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Review

Review of anti-inflammatory, immune-modulatory and wound healing properties of molluscs

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6-bromoisatin (Pubchem CID 95716)
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Carrageenan kappa (PubChem CID: 11966249)
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Ether (PubChem CID: 3283)
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ABSTRACT

Ethnopharmacological relevance: This review focuses on traditional and contemporary anti-inflammatory uses of mollusc–derived products summarising all the *in vitro*, *in vivo* and human clinical trials that have tested the anti-inflammatory activity of molluscan natural products. Inflammatory conditions, burns and wounds have been an ongoing concern for human health since the early era of civilisation. Many texts from ancient medicine have recorded the symptoms, signs and treatments for these conditions. Natural treatments are well-documented in traditional European medicine, Traditional Chinese Medicine (TCM), Siddha and ancient Mediterranean and African traditional medicine and include a surprisingly large number of molluscan species.
Materials and methods: An extensive review of the *Materia Medica* and scientific literature was undertaken using key word searches for “mollusc” and “anti-inflammatory” or “immunomodulatory” or “wound healing”.
Results: Molluscs have been used in ethnomedicine by many traditional cultures to treat different aspects of inflammatory conditions. We found 104 different anti-inflammatory preparations from a variety of molluscan species, of which 70 were from the well-documented Traditional Chinese Medicine (TCM). This traditional use of molluscs has driven the testing for inflammatory activity in extracts from some species in the phylum Mollusca, with 20 *in vitro* studies, 40 *in vivo* animal studies and 14 human clinical trials performed to substantiate the anti-inflammatory and wound healing activity of molluscs. Some of these studies have led to the approval of mollusc-derived products to be used as over-the-counter (OTC) nutraceuticals, like Lyprinol® and Biolane™ from the New Zealand green lipped mussel *Perna canaliculus*.
Conclusion: Natural products provide important leads for the development of pharmaceuticals, including anti-inflammatory agents. Only a small proportion of the molluscan traditional medicines have been tested to confirm their anti-inflammatory activity and most screening studies have tested crude extracts from molluscs without any chemical characterisation. This highlights the need for further research to strategically identify the anti-inflammatory compounds in molluscan medicines to provide leads for novel anti-inflammatory drugs in the future.

Abbreviations: TCM, Traditional Chinese Medicine; OTC, over-the-counter; i.v., intravenous; i.p., interaperitoneal; p.o., orally; FDA, Food and Drug Administration; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; TNFα, tumor necrosis factor alpha; IL, interleukin; COX, cyclooxygenase; WoRMS, World Register of Marine Species; ROS, reactive oxygen species; NFκB, nuclear factor kappa B; NO, nitric oxide; iNOS, induced nitric oxide synthase; LOX, lipoxygenase; LPS, lipopolysaccharide; PUFAs, polyunsaturated fatty acids; GLME, green lipped mussel extract; HFCM, *hannai* fermented with *C. militaris* mycelia; 5-HETE, 5-hydroxyeicosatetraenoic acid; ORAC, oxygen radical absorbance capacity; AA, arachidonic acid; 5-HT, 5-hydroxytryptamine; ETA, eicosatetraenoic acid; AIA, adjuvant-induced arthritis; PLA2, phospholipase A2; HMLE, hard-shelled mussel lipid extract; PT, partial thickness; RA, rheumatoid arthritis; OA, osteoarthritis; PCT, placebo-controlled trial; NSD, no statistical difference; SD, statistically different; HRBC, human red blood cell; FITC, Fluorescein isothiocyanate; ZKC, Zhikang Capsule; TIMPs, tissue inhibitors of metalloproteinases; MMP, matrix metalloproteinase; CIA, collagen-induced arthritis; IFN-γ, interferon gamma; CINC 1, cytokine-induced neutrophil chemoattractant 1; LTB4, leukotriene B4; TXB2, thromboxane B2; DTH, delayed type hypersensitivity reaction; PFC, plaque forming cell; DNFB, dinitrofluorobenzene; FLH, *Fissurella latimarginata* hemocyanin; TRAP, tartrate-resistant acid phosphatase; ACP, acid phosphatase; ALP, alkaline phosphatase; HpH, *Helix pomatia* hemocyanin; TT, tetanus toxoid

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1. Introduction

Many natural products are sourced from the marine environment due to its phenomenal biodiversity. Marine invertebrates (Porifera, Echinodermata, Cnidarian, Mollusca, Arthropoda) have to date provided a substantial diversity of natural products, including terpenes, alkaloids, aliphatic hydrocarbons, steroids, carbohydrates, amino acids and peptides (Leal et al., 2012). These marine-derived natural products have an extensive array of therapeutic properties, including anticoagulant, antimicrobial, wound healing and immune modulating, antioxidant, anticancer, anti-inflammatory, antihypertensive, and other medicinal properties (Perdicalis et al., 2013; Senthilkumar and Kim, 2013). A number of marine natural products have provided important leads for drug development and many are now used in the formulation of novel drugs (Leal et al., 2012; Riguera, 1997; Senthilkumar and Kim, 2013). For example, Ziconotide, first isolated from the cone snail *Conus magus* (Linnaeus, 1758) venom effectively blocks N-type voltage gated calcium channels (Schroeder et al., 2004) and is effective for the treatment of chronic pain. The drug has now been Food and Drug Administration (FDA) approved and has been commercialised under the name of Prialt® (Atanassoff et al., 2000; Svenson, 2013).

The Mollusca is a phylum of marine invertebrates that are of particular interest as a source of new potential drugs leads. Molluscs encompass 7% of living animals on the planet making them the second largest animal phylum with estimated 100–200 thousand species, of which more than 52 thousand have been described and named (Benkendorff, 2010; Bouchet and Duarte, 2006). The phylum Mollusca also includes eight different classes: Gastropoda, Bivalvia, Scaphopoda, Cephalopoda, Polyplacophora, Monoplacophora, Caudofoveata and Solenogastres (Benkendorff, 2010; Ponder and Lindberg, 2008) which illustrates a significant evolutionary divergence over the past 500 million years. Associated with this vast biological diversity is significant chemical diversity, as molluscs use secondary metabolites to communicate and defend themselves against predators and pathogenic invaders (Benkendorff, 2014). As marine invertebrates, molluscs lack acquired immunity and essentially depend on their innate immunity and bioactive compounds to protect against microbial pathogens (Dang et al., 2015; Hooper et al., 2007) and heal wounds in the microbially-rich marine environment.

Molluscs have been a significant focus in the search for biologically active secondary metabolites, with > 1,145 natural products isolated from molluscan species in the last three decades (Benkendorff, 2010, 2014). Two molluscan derived natural products have been clinically tested and approved by the Food and Drugs Administration (FDA); ziconotide from cone shells for the treatment of severe pain and Brentuximab vedotin for treatment of lymphoma and Hodgkin's disease (Mayer et al., 2010). There are at least 18 other compounds originally found in molluscs and associated cyanobacteria that are currently in clinical trials (Mayer, 2017). However, ~ 52% of the molluscan natural products that have been isolated to date have never been tested for any biological activity (Benkendorff, 2014). Furthermore, < 1% of known molluscan species have been studied for their secondary metabolites, although a large number of molluscan species have been used as a source of traditional medicines (Tables 1, 2), which has provided the stimulus for further research into the therapeutic potential of natural products derived from this phylum.

One of the most common therapeutic applications for molluscs in traditional ethnomedicine appears to be for the treatment of inflammatory conditions (Table 1). Inflammation is associated with and may contribute significantly to the pathogenesis of acute and chronic diseases such as atherosclerosis, obesity, multiple sclerosis, chronic obstructive pulmonary disease, asthma, rheumatoid arthritis, neurodegenerative disease and inflammatory bowel disease (Nathan and Ding, 2010). Inflammation can be described as the rapid response of the body to insults such as injury and infection. The inflammatory reaction is recognised macroscopically by four cardinal signs (which were described by Cornelius Celsus in the first century), that of *calor* (heat), *rubor* (redness), *tumor* (swelling), *dolor* (pain) and loss of function (Alessandri et al., 2013). The process of inflammation generally includes the isolation and removal of the injurious stimuli such as damaged cells, chemical irritants and infection, as well as the initiation of the healing process (De Zoysa, 2012). More specifically the response is a spatially and temporally arranged episode in which cells and mediators collaborate to neutralise and eliminate the damaging stimuli, to allow the restoration of homeostasis (Alessandri et al., 2013; Medzhitov, 2010). Although the inflammatory process promotes the elimination of damaging stimuli, the inflammatory process itself may also contribute to damage of neighbouring tissues and can in some cases increase the severity of pathology (Alessandri et al., 2013; Cara et al., 2000).

Current treatments for inflammatory diseases are primarily based on the use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) (Gunawardena et al., 2014), which is often reflective of the severity and responsiveness of the inflammation to the particular therapeutic regime. NSAIDs modulate their effect by preventing the synthesis of prostaglandins (PGs) *via* the inhibition of cyclooxygenase (COX) enzymes, which catalyse the conversion of arachidonic acid to PGs (Auriel et al., 2014; Seibert et al., 1997; Vane and Botting, 1998). However, current NSAID options have been linked to increased blood pressure, greatly increased risk of congestive heart failure, occurrence of thrombosis and they also can predispose patients to serious gastrointestinal erosion (Aisen et al., 2003; McMurray and Hardy, 2002). These side effects are common to almost all NSAIDs to some degree (Vane and Botting, 1998). Because anti-inflammatory drugs are among the most consumed pharmaceuticals, with over 70 million prescriptions and 30 billion tablets of NSAIDs sold over the counter each year (Maroon et al., 2006), there is an urgent need to search for safer sources of anti-inflammatory drugs. Steroidal anti-inflammatory drugs also have many disadvantages including immunosuppressing effects, as well as the resistance of many diseases to steroidal anti-inflammatory drugs (Barnes, 2006, 2010). Natural products have traditionally provided important leads for the development of pharmaceutical drugs and there is evidence that they could be a potential source of anti-inflammatory agents that could provide the benefit of greater activity and less side effects.

The aim of this review is to explore the traditional use of molluscs as a means for controlling inflammatory conditions, as well as critically analysing evidence from *in vitro* and *in vivo* studies, and human clinical trials, supporting the further investigation of molluscan derived extracts and natural products for anti-inflammatory, immune-modulatory and wound healing properties. This timely review of anti-inflammatory properties of molluscan natural products should help identify priority targets for future bioassay-guided isolation and development of novel potential anti-inflammatory agents.

2. Materials and methods

An extensive review of the scientific literature on molluscs with anti-inflammatory activities, immunomodulatory and wound healing activities was undertaken by searching bibliographic databases: MEDLINE/PubMed, Scopus, Web of Knowledge and Google Scholar. The keywords used in the search were 'anti-inflammatory' or 'immunomodulatory' or 'wound healing' AND 'mollusc'. Reference lists of published research articles were also checked for relevant data. Research articles were selected for inclusion if they tested an extract/s or compound/s isolated from species in the phylum Mollusca for anti-inflammatory or wound healing activity. Studies using *in vitro* assays for anti-inflammatory activity included inhibition of reactive oxygen species (nitric oxide), oxidative enzymes (nitric oxygen synthase, lipoxygenase, cyclooxygenase), cytokines (tumor necrosis factor alpha, interleukins, nuclear factor kappa B), immunoglobulin G or prostaglandins. *In vitro* papers testing for immune-modulation included