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REVIEW ARTICLE

Hemolytic venoms from marine cnidarian jellyfish – an overview

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ABSTRACT

Cnidarian jellyfish are viewed as an emergent problem in several coastal zones throughout the world. Recurrent outbreaks pose a serious threat to tourists and bathers, as well as to sea-workers, involving health and economical aspects. As a rule, cnidarian stinging as a consequence of nematocyst firing induces merely local symptoms but cardiovascular or neurological complications can also occur. Hemolysis is a frequent effect of cnidarian stinging; this dangerous condition is known to be caused by several venoms and can sometimes be lethal. At present, the bulk of data concerning hemolytic cnidarian venoms comes from the study of benthic species, such as sea anemones and soft corals, but hemolytic factors were found in venoms of several siphonophore, cubozoan and scyphozoan jellyfish, which are mainly involved in the envenomation of bathers and sea-workers. Therefore, the aim of this paper is to review the scientific literature concerning the hemolytic venoms from cnidarian jellyfish taking into consideration their importance in human pathology as well as health implications and possible therapeutic measures.

KEYWORDS: Cnidaria, jellyfish, venoms, hemolysis, plankton

INTRODUCTION

Tourists and sea-workers can frequently stumble on free swimming or floating jellyfish or on benthic corals and anemones, which could cause nuisance as well as local and/or systemic pathologies of clinical interest ranging from simple skin rashes to serious atopic or anaphylactic phenomena.

Such events may occur because these organisms own particular stinging cells – cnidocytes – containing thickened-wall capsules – nematocysts – the reservoirs for venoms. Nematocysts have a spiralized thread that, when triggered by a chemical or a mechanical stimulus, throws outside acting as a syringe needle and injecting venom into the prey/attacker. Nematocysts are linked mainly to the offence behaviour of Cnidaria, essentially predators of zooplankton and small fish that they kill using the stinging apparatus.

Due to the emergent problem of jellyfish outbreaks (Condon et al, 2012; Duarte et al, 2013; De Donno et al, 2014) and the associated health and economical consequences (Nastav et al, 2013), the research on this subject has resulted in a great deal of data about the effects of venoms.

Most Cnidaria are relatively harmless to humans and some of them are also used for food, typically in Asian countries (Armani et al, 2013). Nevertheless, some species are known to induce serious dermonecrotic, cardiotoxic and neurotoxic effects with motorial and respiratory symptoms as well as hemolytic effects. Hemolysis consists in red blood cells (RBCs) breaking down with consequent release of hemoglobin. It occurs naturally at the end of RBCs life span, but several factors such as RBC malfunction, blood dilution, hypotony, antigen-antibody reactions, hemolytic toxins, can trigger the process. Some lytic peptides alter cell permeability resulting in ion transport, cell swelling and osmotic lysis, while others are phospholipases inducing degradation of bilayer phospholipids or channel-forming agents embedded into the membrane.

At present, the bulk of data about hemolytic cnidarian venoms derives from benthic sea anemones and soft corals (Mariottini and Pane, 2014); there is less information relating to swimming/floating jellyfish, which are mainly involved in the envenomation of bathers and sea-workers. On this basis, this paper aims to provide an overview of the data concerning the hemolytic properties of venoms from planktonic Cnidarians. Data have been acquired by means of a detailed bibliographical research into scientific databases and arranged taking into account the different class of planktonic Cnidaria.

Hydrozoa

The hemolytic properties of hydrozoans have been studied mainly on the highly toxic Portuguese Man-of-War *Physalia physalis*. The major glycoprotein component of *Physalia physalis* venom is physalitoxin (about 28% of total proteins in the nematocyst venom), a potent 240kDa molecular weight (MW) hemolysin responsible for both hemolytic and lethal activities (Tamkun and Hessinger, 1981). Glycophorins, glycoproteins from the membranes of rat, dog, human and sheep RBCs, were found to neutralize hemolysis from *Physalia physalis* venom, suggesting that glycophorin could be a possible binding site for the hemolytic component of venom (Lin and Hessinger, 1979). Intravenous hemolysis of 1.6% of RBCs was observed *in vivo* in dogs injected with nematocyst venom from *Physalia physalis* (Hastings et al, 1967). Other studies reported *Physalia physalis* venom is unable to hemolyze sheep RBCs (Baxter and Marr, 1974) but causes temperature-dependent hemolysis on rat RBCs showing decrease at low temperatures (Tamkun and Hessinger, 1981). A case of hemolytic reaction associated with acute renal failure (ARF) was observed in a 4 year-old girl stung by Portuguese Man-of-War. The authors stated that no important known factor in the genesis of hemoglobin-induced ARF was documented in the patient, thus they suggested 'the possibility that some other factor contributed to the genesis of this child's ARF' (Guess et al, 1982).

Other hydrozoan jellyfish, *Pandea rubra* (Hydroida: Anthomedusae), *Arctapodema* sp., *Colobonema sericeum*, *Crossota rufobrunnea*, *Halicreas minimum* and *Pantachogon haeckeli* (Trachylina: Trachymedusae), *Aeginura grimaldii*, *Aegina citrea* and *Solmissus incisa* (Trachylina: Narcomedusae) were studied about the capability to induce hemolysis on sheep RBCs; only extracts from *Arctapodema* sp., *Colobonema sericeum*, and *Crossota rufobrunnea* caused cytolysis with ED_{50} values of 110, 190 and 100µg/ml, respectively (Kawabata et al, 2013). Table 1 shows the active concentration of hydrozoan extracts inducing hemolysis on RBCs of different species.

Cubozoa

Box jellyfish venoms are known to be hemolytic (Mariottini and Pane, 2014). Hemolysis was stated to be due to labile basic proteins 42–46kDa peculiar to these organisms, having common structural features of the N-terminal region and a transmembrane-spanning region (TSR1) with highly conserved amino acids (Nagai et al, 2000a; Nagai et al, 2000b, Nagai et al, 2002; Brinkman and Burnell, 2007). Nevertheless, the action mechanisms are in large part unknown (Brinkman and Burnell, 2009).

Table 1. Hemolysis induced by hydrozoan extracts to RBCs from different mammals; (∗) approximate values derived from original graphs; (+) whole venom; (^) hemolysin; n.p.= value not provided; n.a. = not active.

Species	Sensitive RBCs	Concentration inducing 50% hemolysis (µg/ml)	Reference
Physalia physalis			
fractionated venom	rat	0.1 (*) (+) 28 (*) (^)	Tamkun and Hessinger, 1981
crude extract	dog (*in vivo*)	n.p. (1.6% hemolysis)	Hastings et al, 1967
crude venom	sheep	n.a.	Baxter and Marr, 1974
Pandea rubra water-soluble extract	sheep	n.a.	Kawabata, 2013
Arctapodema sp. water-soluble extract	sheep	110	Kawabata, 2013
Colobonema sericeum water-soluble extract	sheep	190	Kawabata, 2013
Crossota rufobrunnea water-soluble extract	sheep	100	Kawabata, 2013
Halicreas minimum water-soluble extract	sheep	n.a.	Kawabata, 2013
Pantachogon haeckeli water-soluble extract	sheep	n.a.	Kawabata, 2013
Aeginura grimaldii water-soluble extract	sheep	n.a.	Kawabata, 2013
Aegina citrea water-soluble extract	sheep	n.a.	Kawabata, 2013
Solmissus incisa water-soluble extract	sheep	n.a.	Kawabata, 2013

The first data about hemolytic compounds from sea wasp *Chironex fleckeri* indicated the occurrence of molecules having MW 10–30kDa (Baxter and Marr, 1969). The fractionation of *Chironex fleckeri* venom yielded a low MW fraction with hemolytic activity and cardiotoxic to Guinea pigs atria (Freeman, 1974). Subsequent studies showed the hemolysis to be due to a protein of 70kDa approximately (Endean et al, 1993) and to another 120kDa protein (Naguib et al, 1988). The proteinaceous nature of the hemolytic components of venom from *Chironex fleckeri* was also suggested by Marr and Baxter (1971) after the observation of the increasing loss of hemolytic activity after incubation with crude trypsin (<1% hemolysis induced by 11.2µg/ml) and crystalline trypsin (<0.4% hemolysis induced by 500µg/ml), which digested the hemolytic component of venom in a proteolytical way. The partial purification of *Chironex fleckeri* venom was carried out by Calton and Burnett (1986) using an antivenom. The minimal amount of crude venom producing hemolysis (0.67mg) increased to 2.0mg using the purified fraction obtained employing a *Chironex*-antivenom immunochromatography column; the eluate showed an intermediate value (1.1mg). On the basis of these results, the authors stated that 'more than one component was responsible for [the writhing, hemolytic and dermonecrotic] activities' (Calton and Burnett, 1986).

Three cytolysins with molecular masses 370kDa (subdivided into CfTX-1 and CfTX-2 subunits having 43 kDa and 45kDa, respectively), 145kDa (subdivided into two major proteins 39kDa and 41kDa), and 70kDa approximately were recently isolated from *Chironex fleckeri* nematocysts and found to be hemolytic to sheep RBCs (Brinkman and Burnell, 2008). The hemolysis induced by *Chironex fleckeri* proteins showed a sigmoidal dose–response curve (Brinkman and Burnell, 2007, 2008).

The characterization of pore-forming proteins CfTX-A (~40 kDa) and CfTX-B (~42 kDa) from *C. fleckeri* and the encoding cDNA sequences have been described recently and the hemolytic activity of crude venom and purified fractions has been evaluated on sheep RBCs (Brinkmann et al, 2014).

The assumption that different MW fractions produce different hemolytic activity (Keen and Crone, 1969a) was confirmed by Brinkman and Burnell (2008) who showed the concentrations of crude venom, 370kDa and 145kDa hemolysins that caused 50% lysis (HU_{50}) were 5, 14 and 7ng protein/ml, respectively and observed a pre-lytic lag phase of approximately 6–7min (Brinkman and Burnell, 2008). The activity of subunits CfTX-1 and -2 was shown to increase with temperature with remarkable differences at 4, 18 and 37°C (Brinkman and Burnell, 2008). This agrees with results from Keen (1970) and Keen (1971) who stated that the hemolysin loses its stability from 35 to 40°C and its activity is reduced *in vivo* at 37°C on rats being the intravascular hemolysis countered by body temperature and serum proteins (Keen, 1970). Other research indicated that incubation of *Chironex fleckeri* venom at 37°C for 2 to 12 h inactivates the hemolytic activity (Torres et al, 2001) and a recent review reports the effectiveness of heat to reduce the activity of venom (Cegolon et al, 2013). The immersion in hot water can be a therapy for envenomations by *Chironex fleckeri* (Baxter and Marr, 1969) as well as for the treatment of pain (Atkinson et al, 2006). Taking into account that the low molecular weight hemolysin of *Chironex fleckeri* is both hemolytic and cardiotoxic (Freeman, 1974), heat may contribute to reduce the cardiovascular toxicity inactivating the hemolytic properties of venom.

Also the release of intracellular potassium as a consequence of hemolysis induced *in vitro* by *Chironex fleckeri* venom decreases at 37°C (Keen, 1970). The activity of *Chironex fleckeri* hemolysin is affected by pH (Keen and Crone, 1969b), having an optimum at pH 6.3–7.0, and decreases remarkably at alkaline values (Baxter and Marr, 1969).

RBCs of different species exhibited different sensitivity to 70kDa haemolysin from *Chironex fleckeri* with a ranking of resistance rat<human<mouse<guinea pig (Keen and Crone, 1969b). Hemolytic fractions of venom prepared by chromatography caused hemolysis of rabbit RBCs after 5 hours treatment. During the same research a specific interaction with an unidentified lipid component of the RBC membrane was excluded using lipid monolayers consisting of cholesterol, lecithin and mixed lipids from rabbit RBCs (Keen, 1972).

In comparison with other jellyfish, the whole nematocyst venom from *Chironex fleckeri* showed activity similar to those from *Carybdea xaymacana* and *Chiropsalmus* sp. against sheep and human erythrocytes, causing 100% hemolysis within minutes, but decreasing remarkably at high dilution factors (over than 10,000) (Bailey et al, 2005). The hemolytic activity of nematocyst venom from

Chironex fleckeri was ascribed to one fraction containing small MW components obtained through capillary elecrophoresis (Bloom et al, 2001).

Sucrose (Keen and Crone, 1969b), EDTA and divalent cations added as 1mM salts (Zn as $ZnSO_4$ and Ni as $NiSO_4$ caused 30 and 40% inhibition, respectively) inhibited hemolysis induced by venom from *Chironex fleckeri* while less efficiency showed $CdCl_2$, $FeSO_4$ (both 65% activity), Co, Mn and Mg compounds (Crone, 1976). *Chironex fleckeri* antiserum completely neutralized the hemolytic activity of sea wasp venom at 28 antihaemolytic units and caused 50% reduction using 14 antihaemolytic units defined as "that amount of serum capable of neutralizing one minimum haemolytic dose (M.H.D.) of the venom then currently in use' (Baxter and Marr, 1974). 56U of antihaemolytic antiserum inactivated the venom from *Chropsalmus quadrigatus* but the antiserum was ineffective against *Chrysaora quinquecirrha* venom (Baxter and Marr, 1974). *In vivo* studies on anaesthetized piglets showed that the hemolytic and cardiovascular effects caused by *Chironex fleckeri* venom can be reduced by the intravenous administration of specific antivenom, while the calcium-channel blocker Verapamil was ineffective (Tibballs et al, 1998). Neutralization of *in vitro* hemolytic activity induced by tentacle extracts of *Chironex fleckeri* was also obtained using monoclonal antibodies (Collins et al, 1993). Bloom et al (2001) described the inhibition of hemolysin from *Chironex fleckeri* by sera of human subjects repeatedly stung by jellyfish, observing that hemolytic activity was unaffected

by pre-incubation of RBCs with phosphatidylserine, gangliosides and sphingomyelin and was scarcely inhibited by phosphatidylcholine and phosphatidylethanolamine. On the contrary, cholesterol reduced the hemolytic activity, contradicting the results of Keen and Crone (1969b) who stated cholesterol or plasma were not able to inhibit the haemolysis induced by *Chironex fleckeri* extracts at a given concentration. The cholesterol-induced inhibition of hemolysis was confirmed recently by Brinkmann and Burnell (2009).

Recently, Yanagihara and Shohet (2012) observed the effectiveness of Zinc gluconate at 10–40U/ml/% to suppress the activity of a hemolytic porin purified from venom of *Chironex fleckeri* which was shown to hemolyse human RBCs within 20min as well as to induce the production of 12nm transmembrane pores and potassium release within 5min.

CARTOX, a heat labile Ca^{2+}-dependent 102–107kDa cytolysin of nematocystic origin, was isolated from *Carybdea marsupialis* collected in the Adriatic Sea and described as a pore-forming protein, haemolytic to sheep but not to human and rabbit RBCs and inhibited by carbohydrates (sialic acid and b-methylgalactopyranoside) and lipids (sphingomyelin and phosphatidylinositol) (Rottini et al, 1995). Subsequently, Sánchez-Rodríguez et al (2006) isolated three cytolysins (220 kDa, 139 kDa and 36kDa) active on human RBCs from nematocyst venom of Caribbean *Carybdea marsupialis*. The cytolytic activity of *Carybdea marsupialis* venom was presumably due to binding to carbohydrates and lipids on the cell surface (Rottini et al, 1995; Gusmani et al, 1997; Chung et al, 2001).

Carybdea rastoni has been described as "one of the most bothersome stinging pests to swimmers and bathers on the Japanese coast" (Nagai et al, 2000a). From tentacles of this jellyfish Azuma et al (1986) partially purified three highly haemolytic proteins (CrTX-I, CrTX-II and CrTX-III) which also induced platelet aggregation. Subsequently, Nagai et al (2000a) sequenced the full-length cDNA of *Carybdea rastoni* (1600bp) encoding two labile hemolytic proteins (CrTX-A mainly nematocystic, and CrTX-B occurring in tentacle tissues) having molecular masses 43 and 46kDa, respectively, and published the first complete amino acid (450) sequence of a proteinaceous jellyfish toxins.

Nagai et al (2000b) isolated two hemolytic toxins (CaTX-A, 43kDa nematocystic, and CaTX-B, 45kDa extra-nematocystic) from tentacles of *Carybdea alata* and published the sequence of the cDNA encoding CaTX-A, the result of which showed a composition of 463 amino acids and 43.7% homology to the CrTX toxins from *Carybdea rastoni*. Recently, based on the partial N-terminal amino acid sequence, similar haemolytic proteins CAH1 (42kDa) and CaTX-A were isolated from the nematocyst venom of *Carybdea alata* by Chung et al (2001) and by Nagai et al (2000b), which were suggested to be the same (Brinkmann and Burnell, 2009).

The activity of venom from this cubozoan is affected in the presence of Mg^{2+}, Ca^{2+} and Zn^{2+} with optimum values of 10mMCa^{2+} or Mg^{2+} (Chung et al, 2001). The protein CAH1, having an apparent mass of 42kDa, seems to be responsible for the hemolysis induced by *Carybdea alata* venom which involves docking or binding with cell surface carbohydrates or phospholipids (Chung et al, 2001).

The whole nematocyst venom from *Carybdea xaymacana* showed considerable hemolytic activity against sheep and human RBCs inducing 100% and over 60% hemolysis at a dilution factor 10,000 and 100,000, respectively (Bailey et al, 2005).

Despite the chirodropid *Chiropsalmus quadrigatus* has been reported to be responsible of three fatal cases in Japan, the hemolytic activity on sheep RBCs of the purified hemolytic protein CqTX-A (ED_{50} = 160ng/ml) revealed it seems less effective than proteins purified from other carybdeid jellyfish known to be less dangerous. The remarkable danger of *Chiropsalmus quadrigatus* in nature was suggested to be due to the length and size of tentacles, which allow the injection of large doses of toxin. The cDNA amino acid sequence (462 amino acids) is similar to *Carybdea rastoni* CrTXs and to *Carybdea alata* CrTX-A (25.2% and 21.6%, respectively) (Nagai et al, 2002). The optimum temperature for *Chiropsalmus quadrigatus* extracts to cause hemolysis on rabbit RBCs was reported as approximately 15°C, the hemolysin was inactivated at 35°C and lost its stability at 35≥40°C (Keen, 1971). The whole nematocyst venom from *Chiropsalmus* sp. exhibited remarkable (100% within minutes) but labile hemolytic activity decreasing to nearly 90% and 35% after dilution at factors 100 and 1,000, respectively and falling to values lower than 10% at dilution factor 10,000 (Bailey et al, 2005).

As for the clinical implications of hemolytic properties of cubozoan venoms, the matter is controversial. In fact, hemolysis has not been demonstrated to be a clinical feature in envenomed humans from cubozoans (Bailey et al, 2005; Tibballs, 2006; Brinkmann et al 2014). This indicates that the activity *in vivo* of cubozoan toxins could preferentially targeted to other cell types, even though '*in vivo* effects in rats suggest that these toxins may be the primary cause of similar effects in humans' (Brinkman et al, 2014).

Furthermore, other studies show that haemolytic activity does not correlate with lethality (Bailey et al, 2005) and monoclonal antibodies which neutralize '*C. fleckeri* induced haemolysis do not protect against the lethal effects of venom in an experimental animal model of envenoming' (Collins et al, 1993).

Considering the constant occurrence of hemolysis *in vitro* and in experimental animal envenoming (Collins et al, 1993; Endean et al, 1993), Bailey et al (2005) suggested that 'there are fundamental differences between jellyfish venom prepared for laboratory studies and that injected into victims, or that there are specific mechanisms present in envenomed humans but not present in experimental animals that inhibit the toxic haemolytic proteins in jellyfish venom'. Furthermore, haemolytic toxins may not be capable of reaching the systemic circulation (Bailey et al, 2005). Table 2 shows the active concentrations of cubozoan extracts inducing 50% hemolysis on RBCs of different species.

Table 2. Hemolysis induced by cubozoan extracts to RBCs from different mammals, birds and fish; (*) human, monkey, dog, cat, horse, sheep, goat, rabbit, guinea pig, rat, mouse, ferret, echidna, chicken, pigeon, trout; (+) MHD = Minimum Hemolytic Dose (see the original paper for details); (^) mixture; (#) hemolysin titre estimated as 'the logarithm of the reciprocal of the highest dilution of extract which produced 50% hemolysis' (Keen, 1971).

Species	Sensitive RBCs	Concentration inducing 50% hemolysis	Reference
Chironex fleckeri			
nematocyst venom	16 species (*)	2560–20MDH/ml (+)	Baxter and Marr, 1969
crude venom	sheep	148ng/ml	Brinkmann et al, 2014
co-purified CfTX-1/2	sheep	161ng/ml	Brinkmann et al, 2014
CfTX-1/2, CfTX-A/B (^)	sheep	22ng/ml	Brinkmann et al, 2014
purified CfTX-A and –B	sheep	5ng/ml	Brinkmann et al, 2014
undiluted venom	human sheep	100% within minutes	Bailey et al, 2005
crude nematocyst venom	human	0.04µg/ml	Bloom et al, 2001
crude venom	sheep	5ng protein/ml	Brinkmann and Burnell, 2008
370kDa hemolysin	sheep	14ng protein/ml	Brinkmann and Burnell, 2008
145 kDa hemolysin	sheep	7ng protein/ml	Brinkmann and Burnell, 2008
crude extract	rabbit	5.2–6.0 (#)	Keen, 1971
Carybdea rastoni			
CrTX-A	sheep	1.9ng/ml	Nagai et al, 2000a
CrTX-B	sheep	2.2ng/ml	Nagai et al, 2000a
Carybdea alata			
CaTX-A	sheep	70ng/ml	Nagai et al, 2000b
CaTX-B	sheep	80ng/ml	Nagai et al, 2000b
Carybdea xaymacana			
undiluted venom	human sheep	100% within minutes	Bailey et al, 2005
Chiropsalmus quadrigatus			
crude extract	rabbit	3.9–5.0 (#)	Keen, 1971
CqTX.A	sheep	160ng/ml	Nagai et al, 2002
Chiropsalmus sp.			
undiluted venom	human sheep	100% within minutes	Bailey et al, 2005

Scyphozoa

Rhizostomeae

Although Scyphozoa are known to be less dangerous than Cubozoa, the hemolytic properties of some species are well known (Mariottini et al, 2008; Mariottini and Pane, 2010).

Stomolophus meleagris, the cabbage head jellyfish, is known to induce strong hemolytic effects on rats at doses ranging from 14 to 19mg/Kg (Toom et al, 1975). Recent purification of the hemolytic protein SmTX from nematocyst venom yielded two protein bands with apparent MWs of ≈45kDa and 52kDa in the SDS-PAGE analysis. The temperature- (optimum at 37°C) and pH-dependent (optimum at pH 5.0) hemolytic activity of SmTX (HU_{50} = 70µg/ml approximately) was observed on chicken RBCs (Li et al, 2013).

Partially purified venom from *Rhopilema nomadica*, elicited remarkable but unstable hemolytic activity against human, rabbit, sheep and guinea-pig RBCs with HU_{50} values 32, 55, 57 and 67ng, respectively. Hemolysis occurred mainly at low temperature (4°C) and decreased remarkably at 20–37°C. The increase of pH and the activity of the enzyme α-chymotrypsin dramatically reduced the hemolytic activity. Some enzymes, lipids and carbohydrates were observed to reduce the toxin-induced hemolysis and high inhibition was caused by phosphatidylserine, N-acetylneuraminic acid and phosphatidylethanolamine (92, 78 and 77% inhibition, respectively) (Gusmani et al, 1997). A protease prepared from oral arms of *Rhopilema esculentum* affected the hemolytic activity of venom having minimal activity at pH 7.5 (Li et al, 2005). After studies on chicken and rabbit RBCs temperature was shown to have a strong effect on the hemolytic activity of venom from *Rhopilema esculentum* (Yu et al, 2007).

Strong hemolytic activity (7µg/ml induced 50% hemolysis to human RBCs) was caused by *Cassiopea xamachana* after fluorescence activated cell sorting of nematocyst from symbiotic algae occurring into the epithelial tissue of tentacles. As this jellyfish is not dangerous in nature, symbiotic algae were supposed to play a role in venom inhibition (Radwan and Burnett, 2001). Torres et al (2001) reported

that *Cassiopea xamachana* venom produced concentration-dependent hemolysis on sheep and, particularly, human RBCs with HU_{50} values of 56 and 6.89µg/ml, respectively. Incubation (2–12hr) at 37°C inactivated the venom while the activity was preserved after 24hr incubation with protease inhibitors. In a comparative study, the HU_{50} values of *Cassiopea andromeda* and *Cassiopea xamachana* venoms on human RBCs were 1 and 11µg protein, respectively. In both venoms the hemolytic activity was inhibited by cholesterol, phosphatidylcholine and gangliosides (Radwan et al, 2001). Subsequently, fractions (I-VI) of crude venom from *Cassiopea xamachana* were tested for hemolytic activity on human RBCs. Hemolysis was induced by all fractions, particularly those containing low molecular mass components (≤10kDa). The authors suggested that fraction VI contributes "to most of cytolysis as well as membrane binding events" (Radwan et al, 2005).

Rhizolysin, a cytolysin having an apparent MW of 274kDa, was isolated from *Rhizostoma pulmo* nematocysts. 5 and 11µg protein, respectively induced 20% hemolysis after 25min at 30°C on human and rat RBCs. Hemolysis was highly affected by pH and inhibited (50 and 20%, respectively) by 50µg/ml cholesterol and sphingomyelin (Cariello et al, 1988). The cytolytic activity of the nematocyst-free fraction obtained from *Rhizostoma pulmo* oral arms caused scarce hemolysis to human RBCs (Allavena et al, 1998). Further HPLC preparation yielded high MW fractions inducing complete hemolysis at 32µg/ml on human RBCs, suggesting that venom has a good capability to bind to membranes (Mazzei et al, 1995).

The venom from the giant jellyfish *Nemopilema nomurai* was shown to have a concentration-dependent hemolytic activity against mammal RBCs starting from 10µg/ml. EC_{50} values for dog, rat, cat, rabbit and human RBCs were 151, 497, 685, 729 and 964µg/ml, respectively (Kang et al, 2009).

The clinical evidence of hemolysis in humans stung by Rhizostomeae is not demonstrated, even though some data, such as that concerning the hemolytic properties of *Stomolophus meleagris*, "may be consistent with at least some of the observed effects of jellyfish stings *in vivo*" and may account in part for the ability of hemolytic proteins in nematocyst venom "to cause toxic effects including erythema and edema" (Li et al 2013). Hemolysis has been also defined as an established mediator of toxicity (Li et al, 2013). Furthermore, hemolysis induced by *Cassiopea* venom has been observed to be coexistent with dermonecrosis and vasopermeability and correlated with mice lethality (Radwan et al, 2001).

Semaeostomeae

Over twenty years ago the venom of a Thai variety of *Aurelia aurita* exhibited hemolytic activity (Pong-Prayoon et al, 1991). Subsequently, the venoms from *Aurelia aurita* from the Red Sea and Chesapeake Bay were shown to have different hemolytic properties (50% hemolysis was induced by 1 and 8µg protein, respectively); in all cases the hemolysis was inhibited by cholesterol, dihydrocholesterol, phosphatidylcholine, phosphatidylserine and gangliosides (Radwan et al, 2001). Extracts from tentacular margins of *Aurelia aurita* caused hemolysis of human sheep and bovine RBCs. Human RBCs resulted the most sensitive reaching 50% hemolysis after treatment with 0.61mg of crude venom protein, while sheep and bovine RBCs never reach 50% hemolysis at the tested concentrations (approximately 0–0.7 mg protein/ml) showing a maximum of about 30%. The different sensitivity was ascribed to the differences in the sphingomyelin content of RBC membranes. The authors suggested that at least two active principles are present and the effect to human RBCs of the second hemolysin becomes apparent only at doses above 0.4 mg/protein/ml (Segura-Puertas et al, 2002). The protein-membrane interaction resulting in hemolysis was recently studied using a chip-based technology with immobilized liposomes (Helmholz, 2010). Proteins from *Aurelia aurita* resulted hemolytic with EC_{50} values of 35.3 and 13.5µg/ml against sheep and rabbit RBCs, respectively. The 50% binding level, indicating the strength of binding (RU_{50}), ranged from 12.4 to 29.9µg/ml and a preference was found for binding to cholesterol and sphingomyelin and less for phosphatidylcholine. The binding of lysins was found to increase as a function of protein concentration (Helmholz, 2010).

The hemolytic properties of the crude venom from the mauve stinger *Pelagia noctiluca* have been described to be time- and dose-dependent, 0.1µg/ml inducing 90% hemolysis on rabbit and chicken RBCs and 35% and 41% on human and eel RBCs, respectively, up to a complete hemolysis of RBCs from all species at 0.5µg/ml (Marino et al, 2007). The hemolysis from *Pelagia noctiluca* venom is countered by osmotic protectants, such as ions Ba^{2+} and Cu^{2+} (70 and 90% inhibition, respectively), but only slightly by antioxidants (22–27% inhibition) and almost negligibly by carbohydrates and proteases (Marino et al, 2008). The venom was observed to act through a pore-forming mechanism (Marino et al, 2009). Notwithstanding the venom from *Pelagia noctiluca* was asserted to be scarcely effective on fish RBCs (Marino et al, 2007), recent results indicate that four proteic fractions induce lysosomal membrane destabilisation of freshwater (*Carassius auratus*) and marine (*Liza aurata*) fish RBCs and sphingomyelin strongly inhibits the activity (Maisano et al, 2013).

The comparison between nematocyst venom from fishing and mesenteric tentacles of *Chrysaora quinquecirrha* showed that fishing tentacles have more hemolytic potency on mouse RBCs and venoms possess different hemolysins (Burnett and Calton, 1976). Subsequently, after observing that lethal doses of nematocyst venom from *Chrysaora quinquecirrha* mesenteric tentacles did not induce hemolysis on rat RBCs, Kelman et al (1984) stated that "hemolysis is probably not a significant factor in the toxicity" of sea nettle venom. The MW (6–10kDa) of the hemolysin of *Chrysaora quinquecirrha* nematocysts was then determined and the occurrence of large quantities of glycine and serine was found; the dose-dependent hemolysis induced by crude venom on human RBCs and an approximately 80% inhibition of hemolysin by glycophorin and sphingomyelin were also described (Long and Burnett, 1989).

Evaluation of the activity of hemolysin from fishing tentacles on various RBCs showed that chicken erythrocytes were more resistant than mammalian erythrocytes (Long-Rowe

and Burnett, 1994). Fractionated venom of nematocysts from *Chrysaora quinquecirrha* fishing tentacle is able to produce nonspecific pores on cell membrane and the hemolysis is caused by only a fraction (Bloom et al, 2001). 63µg protein/ml from fishing tentacles of *Chrysaora quinquecirrha* induced 50% hemolysis on human RBCs, remarkably higher than that caused by fishing (150µg protein/ml) and by mesenteric tentacles (220µg protein/ml) from *Chrysaora achlyos* (Radwan et al, 2000).

A hemolytic labile fraction of venom from *Chrysaora hysoscella* was studied by Del Negro et al (1991) who showed its partial proteinaceous nature and the occurrence of a cationic protein. The dialysed supernatant obtained from the centrifugation of nematocyst fluid was evaluated on mouse, sheep and human RBCs resulting in remarkable hemolysis, mainly on mouse erythrocytes.

Cyanea capillata nematocysts were found to include glycine- and serine-rich hemolytic fractions. The crude venom induced a clear dose-dependent hemolysis on human RBCs that was inhibited up to 40–50% by glycophorin, cholesterol and sphingomyelin (Long and Burnett, 1989). Subsequently, purified cnidocyst extracts from fishing and mesenteric tentacles of *Cyanea capillata* and *Cyanea lamarckii* were shown to induce dose-dependent haemolysis on human RBCs with differences between extracts resulting from small or large specimens and between mesenteric or fishing tentacles (Helmholz et al, 2007). In a subsequent study the crude venom from mesenteric tentacles of large *Cyanea capillata* induced more pronounced RBC lysis (HE_{50}=98µg/ml) than that of small medusae (HE_{50}=177µg/ml) (Helmholz et al, 2012).

The hemolysis induced by tentacle extract devoid of nematocysts from *Cyanea capillata* was studied on sheep, rabbit, mouse, rat and human RBCs and shown to be dose-dependent and to decrease after addition of serum albumin as well as of plasma. The 0.45% erythrocyte suspension was hemolyzed more strongly than 1% whole blood. 20mmol/l antioxidants GSH and ascorbic acid induced a significant ($P<0.05$) inhibition of hemolysis in RBCs exposed to 400µg/ml tentacle extract (Wang et al, 2012). The hemolytic activity of *Cyanea capillata* was ascribed to protein-membrane interactions subsequent to lysin adsorption into membrane lipids and resulted in EC_{50} value of 43.1µg/ml against sheep RBCs and 8.8µg/ml against rabbit RBCs and RU_{50} ranging from 21.0 to 35.4µg/ml. The hemolysin from *Cyanea capillata* bound preferably to cholesterol and sphingomyelin, having less affinity for phosphatidylcholine (Helmholz, 2010). Alkaline denaturation and dialysis induced complete loss of the hemolytic properties of *Cyanea capillata* crude extracts devoid of nematocysts. The cardiovascular toxicity and the hemolysis were stated to be independent and driven by different proteins (Liang et al, 2012).

Extracts devoid of nematocysts from *Cyanea capillata* caused dose-dependent haemolysis in male rat RBCs with almost complete lysis at 800µg/ml. A protective role of blood plasma was concluded (Xiao et al, 2010). Recently, a concentration-dependent increase of hemolysis in the presence of Ca^{2+} and a decrease induced by Ca^{2+} channel blockers (Diltiazem, Verapamil, Nifedipine) was observed in rat RBCs exposed to extracts from *Cyanea capillata* tentacles (Wang et al, 2013).

A concentration-dependent hemolytic activity on chicken RBCs at pH optimum of 7.8 was also observed for *Cyanea nozakii* nematocyst venom. Incubation with sphingomyelin inhibited hemolysis up to approximately 20%. Ca^{2+} increased hemolysis in a concentration-dependent way while Cu^{2+}, Mn^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} reduced and 5mM EDTA completely inhibited the activity (Feng et al, 2010). Somewhat different results were obtained studying the purified hemolysin CnPH. The HU_{50} was reached treating RBCs with approximately 5µg/ml of CnPH while complete hemolysis was observed with 11µg/ml. The hemolysis that resulted was both temperature- and pH-dependent, was enhanced by EDTA and inhibited by Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Ca^{2+} (Li et al, 2011).

Recent data concerning extracts from other scyphozoans, the deep-sea Coronatae *Atolla vanhoeffeni*, *Atolla wyvillei* and *Periphylla periphylla*, showed they are unable to hemolyse sheep RBCs (Kawabata et al, 2013). The active concentrations of scyphozoan extracts inducing hemolysis on RBCs of different species are shown in table 3.

CONCLUSIONS

The encounter with free swimming or floating Cnidaria is a frequent occurrence for tourists or workers who are active in the marine environment. The effects can range from simple nuisance to serious pathological and lethal events. Hemolysis is a frequent effect of a number of cnidarian venoms acting as phospholipases, which induce degradation of bilayer phospholipids, or act as channel-forming agents embedded into the membrane. Literature concerning hemolytic cnidarian jellyfish is widely available and the review of papers published during the last five decades allows us to conclude that;

- hemolytic compounds have been found in all most dangerous species
- hemolytic properties of cnidarian venoms are often connected to other unpleasant effects but are caused by different compounds
- mammalian RBCs are a good tool to study the hemolytic effects of cnidarian venoms
- the sensitivity of human RBCs in some cases differs from that of other mammalian RBCs
- the hemolysis induced by cnidarian venoms can be fought by several compounds that could be used as therapeutical tools
- heat could be mentioned as an approach to contrast the hemolytic effects of venom form various jellyfish species

In conclusion, the research about cnidarian venoms needs to develop towards a better knowledge of venomous compounds and, consequently, to the characterization of fractions responsible for the different toxic activities extracted from dangerous species whose venom composition is at present largely unknown.

Table 3. Hemolysis induced by scyphozoan extracts to RBCs from different mammals, birds and fish; (*) from Red Sea (AaRS); (+) from Chesapeake Bay (AaCB); (^) after Fluorescence Activated Cell Scanner (FACS) preparation; (#) post-Sephadex G-200; (§) value obtained after incubation with 100mg protein/ml; (○) from fishing tentacles; (●) from oral arms; (□) approximate values derived from original graphs; (■) 0.45% RBC suspension; n.a. = not active; n.v. = nematocyst venom; l.s. = from large specimen; s.s. = from small specimens; PH = purified hemolytic fraction

Species	Sensitive RBCs	Concentration inducing 50% hemolysis	Reference
Atolla vanhoeffeni			
water-soluble extract	sheep	n.a.	Kawabata, 2013
Atolla wyvillei			
water-soluble extract	sheep	n.a.	Kawabata, 2013
Aurelia aurita			
crude venom (*)	human	1µg venom protein	Radwan et al, 2001
crude venom (+)	human	8µg venom protein	Radwan et al, 2001
crude venom protein	human	0.61mg/ml	Segura Puertas et al, 2002
lysine	sheep	35.3µg/ml	Helmholz, 2010
	rabbit	13.5µg/ml	
Carybdea marsupialis			
nematocyst venom	sheep	27.91ng/ml	Rottini et al, 1995
Cassiopea andromeda			
crude venom	human	1µg protein	Radwan et al, 2001
Cassiopea xamachana			
crude venom (^)	human	7µg/ml	Radwan and Burnett, 2001
crude venom	human	11µg protein	Radwan et al, 2001
crude venom	human	6.89µg/ml	Torres et al, 2001
	sheep	56µg/ml	
crude venom CxTX	human	40.6% hemolysis (§)	Radwan et al, 2005
fractions I-VI (#)		15.2–76.5% hemolysis (§)	
Chrysaora achylos			
crude venom (○)	human	150µg/ml	Radwan et al, 2000
crude venom (●)	human	220µg/ml	Radwan et al, 2000
Chrysaora hysoscella			
>10KDa MW n.v.	human	>5.0µg protein (□)	Del Negro et al, 1991
	mouse	2.7µg protein approx. (□)	
	sheep	3.5µg protein approx. (□)	
Chrysaora quinquecirrha			
crude venom	human	0.5–0.63mg protein	Long-Rowe and Burnett, 1994
	dog	0.34mg protein	
	pig	0.15mg protein	
	horse	1.29mg protein	
	sheep	0.66mg protein	
	rabbit	1.52mg protein	
	rat	0.28mg protein	
	chicken	2.58mg protein	
crude venom (○)	human	63µg/ml	Radwan et al, 2000
nematocyst venom (○)	human	35µg/ml	Bloom et al, 2001
Cyanea capillata			
nematocyst venom (l.s.)	rodent	98µg/ml	Helmholz et al, 2012
nematocyst venom (s.s.)	rodent	177µg/ml	Helmholz et al, 2012
lysine freshly prepared	sheep	43.1µg/ml	Helmholz, 2010
	rabbit	8.8µg/ml	
lysine stored at -80°C	sheep	>50µg/ml	Helmholz, 2010
	rabbit	>50µg/ml	
tentacle-only extract	rat	150µg/ml (□)(■)	Xiao et al, 2010
tentacle-only extract	human	210µg/ml (□)(■)	Wang et al, 2012
	sheep	156µg/ml (■)	
	rabbit	210µg/ml (□)(■)	
	rat	230µg/ml (□)(■)	
	mouse	260µg/ml (□)(■)	

Cyanea nozakii			
fresh n.v.	chicken	5.08µg/ml approx.	Feng et al, 2010
–80°C stored n.v.	chicken	5.35µg/ml approx.	Feng et al, 2010
CnPH		HU50 approx. 5µg/ml	Li et al, 2011
Nemopilema nomurai			
crude extract	human dog cat rabbit rat	964 µg/ml 151 µg/ml 685 µg/ml 729 µg/ml 497 µg/ml	Kang et al, 2009
Pelagia noctiluca			
crude extract	human rabbit chicken eel	0.1µg/ml (35% hemolysis) 0.1µg/ml (90% hemolysis) 0.1µg/ml (90% hemolysis) 0.1µg/ml (41% hemolysis)	Marino et al, 2007
Periphylla periphylla			
water-soluble extract	sheep	n.a.	Kawabata, 2013
Rhizostoma pulmo			
rhizolysin	human	5µg protein (20% hemol.)	Cariello et al, 1988
	rat	11µg protein (20% hemol.)	
high MW fractions	human	32µg/ml (100% hemolysis)	Mazzei et al, 1995
Rhopilema nomadica			
partially purified venom	human sheep rabbit guinea-pig	32ng 57ng 55ng 67ng	Gusmani et al, 1997
Stomolophus meleagris			
crude venom purified fractions SmTX	chicken	10.5µg/ml 110µg/ml 70µg/ml	Li et al, 2013

COMPETING INTERESTS

None declared.

LIST OF ABBREVIATIONS

RBCs; red blood cells
TSR1; transmembrane-spanning region
HU_{50}; concentration of protein causing 50% lysis
C_{50}: 50% hemolysis

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