

AD-A210 932

PROPHYLACTIC AND THERAPEUTIC USE OF AN ANTI-BREVETOXIN
(PbTx-2) ANTIBODY IN CONSCIOUS RATS

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RUNNING TITLE: Brevetoxin Antibody Treatment In Rats

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REPORT DOCUMENTATION PAGE

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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) SGRD-UIS	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) Prophylactic and therapeutic use of an anti-brevetoxin (PbTx-2) antibody in conscious rats.					
12. PERSONAL AUTHOR(S) Charles B. Templeton, Mark A. Poli, and Rikki Solow					
13a. TYPE OF REPORT Interim		13b. TIME COVERED FROM _____ TO _____		14. DATE OF REPORT (Year, Month, Day) 16 May 1989	
15. PAGE COUNT 28					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	brevetoxin, prophylaxis, therapy antibody, rats		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) C. B. TEMPLETON, M. A. POLI, AND R. SOLOW. Prophylactic and therapeutic use of an anti-brevetoxin (PbTx-2) antibody in conscious rats. Toxicon __, __-__, 198_. -A polyclonal antiserum was investigated for prophylactic and therapeutic use in the treatment of brevetoxin intoxication. Conscious, tethered male rats were pre-treated with 1 ml of anti-brevetoxin IgG (PbAb) or control IgG by a 10 min infusion, then given brevetoxin (25 µg/kg) by a 1 hr infusion. Rats pre-treated with control IgG demonstrated signs of brevetoxin intoxication; these signs were absent in rats pre-treated with PbAb. In therapy studies, rats were infused for 1 hr with 100 µg/kg brevetoxin, followed immediately by 2 ml of either PbAb or control IgG. During toxin infusion, both groups showed signs of brevetoxin intoxication. Rats treated with control antibody died within 6 hr. In rats treated with PbAb, respiratory rates began to return toward baseline almost immediately, and fewer neurological signs developed. After 24 hr, nearly all neurological signs had disappeared and both core and peripheral temperatures had returned to normal. There was a time					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (Include Area Code)		22c. OFFICE SYMBOL

differential between two groups of signs, suggesting high and low accessibility compartments for the antibody. These compartments probably represent central and peripheral nervous system. All animals treated with PbAb survived at least 8 days. These results suggest that PbAb has both therapeutic and prophylactic potential in the treatment of brevetoxin intoxication. Further, because of the differential in efficacy in reversing central and peripheral nervous system signs of brevetoxin intoxication, it provides useful new information on the mechanism of action of this toxin.

ABSTRACT

C. B. TEMPLETON, M. A. POLI, and R. SOLOW. Prophylactic and therapeutic use of an anti-brevetoxin (PbTx-2) antibody in conscious rats. Toxicon __, ____-____, 198_. - A polyclonal antiserum was investigated for prophylactic and therapeutic use in the treatment of brevetoxin intoxication. Conscious, tethered male rats were pre-treated with 1 ml of anti-brevetoxin IgG (PbAb) or control IgG by a 10 min infusion, then given brevetoxin (25 µg/kg) by a 1 hr infusion. Rats pre-treated with control IgG demonstrated signs of brevetoxin intoxication; these signs were absent in rats pre-treated with PbAb. In therapy studies, rats were infused for 1 hr with 100 µg/kg brevetoxin, followed immediately by 2 ml of either PbAb or control IgG. During toxin infusion, both groups showed signs of brevetoxin intoxication. Rats treated with control antibody died within 6 hr. In rats treated with PbAb, respiratory rates began to return toward baseline almost immediately, and fewer neurological signs developed. After 24 hr, nearly all neurological signs had disappeared and both core and peripheral temperatures had returned to normal. There was a time differential between two

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INTRODUCTION

Red tides resulting from blooms of the dinoflagellate Ptychodiscus brevis along the southeast coast of the United States and in the Gulf of Mexico have elicited a great deal of scientific interest since the first documented event over 100 years ago (STEIDINGER AND JOYCE, 1973). Brevetoxins are cyclic polyether neurotoxins produced by P. brevis and liberated into the water column during bloom conditions (TRIEFF, et al., 1975; BADEN 1983; SHIMIZU 1982; TAYLOR AND SELIGER 1979). These toxins can be concentrated in filter-feeding molluscs, making them toxic to humans and other animals (McFARREN, et al., 1965). Aerosolization of toxins along the surf zone can cause irritation of the mucous membranes, bronchoconstriction or dyspnea among beachgoers. In addition, massive fish kills during red tides can make beaches unusable and result in significant public health concerns. For these reasons, there has been a concerted research effort to isolate and characterize the toxins of P. brevis during the past two decades. Recent studies have resulted in the isolation and purification of a family of related brevetoxins (reviewed in STEIDINGER and BADEN, 1984), and the elucidation of

the mechanism of action of these compounds at the molecular level (HUANG et al., 1984; POLI et al., 1986). Our research interest has been the study of the pathophysiology of the brevetoxins in vivo and the prophylaxis and treatment of brevetoxin intoxication.

Previous studies in this laboratory (TEMPLETON et al., in press) characterized the physiological effects of the brevetoxin PbTx-2 in conscious rats. After a 1-hr infusion into the jugular vein, the most dramatic changes observed were precipitous, dose-dependent respiratory depression, decreased core and peripheral body temperatures, cardiac arrhythmias and increased pulse pressures. These rats also exhibited ataxia, depression, head-bobbing, head-tilt, and other uncontrolled muscle movements. While the neurological signs and the decrease in core temperatures suggested central nervous system involvement, a peripherally mediated cause for the profound decreases in respiratory and heart rates could not be ruled out.

POLI and HEWETSON (in press) demonstrated that a goat antiserum raised against PbTx-3 had equal affinity for the structurally related brevetoxin PbTx-2 and was capable of completely inhibiting the binding of [^3H]PbTx-3 to its receptor site in rat brain membranes. Therefore, we evaluated

the potential of this antibody in the prophylaxis and treatment of brevetoxin intoxication. We chose the awake, tethered-rat model to avoid anesthesia effects which alter neurological responses and preclude behavioral assessment.

MATERIALS AND METHODS

Toxin

PbTx-2 was obtained from D. G. Baden (University of Miami, FL) as an HPLC-purified fraction (single peak of ultraviolet absorbance at 208 nm) extracted from unialgal cultures of Ptychodiscus brevis derived from the 1953 isolate of Wilson. A stock solution of 1 mg/ml was kept at -10°C in chloroform. Working solutions were dried under a stream of nitrogen, dissolved in 0.1 M phosphate-buffered saline (PBS), pH 7.4, containing 0.01% Emulphor EL-620 (GAF, Inc., New York), and used immediately. Emulphor EL-620, a nonionic emulsifier, is required to solubilize PbTx-2 in aqueous buffer.

Antibody

Polyclonal antiserum produced by immunization of goats with PbTx-3 conjugated to bovine serum albumin was used in all studies (POLI and HEWETSON, in press). Serum IgG was precipitated by

sequential incubation with 30% and 45% ammonium sulfate. Serum was stirred for 4 hr at room temperature with an equal volume of 60% ammonium sulfate. The precipitated protein was collected by centrifugation for 30 min at 1500 x g and re-dissolved in distilled water. The above step was then repeated with 90% ammonium sulfate and the precipitated protein re-dissolved in distilled water. This solution was dialyzed for 12 hr against 0.1 M PBS, pH 7.4, and then for 24 hr against 0.9% NaCl, pH 8. For the prophylactic study, the maximum binding capacity (B_{max}) of the precipitated IgG solution was determined to be 0.27 μ g toxin/ml by Rosenthal analysis of saturation binding data (not shown). The IgG was concentrated in the therapeutic study to a binding capacity of 0.9 μ g toxin/ml.

Surgical preparation of animals

Surgical preparation was identical for both prophylaxis and treatment studies. After anesthetic induction with 55 mg/kg pentobarbital, male rats (CRL CD SDBR, Charles River Laboratories, Wilmington, MA) were placed on a heated surgical board (Harvard Apparatus Co., Inc., South Natick, MA). Catheters (PE50, Clay-Adams, Parsippany, NJ), were placed in the carotid artery and the jugular vein. These catheters were used to

measure arterial blood pressure, to collect samples for arterial blood gas measurements, and for venous infusions of antibody and toxin. Thermistor probes (Sensortek, Clifton, NJ) were implanted into the peritoneal cavity and subcutaneously over the sternum. Electrocardiogram leads were placed subcutaneously over the manubrium and sixth thoracic vertebra to simulate a V10 configuration. All catheters and wires were routed subcutaneously to the dorsal cervical area and passed through a 20-cm steel spring tether (Alice Chatham King, Carmel, CA; I.D. 3 mm). After being flushed with heparinized saline, the catheters were occluded with stainless steel pins. The rats were then placed in stainless steel cages with the tether passing through the wire mesh top. This allowed sampling and monitoring with minimum interference or excitement. The rats were allowed to recover from anesthesia for 24 hr.

Experimental protocol

For the prophylaxis study, rats were placed into three groups of four rats each. One group was pre-treated with 1 ml of PbAb by 10 min i.v. infusion. A second group was pre-treated with 1 ml of control IgG solution matched for total protein content, and a third group was pre-treated with 1 ml of saline only. Twenty

min after pre-treatment, PbTx-2 (25 µg/kg, in 1 ml of PBS containing 0.01% Emulphor EL-620) was infused over 1 hr into the jugular venous catheter. This sublethal dose of toxin was used to evaluate better the parametric alterations over time. Heart rate, core and peripheral body temperature, lead V10 ECG, and arterial blood pressure were monitored continuously. Respiratory rates were visually counted and recorded at 5 min intervals for 2 hr, then at 15 min intervals for an additional 4 hr. Animals were then observed periodically for 24 hr.

For the therapy study, rats were divided into two groups of six rats each. All animals were given an infusion of PbTx-2 (100 µg/kg in 2 ml of PBS containing 0.01% Emulphor EL-620) for 1 hr. This lethal dose (LD₉₅) was used to test the ability of the antibody to prevent death as well as to improve parameters. Immediately following toxin administration, a 30-min infusion of 2 ml PbAb was begun. Control animals were infused with 2 ml of the control IgG solution, matched for total protein content. Data were collected as above, except that animals were observed for 8 days post treatment.

Data analysis

Parametric data were analyzed by using a Multiple Analysis of Variance for profile comparison and a Dunnett's test to determine differences between the control and each set of succeeding points.

RESULTS

In the prophylaxis study, the first sign demonstrated by intoxicated animals pre-treated with saline or control IgG was a rapid decrease in respiratory rate to 20% of baseline values (Fig. 1). This decrease was apparently accompanied by an increase in tidal volume, since blood oxygen tension remained normal (93-109 mm Hg) throughout the 6 hr. Core (Fig. 2) and peripheral (not shown) temperatures decreased by 1.5 to 2°C during toxin infusion. As previously reported (TEMPLETON et al., in press), numerous cardiac arrhythmias were observed. Arterial blood pressures and heart rates were variable but were not significantly altered from control. Multiple neurological signs were evident including head-bobbing, ataxia, and depression. These results were consistent with our previous characterization of PbTx-2 intoxication at this dose (TEMPLETON et al., in press). Rats pre-treated with PbAb showed no decrease in respiratory rates (Fig. 1), temperatures (Fig. 2), and no ECG abnormalities. Arterial blood pressures (not shown) were not statistically different from control. There were no obvious neurological manifestations and the animals continued to eat,

drink and groom for the entire 6 hr experiment.

In the therapy study, rats administered PbAb after toxin infusion initially exhibited all of the characteristic signs of PbTx-2 intoxication described above. However, by the end of the PbAb infusion, respiratory rates had begun to return toward baseline values (Fig. 3). Core (Fig. 4) and peripheral (not shown) temperatures did not immediately return to baseline, but no further decrease was observed. Behavioral signs were still largely evident 6 hr post toxin, but after 24 hr, nearly all neurological signs had disappeared and both core and peripheral temperatures had returned to normal. Only one of the six rats infused with control antibody survived longer than 4 hr. All six PbAb-treated rats survived the entire 8 days.

DISCUSSION

These experiments demonstrate that PbAb protects rats from the pathophysiological effects of i.v. PbTx-2. The polyether backbone portion of the brevetoxin molecule appears to be responsible for the cross-reactivity of the antibody (POLI and HEWETSON, in press) and, therefore, this serum should protect against brevetoxins sharing the B-type backbone structure. This hypothesis is currently under investigation.

The ability of 2 ml of PbAb (calculated B_{max} : 0.9 $\mu\text{g/ml}$) to reverse the pathophysiological effects of PbTx-2 (100 $\mu\text{g/kg}$, LD_{95}) was somewhat unexpected. Pharmacokinetic experiments (POLI et al., in press) have demonstrated the half-life of distribution of $[^3\text{H}]\text{PbTx-3}$ to be 20-30 sec. Within minutes after the intravenous infusion, only trace amounts of toxin remain in circulation. The bulk of the administered toxin distributes to the skeletal muscle, liver, and gastrointestinal tract. We believe the circulating toxin to be the primary source of toxin interacting with specific receptor sites in the peripheral and central nervous tissue, and that the

neutralization of this toxin is sufficient to interrupt the pathophysiology of intoxication. The toxin in the liver and gastrointestinal tract likely reflects metabolism and biliary excretion (POLI et al., in press). The significance of the toxin pool in the skeletal muscle is still under investigation.

Central nervous system signs of PbTx-2 intoxication consisted of head-bobbing, depression, and ataxia. In some, an apparently involuntary muscular contraction occurred, usually in the hindquarters, which caused the animals to lunge violently across the cage in one coordinated motion. Both hindquarters contracted simultaneously, indicating the initiating impulse originated at the level of the spinal cord or higher. The decrease in core temperatures also suggests central nervous system involvement. This may be due to decreased metabolism, resulting in decreased oxygen utilization and heat production. When core temperatures were minimal, the animals appeared weak and listless. During the entire experimental period, the peripheral temperature remained lower than the core temperature and the differential between the two remained constant. This suggests that the drop in core temperature was not due to peripheral vasodilation.

We believe the central and peripheral effects of PbTx-2 intoxication can be distinguished by the difference in efficacy

of PbAb in reversing the clinical signs. The first sign of intoxication in control animals was a precipitous decrease in the rate of breathing. Infusion of PbAb resulted in immediate improvement; breathing rate began to increase toward baseline levels by the end of the infusion period. This was likely due to the neutralization of toxin in the circulating blood by PbAb, thereby shifting the binding equilibrium between circulating toxin and peripheral receptor sites.

Signs of central nervous system involvement did not improve as rapidly after antibody treatment as did peripheral signs. Infusion of PbAb prevented further decrease in core and peripheral body temperatures, which remained depressed for the 6 hr experiment, but returned to baseline by 24 hr. Similarly, behavioral signs persisted throughout the experimental period and did not greatly diminish until 24 hr post-treatment. This may reflect the inability of PbAb to penetrate the blood-brain barrier and compete for toxin binding in the central nervous system. Diminution of signs after 24 hr is consistent with the time course of brevetoxin metabolism and excretion in rats (POLI, in press).

The ability of this antibody preparation to differentiate peripheral from central toxin effects yields important

information on the mechanism of PbTx-2 toxicity. In the therapy study, all animals treated with PbAb survived a lethal dose of PbTx-2. Assuming minimal penetration of the blood:brain barrier by circulating IgG, this suggests that the lethal effects of intoxication are peripherally mediated. Interruption of the peripheral pathophysiology allowed the animals to metabolize and excrete the toxin with no apparent permanent central nervous system effects. Sufficient respiratory compensation, in terms of increased tidal volume, maintained normal blood gases and pH throughout the period of intoxication. BORISON, et al., (1980) demonstrated that crude extracts of P. brevis cause respiratory failure in cats when administered a bolus injection intravenously. They suggested a transient peripheral effect on the vagus nerve slowed the respiratory rate, but that a direct central effect resulted ultimately in the asphyxial arrest of breathing. BADEN et al., (1982) showed that aerosol exposure to the brevetoxin T-17 (PbTx-3) resulted in a dose-dependent bronchoconstriction in anesthetized guinea pigs. While not addressing the question of whether this response was centrally- or peripherally mediated, they noted that cutting the vagi at the neck produced no significant change in the pulmonary response. The results presented here cannot rule out a central mechanism for lethality in brevetoxin intoxication. However, the ability of circulating antibody to prevent death suggests that if a

central mechanism is present, then it must be influenced by signals originating in the periphery. Further investigation of this process is planned including central access of the antibody and equilibration rate of the toxin across the blood:brain barrier.

Acknowledgements - All procedures performed in this study conform to the "Guide to the Care and Use of Laboratory Animals", published by the National Institutes of Health, Bethesda, MD. All research facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

The opinions of the authors in no way reflect the opinions of the Department of the Army or the Department of Defense.

REFERENCES

- BADEN, D. G., MENDE, T. J., BIKHAZI, G., and LEUNG, I. (1982) Bronchoconstriction caused by Florida red tide toxins. Toxicon 20(5), 929-932.
- BADEN, D. G. (1983) Marine food-borne dinoflagellate toxins. Int. Rev. Cyt. 82, 99-150.
- BORISON, H. L., ELLIS, S., and MCCARTHY, L. E. (1980) Central respiratory and circulatory effects of Gymnodinium breve toxin in anaesthetized cats. Br. J. Pharmacol. 70, 249-256.
- HUANG, J. M. C., WU, C. H., and BADEN, D. G. (1984) Depolarizing action of a red tide dinoflagellate brevetoxin on axonal membranes. J. Pharmacol. Exp. Ther. 229, 615-621.
- McFARREN, E. F., TANABE, H., SILVA, F. J., WILSON, W. B., CAMPBELL, J. E., and LEWIS, K. H. (1965) The occurrence of a ciguatera-like poison in oysters, clams, and Gymnodinium breve cultures. Toxicon 3, 111-123.
- POLI, M. A. and HEWETSON, J. F. An anti-brevetoxin antiserum as a potential tool in the management of neurotoxic shellfish poisoning. In: Toxins from Marine and Aquatic Environments (HALL, S., Ed.). Washington D.C.: American Chemical Society. (in press).

- POLI, M. A., MENDE, T. J., and BADEN, D. G. (1986) Brevetoxins, unique activators of voltage-sensitive sodium channels, bind to specific sites in rat brain synaptosomes. Molec. Pharmacol. **30**, 129-135.
- POLI, M. A., TEMPLETON, C. B., PACE, J. G., and HINES, H. B. Detection, metabolism, and pathophysiology of the brevetoxins PbTx-2 and PbTx-3. In: Toxins from Aquatic and Marine Environments (HALL, S. Ed.). Washington, D.C.: American Chemical Society (in press).
- SHIMIZU, Y. (1982) Recent progress in marine toxin research. Pure Appl. Chem. **54**, 1973-1980.
- STEIDINGER, K. A. and BADEN, D. G. (1984) Toxic marine dinoflagellates. In: Dinoflagellates, pp. 201-261 (SPECTOR, D. L., Ed.). New York: Academic Press.
- STEIDINGER, K. A. and JOYCE, E. A. (1973) Florida red tides. Fla. Dept. Nat. Res. Ed. Ser. No. 17.
- TAYLOR, D. L. and SELIGER, H. H. (Eds.) (1979) Toxic Dinoflagellate Blooms. New York: Elsevier North Holland.
- TEMPLETON, C. B., POLI, M. A., and LeCLAIRE, R.D. (1989) Cardiorespiratory effects of brevetoxin (PbTx-2) in conscious, tethered rats. Toxicon (in press).
- TRIEFF, N. M., RAMANUJAM, V. M. S., ALAM, M., and RAY, S. M.

(1975) Isolation, physicochemical, and toxicological characterization of toxins from Gymnodinium breve Davis. In: Proc. First Intl. Conf. Toxic Dinoflagellate Blooms, pp. 309-321 (LoCICERO, V. M., Ed.). Wakefield: Mass. Sci. Tech. Found.

FIGURE LEGENDS

FIG. 1. Mean respiratory rate ($\pm$ SEM) of rats (n=4) after 10 min PbAb infusion and 20 min delay. Vertical solid line at 60 min marks the end of toxin infusion (25 μ g/kg). ——— = no Ab pretreatment; ----- = control Ab pretreatment; = PbAb pretreatment; * = significant from value at time 0 ($p < 0.05$).

FIG. 2. Mean core body temperature ($\pm$ SEM) of rats (n=4) after 10 min PbAb infusion and 20 min delay. Vertical solid line at 60 min marks the end of toxin infusion (25 μ g/kg). ——— = no Ab pretreatment; ----- = control Ab pretreatment; = PbAb pretreatment; * = significant from value at time 0 ($p < 0.05$).

FIG. 3. Mean respiratory rate ($\pm$ SEM) of rats (n=6) after 60 min infusion of PbTx-2 (100 μ g/kg) and 30 min infusion of PbAb. Vertical solid line at 60 min marks the end of toxin infusion and vertical solid line at 90 min marks the end of antibody infusion. ----- = control Ab treatment; = PbAb treatment; * = significant from value at time 0 ($p < 0.05$).

FIG. 4. Mean core body temperature ($\pm$ SEM) of rats (n=6) after 60 min infusion of PbTx-2 (100 μ g/kg) and 30 min infusion of PbAb. Vertical solid line at 60 min marks the end of toxin

infusion and vertical solid line at 90 min marks the end of antibody infusion. ----- = control Ab treatment; = PbAb treatment; * = significant from value at time 0 ($p < 0.05$).







