AD NUMBER

AD402152

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BDRLO ltr, 27 Sep 1971

TECHNICAL MANUSCRIPT 55

THE INFLUENCE OF SUBLETHAL X-IRRADIATION ON THE IMMUNITY OF GUINEA PIGS ADMINISTERED LIVE TULAREMIA VACCINE

APRIL 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY
U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

The work reported here was performed under Project 4B11-02-066, "Bacterial and Fungal Agent Research," Task -01, "Selection of Candidate Bacterial and Fungal Agents." The expenditure order was 2070. This material was originally submitted as manuscript 5101.

John Edward Nutter
Maurice Louis Guss

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C022301A068 April 1963

ABSTRACT

Reports from this laboratory have demonstrated that exposure of guinea pigs to sublethal X-irradiation three days before the administration of innocuous respiratory doses of live tularemia vaccine resulted in maximal mortality (23 per cent) in comparison with other X-irradiation schedules investigated. However, agglutinin production and development of immunity was not markedly altered in these animals by the X-irradiation procedure. The present study was performed to determine if the subcutaneous route of vaccination of irradiated animals would result in a decrease in the mortality rate and yet provide resistance to subsequent virulent challenge. Guinea pigs received 140 roentgens (r) of whole-body X-irradiation at intervals ranging from 12 days before to 3 days after subcutaneous vaccination with 10^5 viable cells of live tularemia vaccine. Serological studies of animals from each group were performed at nine post-vaccination intervals. The over-all mortality rate attributable to the combined irradiation-vaccination was fourfold less than that observed among irradiated animals vaccinated by the respiratory route. Three weeks following vaccination 20 animals of each irradiated-vaccinated group and the various control groups were challenged by a respiratory exposure to virulent P. tularensis SCHU S4. The serological study demonstrated that there was no significant inhibition of agglutinin production among the irradiated-vaccinated groups, regardless of the time of irradiation. All vaccinated animals, both irradiated and nonirradiated, developed comparable resistance to virulent challenge.

This laboratory previously reported the results of studies on the effects of combined exposure of guinea pigs* to sublethal X-irradiation and normally innocuous aerosol doses of Pasteurella tularensis vaccine strain LVS. The present study concerns the effect of sublethal X-irradiation on the course of immunization of the subcutaneously vaccinated guinea pig.

Guinea pigs received the whole-body X-irradiation from a 1000-KVP Maxitron machine. Exposures were made in a plastic wheel cage at a distance of 100 cm. Total dose delivered was 140 roentgens (r) at dosage rates of 63 to 67 r per minute, the maximum sublethal dose for the 325- to 375-gram male, Hartley-strain guinea pigs employed in this study.

The vaccine employed was 10^5 viable cells of LVS grown in a modified partial casein hydrolysate medium. The vaccine was administered subcutaneously in the inguinal region and the organisms were contained in 0.2 ml of gelatin-saline diluent. Ten groups of 70 animals each were exposed to 140 r of whole-body irradiation at one of the following intervals: 12 days, 6 days, 3 days, 1 day, 12 hours, or 1 hour before vaccination and 4 hours, 12 hours, 1 day, or three days after vaccination. Control groups for each phase included 70 nonirradiated vaccinated, 25 irradiated nonvaccinated, and 25 untreated normal animals.

At nine intervals, from one to 25 days post-vaccination, five animals of each vaccinated group were sacrificed following collection of a blood sample by cardiac puncture. Serum was separated and a slide test for P. tularensis agglutinins was performed on the individual serum samples. Following the slide agglutination tests, equal aliquot pools were prepared from the five serum samples of each group and a quantitative agglutination test was performed.

Twenty-three days after vaccination, 20 animals of each test and control group were exposed to an aerosol challenge with the highly virulent SCHU S4 strain. The inhaled dose per animal was 18 to 97 cells contained in a small-particle aerosol. Following virulent challenge the animals were observed for 30 days in order to delineate differences in resistance.

Table I presents the mortality response of the guinea pigs to the combined vaccination and irradiation procedures. During the period between vaccination and virulent challenge, eight animals not involved in the sacrifice schedule died. No LVS organisms were cultured from the lung, liver, or spleen of these animals. Post-mortem examination of these animals revealed hemorrhagic involvement of the lung and subcutaneous

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* Animals maintained in compliance with the "Principles of Animal Care" as promulgated by the National Society for Medical Research, Biomedical Purview 1:14. 1961.

TABLE I.
MORTALITY RESPONSE OF GUINEA PIGS FOLLOWING SUBLETHAL X-IRRADIATION AND SUBCUTANEOUS VACCINATION WITH *PASTEURELLA TULARENSIS* LVS

ANIMALS VACCINATED	NO. DEAD/NO. TESTED	PER CENT DEAD
After irradiation		
12 Days	0/40	0
6 Days	3/40	7.5
3 Days	0/40	0
1 Day	1/40	2.5
12 Hours	3/40	7.5
1 Hour	0/40	0
Before irradiation		
4 Hours	1/40	2.5
12 Hours	0/40	0
1 Day	0/40	0
3 Days	0/40	0
Vaccinated Nonirradiated Controls	0/80	0
Nonvaccinated Irradiated Controls	0/50	0
Normal Controls	0/50	0

tissue of five of the eight animals. Seven and one-half per cent of the guinea pigs irradiated six days and twelve hours before vaccination and 2.5 per cent of the groups irradiated one day before and four hours after vaccination died. A maximum mortality of 7.5 per cent at two irradiation intervals with subcutaneous vaccination is in contrast to a maximum mortality of 25 per cent when guinea pigs were irradiated three days before aerosol exposure to LVS in previous studies.

Table II presents the results of the serological study of animals irradiated before vaccination. Listed in the table are the agglutinin titers obtained from the equal aliquot serum pools of each group at the nine sampling intervals. The only significant differences in titer between test animals and vaccinated nonirradiated controls occurred among animals irradiated 12 days before vaccination. Six days following vaccination the 12-day animals had an agglutinin titer of 1:20, whereas vaccinated nonirradiated controls did not possess demonstrable agglutinins. This same difference was demonstrable 12 days following vaccination when the 12-day animals had a titer of 1:160 and the vaccinated nonirradiated control group had a titer of only 1:40.

Table III presents the results of the serological study of animals irradiated following vaccination. All serum samples were negative for agglutinins through the sixth day following vaccination. The only significant difference in titer between test animals and vaccinated nonirradiated controls occurred nine days following vaccination. At this interval the titer of sera collected from animals irradiated four hours following vaccination was 1:40; that of sera collected from vaccinated nonirradiated controls was 1:160.

Table IV presents the mortality rate subsequent to the virulent aerosol challenge of animals irradiated before vaccination. There were no marked differences in mortality rates of vaccinated irradiated and vaccinated nonirradiated guinea pigs. Analysis of the average day of death data among animals of the test groups demonstrated that there was no marked prolongation or shortening of the time of death when compared with the vaccinated nonirradiated group.

Table V presents the mortality rate subsequent to the virulent aerosol challenge of animals irradiated after vaccination. There were no marked differences in mortality rates of animals vaccinated and irradiated and of the vaccinated nonirradiated controls. In addition, the average time of death among animals of both the test and vaccinated control groups demonstrated a homogeneous response regardless of the experimental procedure prior to virulent challenge.

In summary, this study has demonstrated that sublethal whole-body X-irradiation administered at ten intervals ranging from twelve days before to three days after subcutaneous vaccination with the live tularemia vaccine did not alter the agglutinin response or the development of immunity to virulent challenge. These findings are similar to the

previously reported results of the effect of sublethal X-irradiation on the course of agglutinin production and the development of immunity when LVS was administered to guinea pigs by the respiratory route. A comparison of results of the aerosol and subcutaneous vaccination studies revealed one significant finding. Mortality due to the irradiation vaccination procedures alone was higher when the guinea pigs were vaccinated by the respiratory route than when vaccinated subcutaneously, a fourfold difference when the total number of deaths at the same intervals were compared.

TABLE II. AGGLUTININ RESPONSE OF GUINEA PIGS EXPOSED TO SUBLETHAL X-IRRADIATION, THEN VACCINATED SUBCUTANEOUSLY WITH PASTEURELLA TULARENSIS VACCINE STRAIN LVS

Time Lapse [a]	DAYS FOLLOWING VACCINATION								
	1	3	6	9	12	15	18	21	25
12 Days	Neg	Neg	1:20	1:40	1:160	1:320	1:160	1:320	1:160
6 Days	Neg	Neg	Neg	1:20	1:80	1:320	1:320	1:160	1:160
3 Days	Neg	Neg	Neg	1:10	1:80	1:80	1:640	1:160	1:160
1 Day	Neg	Neg	Neg	1:10	1:40	1:160	1:320	1:320	1:320
12 Hours	Neg	Neg	Neg	1:20	1:40	1:80	1:320	1:320	1:320
1 Hour	Neg	Neg	Neg	1:10	1:80	1:80	1:320	1:320	1:640
Vaccinated Nonirradiated Controls	Neg	Neg	Neg	1:20	1:40	1:160	1:320	1:160	1:320

a. Indicates the time lapse after irradiation and before vaccination.

TABLE III. AGGLUTININ RESPONSE OF GUINEA PIGS VACCINATED SUBCUTANEOUSLY WITH *PASTEURELLA TULARENSIS* VACCINE STRAIN LVS, THEN EXPOSED TO SUBLETHAL X-IRRADIATION

Time Lapse [a/]	DAYS FOLLOWING VACCINATION								
	1	3	6	9	12	15	18	21	25
3 Days	Neg	Neg	Neg	1:160	1:160	1:160	1:320	1:640	1:640
1 Day	Neg	Neg	Neg	1:80	1:160	1:160	1:320	1:640	1:640
12 Hours	Neg	Neg	Neg	1:80	1:80	1:160	1:320	1:640	1:640
4 Hours	Neg	Neg	Neg	1:40	1:80	1:160	1:320	1:640	1:1280
Vaccinated Nonirradiated Controls	Neg	Neg	Neg	1:160	1:160	1:160	1:320	1:640	1:640

a. Indicates the time lapse after vaccination and before irradiation.

TABLE IV. MORTALITY OF GUINEA PIGS EXPOSED TO SUBLETHAL X-IRRADIATION BEFORE SUBCUTANEOUS VACCINATION WITH PASTEURELLA TULARENSIS LVS FOLLOWING AEROSOL CHALLENGE WITH SCHU S4

Time Lapse [a]	PER CENT DEAD		AVERAGE DAY OF DEATH
	15 Days	30 Days	
12 Days	40	45	23.6
6 Days	35	50	21.8
3 Days	60	80	17.5
1 Day	30	60	20.8
12 Hours	50	70	20.1
1 Hour	45	60	19.9
Vaccinated Nonirradiated Controls	45	60	20.4
Nonvaccinated Irradiated Controls	95	95	7.6
Normal Controls	90	90	7.4

a. Indicates time lapse after irradiation and before vaccination.

TABLE V. MORTALITY OF GUINEA PIGS EXPOSED TO SUBLETHAL X-IRRADIATION AFTER SUBCUTANEOUS VACCINATION WITH PASTEURELLA TULARENSIS LVS FOLLOWING AEROSOL CHALLENGE WITH SCHU S4

Time Lapse [a]	PER CENT DEAD		AVERAGE DAY OF DEATH
	15 Days	30 Days	
3 Days	10	45	18.0
1 Day	15	40	17.0
12 Hours	35	50	17.0
4 Hours	10	30	17.0
Vaccinated Nonirradiated Controls	20	45	17.1
Nonvaccinated Irradiated Controls	100	-	5.7
Normal Controls	100	-	5.6

a. Indicates time lapse after vaccination and before irradiation.