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MINIREVIEW

Marine Snails and Slugs: a Great Place To Look for Antiviral Drugs

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Molluscs, comprising one of the most successful phyla, lack clear evidence of adaptive immunity and yet thrive in the oceans, which are rich in viruses. There are thought to be nearly 120,000 species of Mollusca, most living in marine habitats. Despite the extraordinary abundance of viruses in oceans, molluscs often have very long life spans (10 to 100 years). Thus, their innate immunity must be highly effective at countering viral infections. Antiviral compounds are a crucial component of molluscan defenses against viruses and have diverse mechanisms of action against a wide variety of viruses, including many that are human pathogens. Antiviral compounds found in abalone, oyster, mussels, and other cultured molluscs are available in large supply, providing good opportunities for future research and development. However, most members of the phylum Mollusca have not been examined for the presence of antiviral compounds. The enormous diversity and adaptations of molluscs imply a potential source of novel antiviral compounds for future drug discovery.

Mollusca is the second-most-diverse phylum, with nearly 85,000 described species (1, 2) and a total world estimate of 120,000 to 200,000 species (3). Molluscs are characterized into eight classes, including Gastropoda, Bivalvia, Scaphopoda, Cephalopoda, Polyplacophora, Monoplacophora, Caudofoveata, and Solenogastres, which can be found in a wide range of ecological niches from marine (52,525 described species) and freshwater (7,000) to terrestrial (24,000) habitats (1, 2). In the food chain, molluscs may be herbivores, carnivores, detritivores, scavengers, filtered feeders, or symbiotic photo- and chemoautotrophs (4). The enormous and very successful adaptation and radiation of molluscs suggest that they possess highly efficient pathways to counter infectious diseases.

Their resistance to infectious disease is especially striking in oceans, where viruses are hyperabundant: in marine environments, virus numbers commonly reach 10^7 particles per milliliter, outnumbering bacteria and archaea by a factor of 10 (5–7). Viral infections are common, with an estimate of 10^{23} infections (of bacteria) occurring every second in the world's oceans (7). Despite living under such virus-rich conditions, many molluscs achieve extraordinary life spans; for example, individual examples of the ocean quahog, *Arctica icelandica*, an edible clam from the north Atlantic, reportedly attain ages of over 400 years (8).

To combat viral infection, molluscs, like other marine invertebrates, are generally believed to rely on innate immunity since there is as yet no clear evidence of adaptive antiviral immunity (9–11). While a system for somatic diversification of fibrinogen-related proteins (FREPs) has been linked to the resistance of *Biomphalaria* snails to the trematode parasites schistosomes (12), it is unknown if FREPs play any role in antiviral defense. The innate immune system in molluscs is provided by physical barriers (e.g., shell, skin, and epithelium), as well as by a variety of immune mechanisms that include antimicrobial compounds (13). Antimicrobial compounds are constitutively expressed or rapidly induced to counter invading microorganisms (14, 15).

To date, over 1,120 secondary metabolites have been isolated from just over 270 species of marine mollusc (~0.3% of named mollusc species) (4, 16). Less than 50% of these molluscan natural products have been examined to date to ascertain if they might be pharmacologically useful; however, of those tested, a wide range have been reported to possess such properties, including anticancer, antimicrobial, neurotoxic, and specific receptor binding activities (4, 16). In the last couple of decades, the discovery and development of novel anticancer agents have been the main focuses for researchers of marine natural products and several molluscan lead compounds have entered final-stage clinical trials (17). By comparison, only 6% of molluscan natural products have been tested for antimicrobial activity (16), and of these, only a few have been examined for antiviral activity.

MOLLUSCS MAKE ANTIVIRAL COMPOUNDS THAT ARE ACTIVE AGAINST A VARIETY OF HUMAN VIRUSES

Molluscs make antiviral compounds that are active against a range of human viruses, which may reflect the inability of their innate immunity to tailor responses to specific viral pathogens. The antiviral properties of molluscan compounds against their own viruses are largely untested. The lack of proliferative cell lines derived from molluscs presents great difficulties in reproducing molluscan viruses *in vitro* (18). While *in vivo* infection systems have been developed for some molluscan species, such as the use of an experimental immersion challenge system for the study of abalone herpesvirus infections in Australian abalone (19), there a only few such models for infecting molluscs. The extent to which the antiviral compounds discussed here are a true reflection of molluscan antiviral immunity remains speculative. It has been shown that antiviral activity against herpes simplex virus 1 (HSV-1) does not correlate with higher resistance to abalone herpesvirus (20).

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TABLE 1 Antiviral extracts and compounds found in gastropod and bivalve molluscs, with suggested modes of action[a]

Mollusc common name and species	Antiviral extract or compound	Virus target(s)	Suggested mode of action	Reference(s)
Gastropod				
Abalone *Haliotis laevigata* and *H. rubra*	Lipophilic extract from the digestive gland	HSVs	Antiviral activity occuring postentry	30
	Hemolymph plasma	HSV-1	Prevention of viral attachment and entry into cells	30
Abalone *H. rufescens*	Aqueous extract from canned abalone	Polyomavirus, influenza A virus, and poliovirus	Unknown	21, 22
Periwinkle *Littorina littorea*	Peptide extract from whole organism (littorein)	HSV-1	Unknown	34
Snail *Buccinulum corneum*	Kelletinin A [ribityl-pentakis (p-hydroxybenzoate)]	Human T-cell leukemia virus type 1	Inhibition of viral transcription and DNA/RNA synthesis (i.e., by inhibition of virus DNA polymerase α and reverse transcriptases)	40, 42
Veined rapa whelk *Rapana venosa*	Glycosylated functional unit of hemocyanin/RtH2	Respiratory syncytial virus, HSV-1 and HSV-2, and EBV	Prevention of virus attachment to cells by interaction with specific regions of HSV glycoproteins	27, 32, 39, 43, 39
Snail *Helix lucorum*	Hemocyanin extract	EBV	Inhibition of viral DNA replication	44
Whelk *Buccinum undatum*	80% SPE fraction from the acidic extract of whole organism	HSV-1	Unknown	33
Bivalves				
Clam *Mya arenaria*	Water and ammonium sulfate extract/paolin	Amphibian virus LT-1, adenovirus type 12, HSV	Inhibition of viral infection (LT-1) at intracellular level; inhibition of tumors in hamsters by adenovirus 12	24, 26
Clam *Mercenaria mercenaria*	Partially purified ammonium sulfate extracts	Moloney and Friend murine leukemia viruses	Unknown	45
Clam *Ruditapes philippinarum*	80% SPE fraction from the acidic extract of whole organism	HSV-1	Unknown	33
Cockle *Cerastoderma edule*	80% SPE fraction from acidic extract of whole organism	HSV-1	Unknown	33
Mediterranean mussel *Mytilus galloprovincialis*	Mytilin	White spot syndrome virus	Inhibition viral transcription	29
	Defensin	HIV-1	Unknown	38
Mussel *Crenomytilus grayanus*	Lectin	HIV	Blocking of viral entry	37
Oyster *Crassostrea virginica*	Acetic acid extract/paolin 2	Poliovirus type 1	Unknown	41
Oyster *C. gigas*	Hemolymph plasma and peptide extracts from the whole animal	HSV-1, infectious pancreatic necrosis virus, human adenovirus, simian rotavirus	Inhibition of viral attachment to the cell surface	35, 36, 46–48
Oyster *C. rhizophorae*	Hemolymph plasma and peptide extracts from the whole animal	HSV-1, human adenovirus, simian rotavirus	Inhibition of viral attachment to the cell surface	46
Oyster *Ostrea edulis*	80% SPE fraction from the acidic extract of gills and mantle	HSV-1	Unknown	33

[a] HIV-1, human immunodeficiency virus type 1; SPE, solid-phase-extraction.

Antiviral activity, mostly against human viruses, has been reported so far in at least eight gastropod species, including abalone (*Haliotis laevigata*, *H. rubra*, and *H. rufescens*), periwinkle (*Littorina littorea*), snail (*Buccinulum corneum* and *Tegula gallina*), veined rapa whelk (*Rapana venosa*), and whelk (*Buccinum undatum*), and nine bivalve species, including the clams *Mercenaria mercenaria*, *Mya arenaria*, and *Ruditapes philippinarum*, cockle (*Cerastoderma edule*), mussels (*Mytilus galloprovincialis* and *Crenomytilus grayanus*), and (oysters *Crassostrea virginica*, *C. gigas*, and *Ostrea edulis*) (21–41) (Table 1). This leaves approximately

100,000 species of molluscs unexamined for the presence of antiviral compounds.

STRUCTURES AND MECHANISMS OF ACTION OF MOLLUSCAN ANTIVIRAL COMPOUNDS

To date, most characterized extracts from mollusc species with *in vitro* antiviral activity have been peptides or glycopeptides, for example, kelletinin A from *Buccinulum corneum* (40), glycosylated functional unit RtH2 of hemocyanin from *Rapana venosa* (27, 32, 39), mytilin and defensin from *Mytilus galloprovincialis* (29, 38), and lectin from *Crenomytilus grayanus* (37). Their modes of action are not fully characterized; however, they appear to include direct inactivation of virus and prevention of viral attachment to or entry into host cells or inhibition of viral transcription and DNA or RNA synthesis (Table 1).

Molluscs have been found to have broad antiviral activity against viruses from different families. For example, oyster hemolymph has been shown to contain compounds active against T3 coliphage (family *Podoviridae*) (49), herpes simplex virus type 1 (*Herpesviridae*), infectious pancreatic necrosis virus (*Birnaviridae*) (35), and human adenovirus type 5 (*Adenoviridae*) (50). The means by which these species have come to have antiviral mechanisms that are sufficiently universal to interfere with human viral systems remain open for speculation.

Molluscan hemocyanins, copper-containing oxygen transport macromolecules, have also been observed to be multifunctional, with innate immune functions, including antiviral activity, as demonstrated against the herpesviruses herpes simplex virus (HSV) and Epstein-Barr virus (EBV), in the abalone *H. rubra* (51) and the veined whelk *Rapana venosa* (32, 43). These observations were extended to include *Rapana venosa* activity against HSV-1 and HSV-2 (39). Hemocyanin of the land snail *Helix lucorum* also has activity against EBV (44). Hemocyanin antiviral activity has been attributed to glycosylated sites (27, 32, 51, 52). It was previously suggested that hemocyanin carbohydrate chains interact with surface-exposed amino acid or carbohydrate residues of the viruses through van der Waals interactions (53). Despite hemocyanins having been found to be associated with arthropod antiviral immunity due to their increased expression upon viral infection (54, 55) and the finding that their antiviral activity is effective against their own arthropod viruses (56, 57), the role of hemocyanins in molluscan immunity remains unclear. The establishment of mollusk-derived cell lines is required to test the activity of hemocyanins against molluscan viruses. Further studies are needed to determine if there is upregulation of hemocyanins in molluscs upon viral infection *in vivo*.

Cavortin, the major hemolymph protein of the Pacific oyster, *C. gigas*, has been shown to have activity against HSV, with this effect exerted after entry of the virus into cells (58). This protein has amino acid sequence similarity to the major hemolymph protein of the eastern oyster *C. virginica*, which is considered to have a domain resembling superoxide dismutase, which has been shown to have antiviral properties (59–62), but the protein reportedly lacks superoxide dismutase activity (63–65). The mechanism for antiviral activity of cavortin remains unclear.

Molluscan natural products encompass a wide variety of chemical classes that extend well beyond the proteinaceous compounds that have been tested for antiviral activity to date (e.g., terpenes, alkaloids, polyproprionates, fatty acid derivatives, sterols, and macrolides) (4). Numerous successful antivirals are either marine derived or analogs of marine compounds. Indeed, vidarabine (adenine arabinoside, or "ara-A"), acyclovir, and zidovudine (azidothymidine) have been commercially synthesized with semisynthetic modifications from or are structural analogs of the arabinosyl nucleosides isolated from the sponge *Cryptotethia crypta* (66, 67). Under selective pressure, some human viruses have become resistant to a number of commercial drugs. It is thus always desirable to explore new antivirals with barriers for viral resistance. The remarkable diversity and successful adaptation of molluscs, especially in habitats with high viral loads, imply a potential source of novel antiviral compounds, with diverse mechanisms of action, for human therapeutic use.

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