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TOXICOLOGY OF *PHYSALLA'S* (PORTUGUESE MAN-O' WAR) VENOM

JUNAID MAHMOOD ALAM and RASHID QASIM
Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

The venomous pelagic coelenterate *Physician* species (Blue bottle) or the Portuguese man-o'war has recently been studied in detail due to their hazardous effects on community. It was observed that the venom of the animal is lethal to man and can also produce coetaneous stings of varying severity. *Physalia* species also pores significant cardio toxicity to man, rats, mice and are lethal to lower animal. This effect has been attributed to the abnormality in ionic transport across membranes. The Cutaneous pain and musculotoxic action in human produced *by Physalia* venom may be induced by one or several high molecular weight polypeptides. Blockage of specified neurons and neuromuscular junction besides attention in end plate potential (EPP) and end plate current (FPC) was also attributed to high molecular weight toxic fractions. Survey of enzymatic contents of *Physalia* venom reveals the presence of a wide variety of enzymes whose presence and actions were similar to that of complex enzyme mixture of snake venom Besides these clinical manifestations neurotoxicity cytotoxicity and other psychopharmacological effects also demonstrated.

Introduction

Physalia species (Blue bottle) or the Portuguese man-o'war is one of the floating Siphonophores which has been named "Pleuston" by the Russian ecologist Savilov (Southcott, 1979). Each specimen of *Physalia* species is not a single animal but a group of organisms. The stinging abilities of *Physalia* like that of other coelenterates has been known for centuries and an early account of them is mentioned in Hakluyt's Voyages for 1579 (Southcott, 1979).

Physalia species has a distribution throughout the tropical and semitropical regions including Pakistan. Most abundantly it is found around the coastal areas of Australia, Florida (America), Gulf of Mexico as well as the coastal areas of Pakistan during summer months and remain a continuous health hazard for the community. Many deaths have been attributed to the painful sting of *Physalia* (Burnett and Gable, 1989; Stein *et al,* 1989; Williamson 1990) but sting injuries and nonfatal envenomation cases are more common.

Due to its hazardous effects on the community, the *Physalia* species, most specifically its venom, has been studied with interest in recent years. Although its lethality to man has been documented recently, lethality to mice, rats, rabbits and other lower animals has long been known to scientists and researchers. This lethality has been attributed to the presence of high molecular weight polypeptides (Burnett and Colton, 1977; Menendez et al, 1990; Tamkun and Hessinger, 1981), a well diversified enzymatic content (Burnett and Colton, 1974a,b) and other physic-pharmacological factors (Burnett

and Calton, 1977). Similarly cardiotoxicity and neurotoxicity has been known to be persistent with the venom altering the calcium ion transport across the membrane. In view of the importance of *Physalia's* venom as a tool to investigate pathological, physiological and pharmacological activities, the present studies have been carried out in our laboratory. This work deals with the chemistry and toxicology of *Physalia* species which gives base line data to initiate a broader investigation of the same in Pakistan.

Materials and Methods

Physalia species was collected along the Clifton and Sandspit beaches of Karachi during the summer months of 1990. The tentacles containing nematocysts were excersized manually and nematocysts were isolated by the method of Endean *et at.* (1969). Nematocyst pellets were washed continuously and centrifuged four to five times at 2000 to 3000 g (Long and Burnett,1989) until pellets were clear of tentacular debris. The pellets were frozen at -10 °C in ampoules. When required the contents of ampoules were centrifuged at 1500 rpm. and extracted by the method of Endean *et al* (1969).

Protein, lipid, carbohydrate and cholesterol contents of venom were measured by the method of Lowry *et al.* (1951), Bligh and Dyer (1959), (Dubois *et al.* (1956) and Stillway (1971) respectively. Molecular weight determination of crude and partially purified venom was carried out by the method described by Weber and Osborn (1969) on polyacrylamide gels using molecular weight standards (Sigma). Crude venom was applied on to a column (90 cm x 2.0 cm) with G-50 and G-75 Sephadex gels (Sigma), void volume was determined by Blue dental. The eluant used was 0.1M sodium phosphate buffer (pH 6.0 and 6.8). Fraction volumes of 2 ml were collected and tested for hemolytic activity after spectral absorbance at 280 um.

Hemolytic activity of venom was determined by the methods described by Alain and Qasim (1991). Cardiotoxicity and neurotoxicity were analysed by using appropriately diluted venom aliquots; i.p injection was administered in juvenile and adult Albino rats and mice and the animals were monitored for any ontoward signs or unusual activities for 1,2,4,8 and 24 hours. lethality and dermonecrotic effects were examined by the methods of Burnett and Calton (1974a) and Endean *et at.* (1969).

Results and Discussion

Venom apparatus

The stinging unit of the *Physalia* is the nematocyst which is formed within the interstitial cell the endoblast (Russell, 1965). *Physalia* possesses only one type of nematocyst which has two size ranges (Burnett and Calton, 1977; Hulet *et al,* 1974; Lane and Dodge, 1958; Southcott, 1979) and our results indicate the same. The mechanism for the discharge of nematocyst involves different factors which include permeability of the capsule wall, increasing hydrostatic pressure and change in pH (Russell, 1965).

Table 1

Major chemical and toxicological properties of *Physalia* nematocyst venom

Nematocyst	Two size groups	This study; Burnett and Calton, 1977; Southcott, 1979.
Major chemical content	Primarily proteinaceous 10%-40%	This study; Burnett and Calton, 1977; Russell, 1965.
Approx. molecular weight of mouse lethal fraction	150,000 and 212, 000	Burnett and Calton, 1977; Russell, 1984.
Enzymatic content	ATPase, RNase, DNase, fibrinolysin, AMPase, non-specific aminopeptidase.	Burnett and Calton, 1974.
Free fatty acid and cholesterol	Present	This study, Middlebrook and Lane, 1968; Stillway, 1974.
Cardiotoxicity	Present	This study; Burnett and Calton, 1977; Burnett and Gable, 1989; Williamson *et al.*, 1988.
Dermonecrotic activity	Present (mild to severe)	This study.
Factor producing smooth muscle contraction	Present	This study; Burnett and Calton, 1977.
Action on Na^+ transport in frog skin	Present	Larsen and Lane, 1970.
Hemolysin	Present (mild to severe)	This study; Tamkun and Hessinger, 1981.
Fibrinolytic activity	Present	This study; Burnett and Calton, 1974a,b.
Human cutaneous pain and papular lesions	Present	This study; Burnett and Gable, 1989; Russell, 1965.
Kinin-like mediators of inflammations	Present	Burnett *et al.*, 1975.

While several scientists extensively examined the possibility of a perfect method for the isolation and purification of *Physalia* nematocyst from cellular debris, Meglumine diatrizoate gradient centrifugation gives reproducible results (Burnett and Calton, 1973). Lane and Dodge (1958) isolated *Physalia* nematocysts by autolysis but comparative study of various techniques reveals that more lethal activity was found in nematocyst suspension prepared by blending and centrifugation than by autolysis (Burnett and Calton, 1977; Calton and Burnett, 1973b). Results reveal that *Physalia* nematocysts are more resistant to physical and chemical treatment than those of jellyfish (Burnett and Calton 1977; Russell 1965 and 1984).

Isolation and partial purification of nematocyst's venom

Active toxic material or venom was separated from the isolated nematocysts of Physalia by sonic treatment, ultracentrifugation, grinding and homogenization of disrupted nematocyst preparations (Burnett and Goldner, 1970a, 1971; Walker, 1977) and by "amnion milking technique (Barnes, 1967). Many differential techniques and modifications of certain previous methods for isolation have also been successfully implicated by several workers (Burnett and Calton 1977; Calton and Burnett, 1973b; Calton *et al,* 1978; Endean *et al,* 1969).

For purification of venom more advanced and practically applicable techniques have been introduced by the passage of time which provides better results and resolutions. These include the classical gel filtration column chromatography (Burnett and Calton, 1977; Burnett and Goldner, 1970a,b and 1971; Calton *et al,* 1978; Menendez *et al,* 1990), preparative gel electrophoresis, isoelectric focusing and d[i]scontinuous membrane partition chromatography (Burnett and Calton, 1974a,b, 1976a).

Chemical content

Major chemical and toxicological properties of *Physalia* venom are summarized in Table 1. Differential studies carried out on *Physalia* venom show that the venom is a viscous fluid, stable on lyophilization, three cycle freeze-thaw and on storage at -29°C to -90°C (Burnett and Calton, 1977; Calton and Burnett, 1973b; Lane and Dodge, 1958). The lethal activity of venom is sensitive to trypsin (Lane and Dodge, 1958). Chemical examination reveals that the nematocyst contents are primarily pertinacious, i.e. 10-40% protein in nature (Lane and Dodge, 1958). Some of the lipids present were free fatty acids and cholesterol (Burnett and Calton, 1977; Middle brook and Lane, 1968; Stillway, 1974). The carbohydrate content was found to be 2-6%.

Studies carried out on enzymatic content of venom indicate the presence of hemolysin and fibrinolysin. Previously several enzymes were determined by a number of scientists using specific methods. ATPase, nonspecific amino peptidase, RNase, DNase, AMPase, hemolysin and a fibrinolysin were present in physiological doses *of Physalia's* venom (Burnett and Calton, 1974; Tamkun and Hessinger, 1981). A small quantity of

phospholipase was also found in autolysed tentacles (Stillway and Lane, 1971). The presence of these enzymes may justify the action of *Physalia* venom on sarcoplasmic reticulum and mitochondria which was similar to that of complex enzyme mixture of snake venom (Calton and Burnett, 1973a; Calton *et al*, 1973). The amino acid analysis performed by Lane (1974) on the crude nematocyst contents of *Physalia* depicits the presence of high levels of glutamic acid and low levels of aromatic amino acids.

Attempts have been made in our laboratory to isolate and partially purify the *Physalia* venom components by the combination of classical gel filtration chromatography and high performance liquid chromatography (HPLC). The results indicate that gel filteration with most of the Sephadex gel matrix is not applicable for isolation or purification of the lethal contents of *Physalia* venom without any additional precautions because of its adsorption to the gel material. However, several scientists have successfully isolated few polypeptides of low to high molecular weights from crude as well as purified venom (Burnett and Calton 1974 ab; Calton and Burnett, 1973 b; Calton *et. al.,* 1973). A polypeptide fraction of molecular weight 150,000 was found to be lethal to mice (Burnett and Calton, 1974b).

Isoelectric focusing revealed that the lethality of venom towards mice was associated with a fraction having a pl of 6.5 and smaller fraction at of 4.5 (Burnett and Calton, 1974b). More recently a hemolytic protein "Physalitoxin" having molecular weight of 212,000 and another polypeptide of molecular weight 220,000 with effects on neuromuscular junction were isolated and purified (Menendez *et al.,* 1990; Tamkun and Hessinger, 1981). Gel electrophoresis carried out with crude and partially purified *Physalia* venom shows the presence of seven to eight fractions having molecular weights ranging from 8000 to 150,000. The results are in good agreement with previous studies as preparative gel electrophoresis gives at least nine lethal fractions (Burnett and Calton, 1977), while eight lethal polypeptides were isolated by paper chromatography (Lane *et al.,* 1961).

Lethality and dermonecrosis

Experiments with a number of animals both vertebrates and invertebrates and also their muscle and tissue preparations show that *Physalia* venom can induce a variety of specific physiological, pathological, psychopharmacological and neurological disorders. It was found that *Physalia* venom is lethal to mice, rats, fidder crab and rabbits (Lane and Dodge, 1958) but seems to produce less dermonecrosis in man and experimental animals than the sea nettle (Calton and Burnett, 1973b). Intraperitoneal, intracisternal and intravenous administration of venom produces paralysis in fish, frog, mice, and rats (Russell, 1965).

In some cases the papules formed following the contact with *Physalia* nematocyst may also be surrounded by erythamatous zone. In some cases the papules may be very close indicating the multiple discharge of nematocysts (Burnett and Gable, 1989; Minton, 1974; Russell, 1965; Stein *et al,* 1989). It was also observed that the

papules develop and increase in size very rapidly with pain and burning sensation. Joints muscle cramps and spasm may persist for several hours following the injury (Minton, 1974; Russell, 1965). Several types of systemic manifestation may also develop following Physalia envenomation which includes nausea, lacrimation and nasal discharge; increased perspiration and vertigo have been reported in many cases (Burnett and Gable, 1989; Russell, 1965; Williamson *et al*, 1988).

Cardiotoxicity and neurotoxicity

Abnormal heart rate was recorded after intraperitoneal injection of venom in rats and mice. Marked signs of paralysis, frothing, hurddling and convulsion leading to death depicits the neurotoxin effects of *Physalia* venom. A number of researchers have studied cardio toxic and neurotoxic effects in several experimental animals using either *Physalia* crude nematocyst extracts or purified toxic fractions (Lane, 1967; Lane and Larsen, 1965; Larsen and Lane, 1966; Menendez *et al*, 1990).

Earlier studies by several scientists on neurogenic crustacean heart and the myogenic heart of rat and dog show a dose dependent electrocardiography effect of coelenterate's venom (Hastings *et al*, 1967; Lane and Larsen, 1965). It was observed that PR interval was shortened in dog followed by P suppression and appearance of an ectopic pace maker near AV node (Burnett and Calton, 1977). Abnormal heart rate was also recorded after intraperitoneal injection of venom in rats. A marked elevation of serum potassium occurred within minutes after i.v. administration of venom in dogs (Hastings *et al,* 1967). The elevation of serum K levels was attributed to the interuption of active ionic transport within the body (Burnett and Calton, 1977).

The nicotinic anticholinergic effects of *Physalia* toxin P were also tested by Menendez et al (1990) on neurons of land snail and frog neuromuscular junction. The toxin was found to have the ability to reversibly block NC responses of snail neuron end plate potential (EPP) and cholinergic potential of frog neuromuscular junction in a dose dependent manner. It also blocks end plate current (EPC) and reduces the maximal responses to iotophoretically applied AC h (Menendez *et al,* 1990)

Effects on other membranes

Hemolytic and fibrinolytic activities *were* also observed by both crude and purified venom materials. Susceptibility of various species of experimental animals' RBC and different age group towards hemolytic properties was indicated by the venom extracted by various techniques. Minimum dose of percent hemolytic (50%) in terms of protein content was found to be 1.0 mg/ml for human RBC; mg/ml for rat RBC 0.5 and 0.7 mg/ml for rabbit RBC. It was noted that more reproducible results were obtained by using cells from an O^+ donor (Alam and Qasim, 1991). Several experimental analyses reveal that *Physalia* venom contains a "hemolysin" which is detectable in 10-15 i.v. mouse LD_{100} doses (Burnett and Calton, 1977). Earlier studies showed the injurious

effects of venom on sarcoplasmic reticulum and mitochondria (Calton and Burnett, 1973a; Calton *et al.,* 1973). A hemolytic protein "Physalitoxin" was obtained from *Physalia* venom which was found to be lethal to mice at 0.2 mg/kg body weight level while LD50 for crude venom was 0.14 ms/kg body weight (Tamkun and Hessinger, 1981). A fibrinolysin was also detected and quantitated which showed its lyric effects on fibrin clot substrate (Burnett and Calton, 1974 a,b).

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