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Biotechnological potential of marine sponges and their associated bacteria as producers of new pharmaceuticals (Part I)

Abstract

Throughout human history natural products have provided the basis for medicinal treatment. About 60 years ago the advent of SCUBA diving techniques granted access to a thitherto untapped source of pharmacologically highly active natural products: the marine environment. In the oceans not plants but sessile animals, particularly sponges, have proven to be the most fruitful organisms in this context. Sponges have evolved a vast arsenal of chemical weapons to defend themselves against various threats from their environment. These molecules remarkably often show potent activities in pharmaceutical assays. Several sponge-derived compounds are already in clinical trials as agents against cancer, microbial infections, inflammation and other diseases. However, in many cases drug development is severely hampered by the limited supply of the respective compounds, as they are often present only in minute amounts in the sponge tissue. Big hope to circumvent this obstacle lies on the supposition that sponge-associated bacteria and not the sponges themselves are in many cases the true producers of the pharmaceutically relevant agents. Once these microorganisms are determined and cultivated outside the sponge tissue, they could be fermented for large-scale compound production. This in future could substantially increase the number of powerful sponge-derived drugs on the market.

Natural products – Medicine provided by Mother Nature

Throughout human history, natural products have served as medicine to treat various human diseases. Traditionally, terrestrial plants represented the major source of these health-restoring or health-supporting chemicals. Among the most ancient documented examples of nature-derived drugs are Mesopotamian clay tablets that date from 2600 B.C. These early documents describe about 1,000 plant derived substances, many of which are still in use today. Also the Egyptians, the Chinese, the Greeks and the Romans have contributed substantially to the rational development of the use of natural products as medicinal drugs in ancient times. In the first century A.D. Dioskurides, supposedly a military physician with the Roman legions, composed his *Materia Medica*, an assemblage of data about the medicinal properties of over 1,000 natural products (Riddle 1985). It was translated from Greek into Latin, Arabic, as well as Armenian and influenced pharmacy and medicine in many countries until the sixteenth century.

Despite the considerable progress achieved by modern synthetic chemistry, natural products are nowadays still an indispensable source for pharmaceuticals. According to estimates by the World Health Organization (WHO) approximately 80% of the world's inhabitants rely mainly on traditional medicines (Farnsworth et al. 1985). Plant products play also an important role in the health care of the remaining 20% of the population, mainly residing in developed countries. A study from 1997 using US-based prescription data from 1993, demonstrated that over 50% of the most-prescribed drugs in the US had a natural product either as the active component, or as a template in the synthesis of the agent (Grifo et al. 1997). Well-known recent examples of plant-derived drugs include the anti-cancer agents paclitaxel (Taxol®) from the yew tree *Taxus brevifolia*, etoposide (Vepesid®) derived by partial synthesis from the natural product podophyllotoxin isolated from the Mayapple *Podophyllum peltatum*, and irinotecan (Camptosar®) which was obtained by optimizing the structure of the alkaloid camptothecin from Camptotheca acuminata, the common names of which are "Cancer Tree" and "Tree of Life".

Since the discovery of the penicillins, terrestrial microorganisms have proven to be probably even more important producers of pharmaceutically relevant natural products than terrestrial plants. Not only antibiotics but also anti-cancer agents, antifungals, antivirals, immunosuppressant, anti-

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malarials, etc. have been isolated from laboratory cultures of bacteria and fungi (Newman et al. 2000).

The advent of SCUBA diving techniques approximately 60 years ago and their subsequent utilization by natural products chemists and biologists granted access to a thitherto almost completely undiscovered source of natural products: the marine environment, which since has turned out to be a virtually inexhaustible resource of unique and unprecedented compounds with pronounced biological activities (Faulkner 2000a, Newman and Gragg 2004, Proksch et al. 2002). There are two major reasons for the fundamental dissimilarities that are often encountered when comparing the chemical structures of terrestrial and marine natural products. Firstly, the biosynthesis of compounds takes place under fundamentally different physical and chemical conditions in the two environments, which gives rise to greatly different biosynthesis chemistry. Secondly, the major producing organisms in the two environments differ considerably: while in the terrestrial environment plants and microorganisms are the main producers of natural products, in marine habitats animals, namely invertebrates such as sponges, tunicates and bryozoans (and, as recently more and more often revealed, their associated microbial communities) are the richest sources of new and bioactive chemicals.

There are now significant numbers of active molecules that have come from marine sources, or have been synthesized based on prototypical compounds of marine origin. Many of these either in or approaching Phase II/III clinical trials in cancer, analgesia, allergy, and cognitive diseases. A substantial number of other potential agents are following and are tested in preclinical trials against these and other diseases (Newman and Cragg 2004).

Chemical weapons of sponges to fight human diseases

Sponges are far more than the bleached skeleton of the sponge species *Spongia officinalis* that is commonly known as bath sponge. There are more than 9000 extant (presently living) species in almost all aquatic habitats all over the world (Brusca and Brusca 1990). They are versatile in color (often even bright colored yellow, red, and orange) and range in size from a few millimeters to 2 m in diameter. Sponges can have a massive and bulky, reticulate or encrusting shape, depending to a large extent on environmental factors such as currents and levels of wave and sunlight exposure.

Sponges are very old in evolutionary terms. They emerged 600-800 million years ago and thus are amongst the oldest, if not the oldest metazoans (Müller 1998). They have settled predominately (but by far not exclusively) in the marine environment and are most abundant and diverse on tropical reefs. However, they can also be found in high numbers in temperate as well as polar zones, while in these regions species diversity is considerably lower compared to the tropics. With very few exceptions (e.g. Vacelet et al. 1995) sponges are filter feeders, creating a water flow through their body and filtering organic material (e.g. bacteria) from the seawater (Bergquist 1978).

Sponges, similar to terrestrial plants, are sessile organisms and as such face various analogous environmental challenges. As they require a "foothold" on a nonmoving, fixed substrate (in the case of sponges this usually is rock or coral), flight behavior as a possible defense is eliminated. For this reason, and also because sponges for the most part lack efficient mechanical defense mechanisms (e.g. shells or stings), one could assume that they are an easy prey for various predators (fishes, sea slugs, sea stars, turtles, etc.). Moreover, again due to their sessile way of living, sponges have to compete with their neighbors for limited space and to prevent overgrowth (Bell and Barnes 2003). This holds especially true in habitats with extremely high spatial competition such as coral reefs. Finally, sponges have to prevent biofouling, the settlement of fouling organisms (bacteria and fungi as well as larvae and spores of various marine invertebrates and algae) on their outer surface, as this would block the tiny pores through which they pump in the surrounding seawater (Becerro et al. 1994, Engel et al. 2002).

To successfully fight these threats, sponges have developed a huge arsenal of chemical weapons: Feeding scars are seldom found on sponges as compounds stored in the sponge deter most potential predators (Schupp et al. 1999, Wadell and Pawlik 2000, Becerro et al. 2003, Thoms et al. 2004). In this context it is interesting to note that there are some specialists (particularly certain sea slug species) that not only tolerate the sponge defense metabolites but even sequester them in their own body for defense against their predators (Avila 1995, Cimino et al. 1999, Thoms et al. 2003b, Thoms et al. in press). Several studies have shown that certain sponges can efficiently prevent overgrowth by other organisms as well as biofouling and proliferation of bacterial pathogens within the sponge tissue by storing and secreting powerful antimicrobial and cytotoxic compounds (Engel and Pawlik 2000, Engel

et al. 2002, Fusetani 2004, Paul and Puglisi 2004). As each sponge species often contains a large variety of different and often species-specific chemical compounds the over 9000 known sponge species represent a huge resource of new natural products (Faulkner 2000a). Moreover, most of the secondary metabolites in sponges have undergone a selection process towards biological activity in the course of evolution and, as they have to overcome dilution effects of seawater en route to their target, they are often extremely potent in order to be efficient in the marine environment.

To date, more than 4000 new natural products have been isolated from sponges (more than 10000 from marine organisms in general) (MarinLit 2003). Many of them proved to possess pronounced biological activities and it has been shown that these activities remarkably often are related to pharmaceutically interesting properties of the compounds, attracting the interest of many natural products researchers (Kobayashi 2000, Proksch 2002, Blunt et al. 2004, Newman and Cragg 2004, Simmons et al. 2005).

Examples of natural products from sponges and their synthetic derivatives in clinical trials

While already in the 1950s structurally unusual nucleosides had been isolated from the Caribbean sponge *Tethya crypta* that later served as lead structures for the development of the nowadays commercially important anti-viral drug Vidarabine® and the anticancer drug Ara-C, the discovery of prostaglandins (compounds involved in inflammatory diseases, fever, and pain) in the coral *Plexaura homomalla* in 1969 is usually considered to be the "take-off point" of serious search for drugs in marine invertebrates (Weinheimer and Spraggins 1969). However, in the early days, until about the late 1970s, marine natural product research evolved slowly, since equipment such as High Performance Liquid Chromatography (HPLC), which nowadays is an indispensable tool for natural product isolation, was not generally available in research labs. Also high-field NMR and mass spectrometry, both techniques that are essential for structure elucidation of chemical compounds advanced considerably in recent years, thereby substantially accelerating progress in natural products research.

The field was considerably advanced when in the early 1980s the U.S. National Cancer Institute (NCI) started funding the search for anti-cancer agents on a large scale. It is predominately due to this major source of funding that so far the majority of pharmacological activities reported for marine metabolites have been anticancer agents. During the last two decades more and more other activities such as anti-viral, antibiotic, anti-inflammatory, analgetic, and anti-malaria activity were also discovered. An excellent and very comprehensive overview about the recent advances in marine natural product research is given by Newman and Cragg (2004). In the following, a small selection of very promising pharmaceuticals derived from sponges is presented.

Halichondrin B is one of a series of compounds originally isolated from the Japanese sponge *Halichondria okadai* that was reported for the first time in 1985 (Hirata and Uemura 1986). Subsequently, a number of sponges from other areas of the Pacific and the Indian Ocean were reported to contain one or more of this class of compounds, too. Pharmaceutical assays revealed that halichondrin B and derivatives thereof possess very potent anticancer activity (Hart et al. 2000). Although there was enough halichondrin B available for initial experiments on its pharmacology, it was too scarce for further drug development. In 1992, the U.S. National Cancer Institute (NCI) issued a request for groups that could provide a variety of scarce natural products from natural sources. The University of Canterbury, together with the New Zealand National Institute for Water and Atmospheric Research (NIWA) successfully applied to the NCI for large-scale recovery and isolation of halichondrin B from the deep water sponge *Lissodendoryx* sp. which contains the natural product at comparatively high concentrations.

The program was conducted as a joint venture with the New Zealand government and after an environmental assessment of the collection area, the NZ government gave permission to collect 1 metric tonne of *Lissodendoryx* sp. from the Kaikoura shelf at depths of 100 m and deeper. By extensive workup, 300 mg of halichondrin B were isolated from these samples.

Subsequent mariculture experiments with *Lissodendoryx* sp. also conducted by the National Institute for Water and Atmospheric Research demonstrated that the sponge could successfully be cultured at 10 m depth and still produces the halichondrins at levels roughly comparable with those found in sponges collected at their natural habitat. Concomitant attempts to synthesize the complex molecule of halichondrin B were successful at Harvard University (Aicher et al. 1992). Based on these results, variants of halichondrin B, smaller molecules that were more stable but nevertheless

maintained the pharmaceutical activity of the original compound, were then synthesized. One of these molecules, coded E7389, has entered Phase I clinical trials in 2002. So far these tests revealed that E7389 could become a highly interesting pharmaceutical to treat non-small cell lung cancer (NSCLC) (Newman and Gragg 2004).

Discodermolide was isolated from the Caribbean sponge *Discodermia dissoluta* and reported in 1990 by scientists at the Harbor Branch Oceanographic Institute, Florida (Gunasekera et al. 1990). Pharmacological assays revealed that discodermolide could become an even more powerful anticancer agent than the famous Taxol® (see above), as it binds more potently to microtubules in proliferating cancer cells (which is part of the mode of action of Taxol®). Harbor Branch licensed the compound to Novartis as a preclinical candidate, and it is now in Phase I clinical trials as a potential treatment against solid tumors.

Agelasphins were first extracted in 1993 from the sponge *Agelas mauritianus*. In pharmaceutical assays these compounds proved to possess pronounced antitumor and potential immunostimulatory properties (Natori et al. 2000). A synthetic derivative of the agelasphins, KRN-7000, entered Phase I clinical trials for cancer immunotherapy in both Asia and Europe in 2001.

Psammaplin A was originally found in a sponge of the genus *Psammaplysilla* in 1987 (Arabshahi and Schmitz 1987, Quinoa and Crews 1987) but was later also extracted from other sponges of the order Verongida. Together with synthetic derivatives, this natural product served as a lead structure for the development of the synthetic compound NVP-LAQ824 (Remiszewski 2003). This agent was extremely potent in anticancer screens by Novartis (then Ciba-Geigy). Currently, it is in Phase I trials at the Dana-Farber Cancer Institute, a private cancer research institution affiliated with Harvard University. Interestingly, another derivate of psammaplin A proved to be highly active against antibiotic resistant strains (methicillin and vancomicin) of the pathogenic bacterium Staphylococcus aureus (Nicolaou et al. 2001).

Manoalide was first isolated from the sponge *Luffariella variabilis* in 1980 and was originally reported as an agent with pronounced antibiotic activity (de Silva and Scheuer 1980). In 1984 manoalide was described also as a potent inhibitor of the enzyme phospholipase A2 which is involved in inflammatory processes (de Freitas et al. 1984). As it was available in relatively large quantities from the source sponge, this compound was considered to be a good candidate for drug development and was licensed to Allergan to be placed into clinical trials as anti-inflammatory drug candidate. It even advanced to Phase II, but the clinical tests revealed that the quantities of the compound that would pass through the patients´ skin during dermal applications were not sufficient to show considerable effects. However, research is still ongoing with synthetic derivatives of manoalide that might serve as replacements for the original compound (de Rosa et al. 2000).

Contignasterol is a natural product accumulated in the tissue of the sponge Petrosia contignata. It was first reported in 1992 (Burgoyne and Andersen 1992). Pharmacological assays revealed that contignasterol as well as a series of synthetic derivatives of this compound inhibit the release of histamine, which is also a mediator in inflammatory processes (Bramley et al. 1995). A chemically modified version of contignasterol (named IPL-576092) successfully completed Phase II clinical trials in 2002 as a novel therapeutic against asthma. IPL-576092 as well as other contignasterol derivatives is also in clinical trials against inflammation of the skin and eye.

(To be continued in the next Issue)