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.

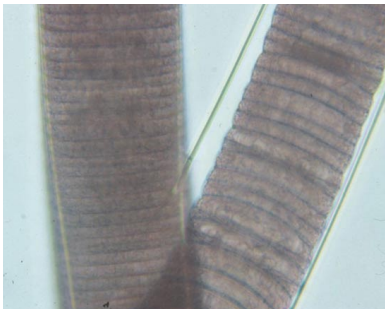

Figure 4. A photomicrograph image of *Lyngbya majuscula.*

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

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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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while preventing interaction of the drug with non-target sites. Liposomes, microscopic vesicles that consist of an aqueous core surrounded by one or more lipid bilayers, represent the most successful advanced drug delivery and targeting system currently available[1] (Fig. 1).

Traditional thermosensitive liposomes have been designed that release their drug loads when heated to temperatures of 42–45°C (Ref. 2), offering the potential to release liposomal contents at a specific heated site. Triggered release occurs because of a lipidic phase transition, from a gel (solid) state at lower temperatures to a liquid (fluid) state at higher temperatures. The increased permeability at the temperature of this gel–liquid phase transition (Tc) is caused by mismatches in molecular packing, especially at interfacial boundary regions of gel and liquid domains.

Low-temperature sensitive liposomes

David Needham and colleagues at the Department of Mechanical Engineering, Duke University, have developed novel LTSLs that contain the anticancer agent, doxorubicin (DOX), and comprise phospholipid bilayers of dipalmitoylphosphocholine (DPPC), which also incorporate the lysolipid monopalmitoylphosphocholine (MPPC) kinetically trapped in the gel phase bilayer. Needham explained that, 'Although MPPC is trapped in the lipidic bilayer, it is soluble in the aqueous medium surrounding the liposomes. Therefore, at the Tc, as the lipid begins to melt, the MPPC readily desorbs from the bilayer, enhancing boundary defect formation and membrane permeability. Entrapped drug rapidly diffuses through the newly-created pores.' Thus, on heating, LTSLs can achieve rapid 'burst' release of the drug. They were shown to release ~45% of their contents in the first 20 s of exposure to 42°C, compared with only 40% release over a period of 30 min for TTSLs (Ref. 3). The presence of MPPC also lowers the Tc, enabling triggered drug release to occur at a lower temperature than for TTSLs.

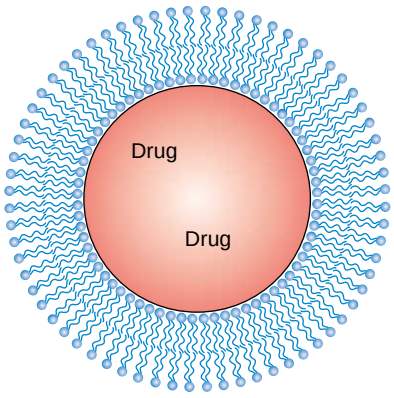

Figure 1. Schematic of a unilamellar liposome with drug entrapped in the central aqueous compartment.

Improved efficacy of LTSLs

In a recent study[4], the researchers used a mouse model bearing a human squamous cell carcinoma to investigate the efficacy, with and without heat treatment (42°C for 1 h), of three different liposomal formulations of DOX:

- NTSLs: non-thermosensitive liposomes;
- TTSLs, which trigger drug release at 42–45°C and release drug over 30 min; and
- LTSLs, which trigger drug release at 39–40°C and release drug within seconds.

At 34°C (i.e. without heat treatment), the most successful formulation was the NTSLs, which slightly extended the delay in tumour growth, compared with controls. Although the NTSLs can accumulate at tumour sites, the formulation was only marginally effective, probably because much of the DOX remained trapped within the liposomal carrier at the tumour site and was thus unavailable to exert its therapeutic effect.

For mice given heat treatment, the most successful formulation was LTSLs, which showed the greatest delay in tumour growth and resulted in the largest number of animals in complete regression at 60 days post-treatment. This group was also the only one that resulted in the release of significant quantities of DNA-bound DOX (i.e. bioavailable and active), indicating that the drug had been successfully released from the carrier. The high efficacy of LTSLs with heat treatment can be attributed to the significant tumour drug levels achievable in a rapid timeframe.

TTSLs were less effective, due probably to timeframe and temperature constraints. For maximum drug release, TTSLs require exposure to 42°C for at least 30 min. However, *in vivo*, the estimated tumour circulation time for liposomes through the heated region is only ~100 s.

Gregory Gregoriadis (School of Pharmacy, University of London, UK), a pioneer in liposome research who first proposed the use of liposomes as drug carriers, comments: 'Liposomes have thus far been successfully engineered to retain the drug and also to evade the body's immune defences. However, little progress has been made to date on the controlled release of the drug from the liposomal carrier. This study shows that it is now possible to trigger drug release, specifically within a tumour tissue, under clinically relevant conditions.'

The next step

Mark Dewhirst (Duke University Medical Center and collaborator on the recent study[4]) comments that further studies will investigate the LTSLs in soft tissue sarcomas using a dog model. Preliminary toxicity studies are also being planned as a prelude to investigatory drug trials in human patients.

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