20030128279

DTIC FILE COPY

AD-A190 153

DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution unlimited - approved for public release
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: U.S. Army Medical Research Institute of Infectious Diseases	6b. OFFICE SYMBOL (If applicable)
7a. NAME OF MONITORING ORGANIZATION: U.S. Army Medical Research and Development Command	
6c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5011	7b. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification): Passive Immunization Against Saxitoxin Administered Intravenously or Via the Respiratory Tract

12. PERSONAL AUTHOR(S): D.A. Creasia and S.R. Davio

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Interim	FROM _____ TO _____	1 May 87	10

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

In this study we compared the pulmonary and systemic toxicities of saxitoxin and examined the ability of anti-saxitoxin rabbit serum to protect mice from saxitoxin-elicited pulmonary toxicity. We found the 24-hr LD_{50} for intratracheally instilled saxitoxin to be 11.2 µg/kg, while that for i.v. toxin was 11.7 µg/kg. When mice were treated with either intratracheal or i.v. saxitoxin at a dose of LD_{99}, a 1:2 dilution of rabbit anti-saxitoxin, injected i.p. 1 hr before toxin treatment, provided 100% protection.

DTIC ELECTE JAN 20 1988 S D E

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) / 22c. OFFICE SYMBOL

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

PASSIVE IMMUNIZATION AGAINST SAXITOXIN ADMINISTERED INTRAVENOUSLY OR VIA THE RESPIRATORY TRACT

D.A. CREASIA and S.R. DAVIO*

Pathophysiology Division,
United States Army Medical Research Institute of Infectious Diseases,
Ft. Detrick
Frederick, MD 21701-5011, U.S.A.

Running Title: Passive Immunization Against Saxitoxin

Corresponding Author: D.A. Creasia, Ph.D.
Pathophysiology Division
USAMRIID, Fort Detrick
Frederick, Maryland 21701-5011

Telephone: (301)663-7181

*Present Address: Stephen R. Davio, Ph.D.
The Upjohn Company
Building 209, Room 649
301 Henrietta Street
Kalamazoo, Michigan 49001

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

88 1 19 015

D.A. CHEASIA and S.R. DAVIS. Passive Immunization Against Saxitoxin Administered Via the Respiratory Tract. Toxicon __, ___-___, 19__. --In this study we compared the pulmonary and systemic toxicities of saxitoxin and examined the ability of anti-saxitoxin rabbit serum to protect mice from saxitoxin-elicited pulmonary toxicity. We found the 24-hr LD_{50} for intratracheally instilled saxitoxin to be 11.2 ug/kg, while that for i.v. toxin was 11.7 ug/kg. When mice were treated with either intratracheal or i.v. saxitoxin at a dose of LD_{99}, a 1:2 dilution of rabbit anti-saxitoxin, injected i.p. 1 hr before toxin treatment, provided 100% protection.

Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

DTIC COPY INSPECTED

INTRODUCTION

Saxitoxin, one of the most lethal non-protein toxins known (DAVIO, 1985), is produced by dinoflagellates of the genus Gonyaulax (SUMMER et al., 1937; SCHANTZ et al., 1966). Gonyaulax dinoflagellates can contaminate shellfish, which, when eaten by humans, have been known to cause numbness, paralysis and death due to respiratory arrest. The primary therapy for saxitoxin poisoning in man is artificial respiration; there is no specific antidote accepted (HALSTEAD, 1967).

In a previous paper, DAVIO (1985) reported that anti-saxitoxin rabbit serum injected i.p. protected mice against the lethal effects of s.c. injected saxitoxin. However, the slower absorption kinetics of s.c. injected saxitoxin could have contributed to the protection afforded by the i.p. injected antiserum by allowing more time for interaction of toxin and antitoxin serum in the circulation. To test this, we passively immunized mice against saxitoxin by i.p. injection of anti-saxitoxin rabbit serum and challenged the mice 1 hr later by either i.v. injection or intratracheal instillation of saxitoxin. The results of these experiments are presented in this report.

MATERIALS AND METHODS

Saxitoxin

The saxitoxin used in this study was obtained from the Edgewood Arsenal, Aberdeen Proving Ground, MD.

RESULTS

Dose-response data comparing the lethality in mice of saxitoxin administered either intratracheally or i.v. are summarized in Table 1. Probit analysis of the data indicate that the LD_{50} for saxitoxin administered intratracheally was 11.2 ± 0.6 μg/kg body weight, while that for i.v. injected saxitoxin was 11.7 ± 0.4 μg/kg. These LD_{50} values are similar and compare favorably with that previously reported by DAVIO (1985) for i.p. injected saxitoxin (12.9 ± 0.5 μg/kg). There were no discernible differences in time to death nor clinical signs of toxicity (data not presented) in any of the saxitoxin-treated mice that could be attributed to the route of administration.

When antiserum was administered to mice 1 hr before saxitoxin treatment, we found that antiserum dilutions of 1:1 and 1:2 offered complete protection against a lethal challenge dose of 20 μg/kg given by either route of administration. These data are summarized in Table 2. Dilutions of 1:4, 1:8 and 1:16 offered partial protection to 20 μg/kg of saxitoxin injected i.v., while only dilutions of 1:4 and 1:8 afforded mice protection from intratracheal instilled saxitoxin.

DISCUSSION

The purpose of our study was to assess whether passive immunization with anti-saxitoxin rabbit serum could protect mice against the lethal effects of saxitoxin given i.v. or by intratracheal instillation. Our investigation showed that rabbit antiserum was equally effective in protecting mice from challenge by either route of administration. The data also tend to confirm results previously reported by DAVIO (1985), who demonstrated protective effects of antiserum to s.c. injected saxitoxin. Thus, we have shown that

antiserum can protect mice from the lethal effects of saxitoxin administered either i.v. or by intratracheal instillation.

The data from this study also show that the lethal effects of saxitoxin can be manifested through respiratory exposure. Although the absorption kinetics of saxitoxin across the pulmonary epithelium were not measured directly in this study, the time to death and the similar LD_{50}s of intratracheally instilled and i.v. saxitoxin suggest a rapid absorption of the toxin from the respiratory tract to the circulatory system.

ACKNOWLEDGEMENTS

The authors thank Ms. Robena C. Fowler for typing this manuscript.

REFERENCES

CARLSON, R.E., LEVER, M.L., LEE, B.W. and GUIRE, P.E. (1984) Development of immunoassays for the PSP - a radioimmunoassay for saxitoxin. In: Seafood Toxins (Ragelis, E.D., Ed.), ACS Symposium Series 262, p. 181. Washington: American Chemical Society.

DAVIO, S.R. (1985) Neutralization of saxitoxin by anti-saxitoxin rabbit serum. Toxicon 23:669.

FINNEY, D.J. (1977) Probit Analysis, 3rd Ed. Cambridge University Press.

HALSTEAD, B.W. (1965). Phylum Protozoa. In: Poisonous and Venomous Marine Animals of the World, 1, p. 182, Washington, DC: United States Government Printing Office.

SAFFIOTTI, U., F. CEFIS, and L.H. KOLB. (1968). A method for the experimental induction of bronchogenic carcinoma. Cancer Res. 28:104.

SCHANTZ, E.J., LYNCH, J.M., VAYVADA, G., MATSUMOTO, K. and RAPOPORT, H. (1966) The purification and characterization of the poison produced by Gonyaulax catenella in axenic culture. Biochemistry 5, 1191.

SOMMER, H., WHEEDON, W.F., KOFOID, C.A. and STOHLER, R. (1937). Relation of paralytic shellfish poison to certain plankton organisms of the genus Gonyaulax. Archs. Pathol. 24, 537.

TABLE 1. ACUTE TOXICITY OF SAXITOXIN TO MICE

Intratracheal Instillation		Intravenous Injection	
Dose (µg/kg)	#Dead/ #Alive	Dose (µg/kg)	#Dead/ #Alive
0 (control)	0/10	0 (control)	0/10
3.0	0/10	3.0	1/10
5.0	2/10	5.0	3/10
10.0	5/10	10.0	4/10
15.0	8/10	15.0	9/10
20.0	10/10	20.0	10/10
LD_{50} = 11.2 ± 0.6 µg/kg		LD_{50} = 11.7 ± 0.4 µg/kg	

TABLE 2. EFFECTS OF SAXITOXIN ANTISERUM ON LETHALITY OF SAXITOXIN* ADMINISTERED EITHER INTRATRACHEALLY OR INTRAVENOUSLY

Intratracheal Instillation		Intravenous Injection	
Antiserum dilution	#Dead/ #Alive	Antiserum dilution	#Dead/ #Alive
1:1	0/9**	1:1	0/10
1:2	0/10	1:2	0/10
1:4	2/10	1:4	3/10
1:8	5/10	1:8	7/10
1:16	10/10	1:16	8/10
(1:32)	N.D.	1:32	10/10
Control serum	10/10	Control serum	10/10

* Saxitoxin dose = 20 µg/kg
** One animal died from trauma induced by the instillation procedure.