altered toxicity values to the therapeutic doses in irradiated animals.

Conclusions. (i) Drug toxicity is altered in irradiated mice. (ii) The toxicity changes are related to both radiation dose and postirradiation time. (iii) The significance of these toxicity changes on drug safety cannot be evaluated without information concerning alterations of therapeutic doses in irradiated animals.

Summary. The acute toxicity (death within 24 hr) of 10 drugs representing four drug classes (anticonvulsants, hypnotics, hypoglycemics, and psychopharmacologics) was studied in irradiated male CF_1 mice 2 hr, 1 and 6 days after 500 rad; 2 hr and 1 day after 1000 rad; and 2 hr after 10,000 rad whole-body doses of mixed gamma-neutron radiations. The LD_{50} value for each drug in irradiated mice was calculated and compared to the LD_{50} value obtained in unirradiated controls. The LD_{50} values for most drugs studied were altered significantly in some of the groups of irradiated mice. All drugs tended to be more toxic in irradiated mice on the sixth day following 500 rads. In general, the anticonvultants were more toxic in mice receiving 1000 and 10,000-rad doses, while the psychopharmacologics were less toxic in the mice receiving 1000 rads but more toxic in those receiving 10,000-rad doses.

The authors express their appreciation to the following persons for supplying many of the drugs used in the study: Dr. A. C. Bratton, Jr., Parke, Davis & Co.; Dr. J. H. Trapold, Sandoz Pharmaceuticals; Dr. Paul O'Connell, The Upjohn Co.; Miss Suzanne Moyer, Smith, Kline & French Labs; Miss Barbara Stearns, The Squibb Institute for Medical Research; Dr. W. E. Scott, Hoffman-LaRoche Inc.

1. Karas, J. S. and Stanbury, J. B., New Engl. J. Med. **272**, 755 (1965).

2. Shipman, T. L., "Proceedings of a scientific meeting sponsored by International Atomic Energy Agency and World Health Organization, Geneva, Oct. 17-21, 1960." Columbia Univ. Press, New York (1961).

3. Doull, J., Radiation Res. **30**, 333 (1967).

4. Frik, W., Strubelt, O., and Wittman, K., Strahlentherapie **127**, 245 (1965).

5. Nair, V., Finer, S., and Shah, D., Proc. Soc. Exptl. Biol. Med. **120**, 246 (1965).

6. Nair, V., Radiation Res. **30**, 359 (1967).

7. Zauder, H. L. and Orkin, L. R., Anesthesiology **21**, 119 (1960).

8. McPherson, C. W., Lab. Animal Care **13**, 737 (1963).

9. International Commission on Radiological Units and Measurements (ICRU) Natl. Bur. Std. (U. S.), Handbook **88**, (1963).

10. Finney, D. J., "Probit Analysis." Cambridge Univ. Press, London and New York (1962).

11. Scheffe, H., Biometrika **40**, 87 (1953).

12. Danysz, A., Proniewski, H., Wisnieueskr, K., Zacaek, T., and Polochi, B., Sb. Ved. Praci **6**, 11 (1963).

Received Oct. 28, 1968. P.S.E.B.M., 1969, Vol. 130.