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RADIOPROTECTION WITH 2-(1-DECYLAMINO) ETHANETHIOSULFURIC ACID IN THE RHESUS MONKEY

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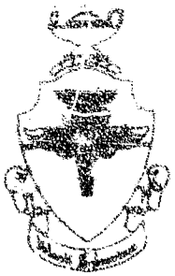

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FOREWORD

This study was carried out under task No. 775703 in the Radiobiology Division. The data were obtained from February to May 1968. The paper was written to fulfill the research requirement of Phase II of the Residency in Aerospace Medicine; it was submitted for publication on 10 July 1968. The statistical analysis of the data was performed by personnel of the Biometrics Branch.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

This report has been reviewed and is approved.

GEORGE E. SCHAFER
Colonel, USAF, MC
Commander

ABSTRACT

An attempt was made to provide radioprotection of the *Macaca mulatta* primates by the use of 2-(1 decylamino) ethanethiosulfuric acid, a long chain, lipid-soluble compound. The animals were divided into seven groups: two drug control groups, one radiation control group, and four treatment groups. Each treatment group received a different dosage of drug or had a different latent period between administration of the drug and total-body irradiation. Clinical parameters were monitored and one animal from three of the treatment groups as well as two drug controls were necropsied and examined for signs of radiation damage or drug toxicity. Survival times were compared between treated animals and controls. Results indicate that this compound, given as described, does not afford the predicted degree of radioprotection.

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I. INTRODUCTION

Previous studies have shown that a degree of radioprotection can be conferred by sulfur or sulfhydryl-containing chemicals, both in rodents (2) and in primates (1, 8).

This report details the morbidity, mortality, and pathologic findings among a group of primates in which protection was attempted by the prior administration of 2-(1 decylamino) ethanethiosulfuric acid. This drug, which was synthesized at the Walter Reed Army Institute of Research and which will hereafter be referred to as WR 1607, differs from previous radioprotective agents by virtue of its decyl chain and resulting lipid solubility. It was postulated that this lipid solubility might enhance the protective effect of the drug on the lymphoid system by preferential distribution.

WR 1607 has previously proved to be of low toxicity in rodents (I.P., LD_{50}: 17 mg./kg.) (15), and to be of value in the radioprotection of rodents (15).

II. MATERIAL AND METHODS

The monkeys used were of the *Macaca mulatta* strain of rhesus monkey and ranged in weight from 6.4 to 8 lb. The animals were standardized by means of fecal smears and cultures, physical examination, and cage acclimatization (4, 18). Additional hematology, chemistry, and temperature and weight studies were also performed and will be reported in a separate study.

The 32 animals were randomly divided into seven groups. Four animals were irradiated at 850 R (LD_{100}: 850 R) (8) as radiation controls, 4 animals were utilized as drug controls (10 mg./kg.), and 4 animals served as a second drug group (20 mg./kg.). The remaining 20 animals were divided into four treatment groups of 5 animals each.

Whole-body irradiation was performed with a Maxitron 300 x-ray unit at 300 kvp, 18 ma., with standard Al-Cu-Sn filtration; half-value layer of copper, 2 mm. Dosimetry was by means of Victoreen ion chambers and rate meters. The dose rate was 18 ± 2 R/min. and the exposure cage was rotated at 3 r.p.m.

The WR 1607 was obtained in powder form from the Walter Reed Army Institute of Research and was placed in solution using Carbonax 200 as the vehicle. Recovery of the drug from solution and subsequent analysis revealed no change in structure.

The first group of treated animals received a dose of 10 mg./kg. administered 1 hour prior to irradiation; the second group received 10 mg./kg. 3 hours prior to irradiation; the third group received 20 mg./kg. 1 hour prior to irradiation; and the fourth group received 20 mg./kg. 3 hours prior to irradiation. All injections were intraperitoneal following sterile preparation.

The dosage and delay period were chosen on the basis of previous toxicity studies conducted by the Woodard Research Corporation (15) and on studies conducted in the Radiobiology Division, USAF School of Aerospace Medicine (7).

Two drug control animals, one from each group, were sacrificed after three days for

TABLE I

Survival times of experimental groups

Group and animal No.	Survival time (days)	Experimental condition
Group I		
33B*	3	Drug control group, 10 mg./kg. WR 1607. No irradiation.
88L	30	
70M	30	
82M	30	
Group II		
1K3*	3	Drug control group, 20 mg./kg. WR 1607. No irradiation.
02P	30	
42N	30	
94J	30	
Group III		
20K	19	Treatment group, 10 mg./kg. WR 1607, 1 hour prior to 850 R.
00N	8	
94M	9	
40L	3	
00P	30	
Group IV		
42P	15	Treatment group, 10 mg./kg. WR 1607, 3 hours prior to 850 R.
12K	10	
36N	10	
52N	9	
48N	12	
Group V		
48K	11	Treatment group, 20 mg./kg. WR 1607, 1 hour prior to 850 R.
34P	13	
46P	12	
44M	6	
28J	12	
Group VI		
10P	30	Treatment group, 20 mg./kg. WR 1607, 3 hours prior to 850 R.
30N	5	
90J	9	
48J	12	
26P	2	
Group VII		
24N	13	Radiation control group. Exposed to 850 R with no treatment.
16N	13	
46J	14	
32K	24	

*Sacrificed at 3 days for tissue study.

necropsy and tissue study. One animal from three of the four treatment groups was also necropsied immediately after death.

All animals were monitored closely for evidence of drug toxicity or radiation damage (3, 6, 12, 14).

The survival time, calculated in days, was used in comparing the treated animals with the untreated, irradiated controls.

Parametric (analysis of variance) and non-parametric (Kruskal-Wallis) (16) statistical tests were performed to determine if there were any significant statistical differences in mean survival time among the five groups of animals.

III. RESULTS

Table I summarizes the survival of the drug controls who received 10 mg./kg. (group I); drug controls who received 20 mg./kg. (group II); treated animals receiving 10 mg./kg. 1 hour prior to irradiation (group III); animals receiving 10 mg./kg. 3 hours prior to irradiation (group IV); animals receiving 20 mg./kg. 1 hour prior to irradiation (group V); animals receiving 20 mg./kg. 3 hours prior to irradiation (group VI); and the 4 irradiated controls who received no treatment (group VII).

Table II shows the results of mean survival times calculated both with the inclusion and exclusion of the 2 thirty-day survivors, groups III and VI.

If the 2 surviving animals are given thirty days as their survival time, then the four mean survival times for the treated groups are all less than the mean survival time for the control group. From these data there is no indication that the treatment increased survival time. (The mean survival time of the control group falls within previously established survival times for untreated animals at 850 R (7).)

Observing 2 thirty-day survivors out of 20 treated animals as opposed to 0 out of 4 control animals does not indicate a statistically significant difference in percent of thirty-day survivors between the treated and control groups.

If the 2 thirty-day survivors are omitted from the analyses, then the statistical testing indicates that the mean survival time for the treated group is significantly ($P < .025$) smaller than for the control group.

Tables III through VIII summarize the clinical history of all groups. The ratings were derived by duration and severity of symptoms using guidelines as expounded by Reid et al. (14).

Symptoms and signs included anorexia, diarrhea, bloody diarrhea, inactivity, abdominal welts at injection site, petechial hemorrhages, dehydration, and epilation. As can be seen, the symptoms noted in the drug controls consisted primarily of mild anorexia, inactivity, and abdominal welts. The signs and symptoms

TABLE II

Mean survival times of experimental groups

Group	Including 30-day survivors (days)	Excluding 30-day survivors (days)
Radiation controls	16.0	16.0
10 mg./kg., 1 hour	13.8	9.8
10 mg./kg., 3 hours	11.2	11.2
20 mg./kg., 1 hour	10.8	10.8
20 mg./kg., 3 hours	11.6	7.0

S.D. — 4.42.

TABLE III

Clinical evaluation of drug controls

Clinical signs and symptoms	10 mg./kg.				20 mg./kg.			
	33B*	1K8	88L	70M	82M	02P	42N	94J
Diarrhea								
Bloody diarrhea								
Anorexia	+		+			+	+	+
Inactivity	+					+	+	+
Epilation								
Petechiae								
Dehydration								
Welt at injection site	+					+	+	+++

*Animal number.

TABLE IV

Clinical evaluation of radiation controls

Clinical signs and symptoms	24N*	16N	43J	32K
Diarrhea	+++	+++	+++	+++
Bloody diarrhea	+++			+++
Anorexia	++	++	++	+++
Inactivity	+++	++	+++	+++
Epilation			++	++
Petechiae				+++
Dehydration			++	
Welt at injection site	+			

*Animal number.

TABLE V

Clinical evaluation of treatment given 10 mg./kg. 1 hour before irradiation

Clinical signs and symptoms	20K*	00N	94M	40L	00P
Diarrhea	+++	+++	+++		++
Bloody diarrhea		+++			
Anorexia	++	+++	+++	+++	++
Inactivity	++++	+++	++	++++	++
Epilation					++++
Petechiae		++++	++++		
Dehydration	+++				
Welt at injection site	+		+		

*Animal number.

TABLE VI

Clinical evaluation of treatment given 10 mg./kg. 3 hours before irradiation

Clinical signs and symptoms	42P*	12K	36N	52N	48N
Diarrhea	+++	+	++	+++	+++
Bloody diarrhea					++
Anorexia	++	++	++	+++	+++
Inactivity	++	++	+	+++	+++
Epilation	+				+
Petechiae	+++				+
Dehydration			+	++	+
Welt at injection site		+			

*Animal number.

TABLE VII

Clinical evaluation of treatment group given 20 mg./kg. 1 hour before irradiation

Clinical signs and symptoms	48K*	34P	46P	44M	28J
Diarrhea	++	++	+++	++	+++
Bloody diarrhea					
Anorexia	+	+++	+	+++	+++
Inactivity	+	+++	+	+++	+++
Epilation			+		
Petechiae	++				
Dehydration		+	+		
Welt at injection site				+	

*Animal number.

TABLE VIII

Clinical evaluation of treatment group given 20 mg./kg. 3 hours before irradiation

Clinical signs and symptoms	10P*	30N	90J	48J	26P
Diarrhea	+++	++	+++	+	+
Bloody diarrhea					
Anorexia	+++	+++	+++	+++	+
Inactivity	++	+++	+++	+++	++
Epilation	+++				
Petechiae	+				
Dehydration	+		+		
Welt at injection site					+

*Animal number.

seen in the treated, irradiated animals are compatible with those normally seen in acute, severe radiation sickness (3).

Table IX describes the pathologic findings in groups I, II, III, IV, and VI. Bone marrow failure and acute atrophy of lymphoid tissue, compatible with radiation damage, are consistent findings in all irradiated animals and indicate little if any protection to the lymphatic system. The lack of positive findings in the gastrointestinal tract may, however, indicate some degree of protection to this system.

No evidence of visceral toxicity attributable to the drug was found in either the drug controls or the irradiated animals.

IV. DISCUSSION

As can readily be seen, the results of this study were somewhat disappointing. The administration of WR 1607 by the indicated route and at the specified dosage and time does not appear to afford the radioprotection in primates that was previously demonstrated in rodents (15).

The signs and symptoms demonstrated by the treated animals differ little from those of the irradiated controls and the degree of lymphoid hypoplasia noted in the pathology reports indicates little or no protection to the lymphoid system.

The fact that the mean survival time of the treated animals is less than that of the controls and, more important, significantly less if the

TABLE IX

Pathologic findings in experimental groups

Animal No.	Experimental condition	Gross findings	Microscopic findings
33B	Drug control; 10 mg./kg. WR 1607; no irradiation.	Abscess (2 cm.) left anterior abdominal wall at level of umbilicus.	Sterile abscess in the anterior abdominal wall. No evidence of visceral drug toxicity.
IK3	Drug control; 20 mg./kg. WR 1607; no irradiation.	Abscess (2 by 3 cm.) in subcutaneous tissue and anterior abdominal wall in the left hypogastrium.	Sterile abscess in left anterior abdominal wall. No evidence of visceral drug toxicity.
94M	10 mg./kg. WR 1607 1 hour prior to 850 R irradiation.	Numerous petechial hemorrhages, excessive serous fluid in chest and pericardium. Occasional subserosal petechiae.	Sterile abscess in left abdominal wall. Acute hematopoietic and lymphoid aplasia.
48N	10 mg./kg. WR 1607 3 hours prior to 850 R irradiation.	Numerous petechial hemorrhages throughout skin and serosal surfaces. Lungs contained intra-alveolar hemorrhage. Cecum contained large irregular ulcer with thickening of the cecal wall.	Marked aplasia of the lymphoid and hematopoietic tissues. Mucosal ulcers of cecum with nematode infestation.
90J	20 mg./kg. WR 1607 3 hours prior to 850 R irradiation.	No remarkable findings.	Acute lymphoid hypoplasia.

thirty-day survivors are not considered, may point to a synergistic action between the drug and irradiation.

These findings, on the other hand, may be attributable to such variables as species difference, route of administration, vehicle, absorption of the lipid-soluble compound from the peritoneal cavity, and an incomplete knowledge of the proper dosage and latent period in the primate.

Because of the low toxicity of this drug and its possible protective effect upon the gut as indicated by the lack of gastrointestinal pathology, further studies in an effort to solve some of the inherent problems would seem to be indicated.

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