Scuba-diving for neuroprotectors

Janet Fricker, Freelance writer

A newly discovered compound from blue-green algae (cyanobacteria) is showing promise in helping researchers develop new neuroprotectant drugs. The studies, which were presented at the *International Congress of Pacific Basin Societies* held recently in Honolulu, Hawaii, could provide important knowledge for the development of new treatments for pain, epilepsy and stroke.

The discovery

The new compound, kalkitoxin (Fig. 1), was developed during a research programme by William Gerwick, Professor of Pharmacy at Oregon State University (Corvallis, OR, USA) aimed at discovering new medicines from marine algae. The discovery of *Lyngbya majuscula* (the source of kalkitoxin) occurred during a scuba-diving field trip off the Caribbean island of Curaco, near Venezuela in 1994 (Fig. 2). The group noticed a collection of cyanobacteria growing like hairs off the sea floor (Fig. 3).

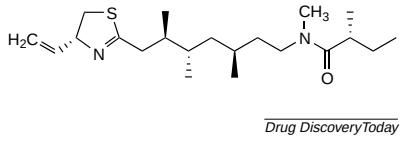

Figure 1. The chemical structure of kalkitoxin.

'Normally in tropical marine environments you see woody algae that have adapted to protect themselves from predation. Then suddenly there was this beautiful, delicate plant which, despite all the predators around, was not being eaten. This gave us the clue that it was likely to have unusual chemistry,' says Gerwick, who speculates that kalkitoxin's natural function is to defend the algae from predation by fish and sea urchins.

Figure 2. The collecting site for *Lyngbya majuscula* (the source of kalkitoxin) in Curaco.

Compound extraction

The team took several litres of the algae preserved at reduced temperature in isopropyl alcohol back to Oregon for testing (Fig. 4). After extracting the oils, ~50 different bioassays were conducted to examine the toxicity of the organic extract to different indicator species. They found that the organic extract of *L. majuscula* exhibited potent toxicity to brine shrimp and goldfish[1], a common indicator of neurotoxicity effects.

Subsequently, they isolated the toxic metabolite from the crude extract by sequential silica gel vacuum-LC and normal-phase HPLC. At each stage of the separation process, they conducted a bioassay on fish to test if the toxic metabolite had been isolated.

Once isolated, they named the neurotoxic metabolite kalkitoxin after the beach Playa Kalki where the algae had been discovered. 'Every collection of *L. majuscula* we have made has been found to be rich in unusual chemicals, many of which are unique to each location. However, some of the collections have overlapping chemistry,' says Gerwick.

Mechanism of action

Kalkitoxin was highly potent in the fish assay with ~50 μl capable of rendering 10,000 litres of water toxic to fish. 'This was an extraordinarily strong effect. Such high potency suggested that we must be hitting a very specific biomolecular receptor, and this led us to look for very specific mechanisms of action,'

Figure 3. Picture of *Lyngbya majuscula* underwater, appearing as reddish hairs.

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says Gerwick. Tom Murray, a neuropharmacologist from the University of Georgia (Athens, GA, USA), isolated rat neurons and showed that the neurotoxic effects of kalkitoxin were inhibited by NMDA-receptor antagonists, indicating involvement of this receptor class.

Working on the neuroblastoma cell line, Gerwick and colleagues examined the effects of kalkitoxin on the responses to a variety of pharmaceutical agents of known effect. One such agent was veratridine, a chemical known to activate sodium channels, leading to sodium influx and cell death, an effect that was inhibited by the prior addition of kalkitoxin. This activity of kalkitoxin at sodium channels suggests it could have a use as a neuroprotector in conditions such as stroke, or in the treatment of epilepsy or as a painkiller.

Molecular structure

A major focus of Gerwick's research has been to determine the molecular structure of kalkitoxin. They found it relatively straightforward to determine the 2D structure of the compound, but considerably more difficult to work out the stereochemistry or the 3D structure. Using one of the most advanced NMR spectrometers currently available and cryoprobe technology (Bruker Instruments, Billerica, MA, USA), as well as chemical fragmentation of kalkitoxin using reagents of known specificity, Gerwick's group reduced the number of stereochemical possibilities from 32 to four.

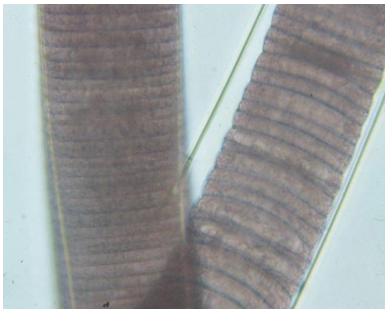

Figure 4. A photomicrograph image of *Lyngbya majuscula.*

The team's Japanese collaborators, headed by Takayuki Shioiri (Nagoya City University, Nagoya, Japan), synthesized the four configurations, and these were then evaluated by NMR, chiroptical techniques and fish toxicity assay. Only one configuration was found to be strongly toxic to fish and have comparable spectroscopic properties to the natural kalkitoxin[1].

Future work

Yuzuru Shimizu, Professor of Pharmacognosy and Chemistry at the University of Rhode Island (Kingston, RI, USA), said that these alga 'are a rich source of new chemical structures and their chemical diversity is comparable to the actinomycetes organisms, which have produced a number of important drugs.' However, he suggests there is still much work to be done: 'Many of the compounds from cyanobacteria have been proved to be useful molecular probes against unique targets, but they are yet to become therapeutic drugs.'

Gerwick's team is now working to define the exact site of action of kalkitoxin on the sodium channel. This knowledge will then enable them to try to decrease the complexity of the molecule to make it easier to synthesize for testing in animal models of disease.

Reference

1 Wu, M. *et al.* (2000) Structure, synthesis and biological properties of kalkitoxin, a novel neurotoxin from the marine cyanobacterium *Lyngbya majuscula*. *J. Am. Chem. Soc.* 122, 12041–12042

Heat-sensitive liposomes for tumour targeting

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A new type of heat-sensitive liposome has recently been developed, specifically for the local control of solid tumours. Researchers from Duke University (Durham, NC, USA) have developed low-temperature-sensitive liposomes (LTSLs) that have been designed to release their drug loads at 39–42°C – a temperature lower than that required to trigger traditional thermosensitive liposomes (TTSLs), and one which is readily achievable in the clinic.

Traditional thermosensitive liposomes

A drug delivery system should ideally deliver the precise concentration of drug exclusively to its site of action, at the correct rate and timing for optimal efficacy,

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