

Anti-oesophageal cancer activity in extracts of deep-water Marion Island sponges

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OESOPHAGEAL CANCER IS ONE OF THE most common causes of cancer-related deaths in South African black males. The limited efficacy of chemotherapeutic agents to treat this disease has prompted a search for potential new chemical entities with anti-cancer properties. We report here on the evidence for anti-oesophageal cancer activity in the methanolic extracts of five species of sponges dredged from a depth of approximately 100 m in the vicinity of Marion Island in the Southern Ocean during the autumn of 2004.

Introduction

Oesophageal squamous cell carcinoma (OSCC) is the second most common cancer observed in South African black males. It has an age-standardized incidence rate of 16.22 per 100 000.¹ This relatively high rate is linked to several extraneous factors including poor diet, the inadvertent ingestion of carcinogenic fungal toxins from contaminated grain and the continuous exposure of many individuals to excessive wood and cigarette smoke.² Early chemotherapeutic intervention with available anti-cancer drugs such as cisplatin and 5-fluorouracil results in complete remission in only 20–30% of cases diagnosed at an early stage.³ The low remission rate coupled with the frequent interruption of treatment due to chronic side effects,³ has prompted our multidisciplinary search for new chemical entities exhibiting *in vitro* activity against the OSCC cell line, WHCO1.

We have recently extended our primary source of new chemical entities with anti-cancer activity, namely, the plethora of bioactive secondary metabolites (natural products) produced by southern African marine invertebrates,^{4,5} to include the compounds synthesized by the deep-

water sponges of the Southern Ocean. Marine invertebrates in general, and marine sponges in particular, are a widely recognized reservoir of structurally novel secondary metabolites.^{6,7} Sponges produce these metabolites often in response to predation as a form of chemical defence or as an inter-species chemical deterrent to provide a competitive advantage in the struggle for space and nutrients in benthic filter-feeding communities.⁶ Serendipitously, a significant proportion of marine sponge metabolites are also cytotoxic to human cancer cells; indeed, sponges are the source of 14 of the 24 compounds of marine origin currently in clinical trials for the treatment of various forms of cancer.⁷

Both the taxonomy of the benthic

sponge fauna of Marion Island and the diversity and potential anti-cancer activity of their secondary metabolites are unknown. Four deep-water marine sponges, dredged during the benthic survey conducted in April 2004 east of the island, have been identified to genus level, while a fifth sponge remains unidentified. Initial anti-oesophageal cancer data (in the form of IC₅₀ values) for the methanolic extracts of these five sponges against the WHCO1 cell line are reported here.

Materials and methods

Researchers from Rhodes University and the University of Cape Town participated in the annual relief voyage (voyage 116) to Marion Island on board the supply and research vessel *SA Agulhas* from 1 April to 6 May 2004.⁸ The ship-based research component of the cruise had three objectives: an interdisciplinary oceanographic survey southwest of Marion Island, a surface sampling survey over the shelf between Marion and Prince Edward Island, and a benthic survey in the vicinity of the main Marion Island base.⁸ The last component involved 13 dredges carried out from the *SA Agulhas* using a steel dredge (1.0 m × 0.4 m × 1.5 m) at various depths from 40–120 m over an area of 16 km² (Fig. 1). Sponges

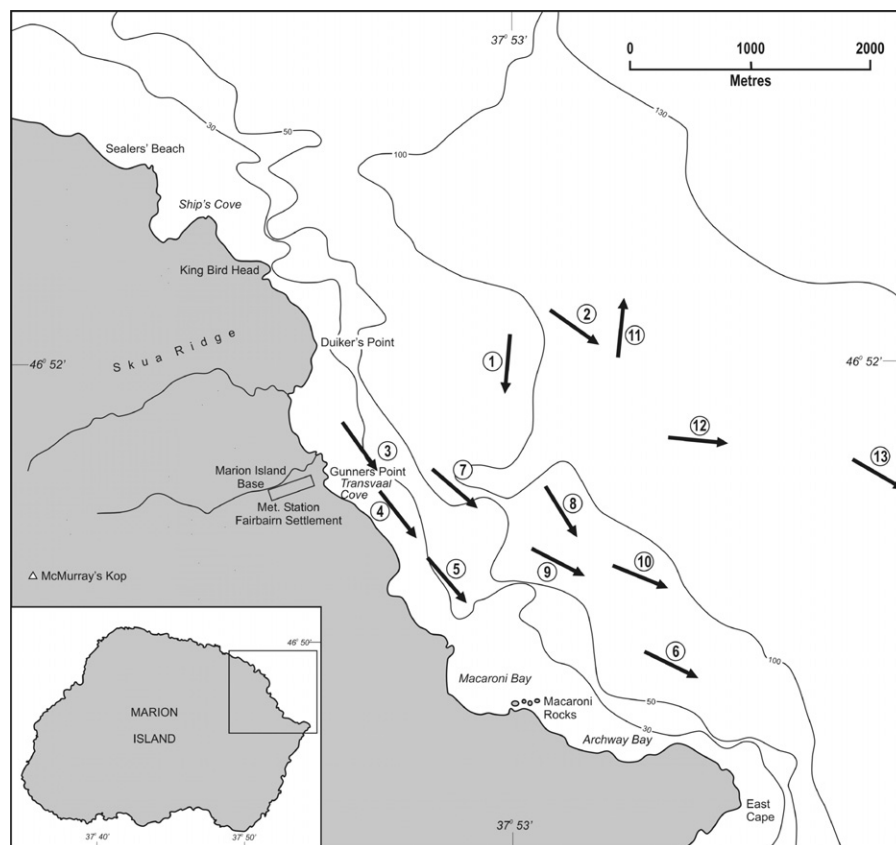


Fig. 1. The locations, directions and approximate distances dredged during the thirteen benthic survey dredges carried out off the coast of Marion Island, April 2004.

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(>50 g) were separated from the benthic detritus, sorted and immediately frozen after collection. Portions of selected frozen sponges (where the wet mass exceeded 100 g) were steeped in methanol for several days and the extracts from these solutions were screened for anti-oesophageal cancer activity.

The human oesophageal cancer cell line WHCO1 was routinely maintained at 37°C, under 5% CO₂. Cells were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Initial screening for cytotoxicity was conducted as described previously.⁹ Briefly, 1500 cells were seeded per well in 90 µl DMEM in 96-well plates. Cells were incubated for 24 hours, test samples were plated at a range of concentrations (1–50 µg/ml) in 10 µl DMEM, with solvent (DMSO) at 0.2%. After 48 hours' incubation, plates were processed according to manufacturer's instructions and read at 595 nm on an Anthos microplate reader 2001. IC₅₀ determinations were carried out using the MTT kit from Roche (catalogue #1465007). Portions of all extracts submitted for anti-oesophageal cancer screening were dissolved in deuterated methanol, after which ¹H nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz to assist in the identification of extracts with similar gross primary and secondary metabolite profiles.

Preliminary results

The geographical locations, directions and approximate distances covered during each of the thirteen dredges conducted off Marion Island are presented in Fig. 1. Both the biodiversity and biomass of sponges in the area surveyed appeared to be poor. No sponges were found in the benthos dredged at depths of 30–70 m, where kelp dominates the inshore (<40 m) and red algae the deeper offshore (40–70 m) benthic communities. The dredges that provided the most sponge material as well as details of the taxonomy, dredge depths and cytotoxic activities of extracts of the five sponges collected are reported in Table 1. Data based on this limited sample must be treated with caution but it appears that one species, *Halichondria cf. paniacea*, is reasonably common at depths of 100–130 m to the east of the island. Interestingly, not all extracts of the various specimens of this species collected exhibited similar activity against oesophageal cancer cells. The

Table 1. Taxonomy, dredge and IC₅₀ (oesophageal cancer cell line WHCO1) data for five species of deep-water Marion Island sponges.

Sponge and voucher number	Dredge no.	Depth (m)	IC ₅₀ WHCO1 (µg ml ⁻¹)
<i>Halichondria cf. paniacea</i> SAF04-001	1	105	0.35
<i>Halichondria cf. paniacea</i> SAF04-006	11	130	0.63
<i>Halichondria cf. paniacea</i> SAF04-008	12	120	379.8
<i>Haliclona</i> spp. SAF04-005	2	110	3.3
<i>Myxilla</i> spp. SAF04-007	12	120	32.3
<i>Latrunculia brevis</i> LAT-1	2	110	26.9
Unidentified (SAF04-004)	2	110	26.5

¹H NMR spectra of the methanolic extracts of each of the *Halichondria cf. paniacea* specimens suggested sufficient congruency in the chemical shifts of clearly identifiable splitting patterns (arising from the protons of the plethora of organic metabolites in the extracts) to support the taxonomic evidence that the extracts were derived from the same species. One explanation for this variable biological activity is that localized stimuli, such as predation or competition with neighbouring species, induced the differential production of secondary metabolites by individuals of the same sponge species.¹¹

Conclusion

The isolation and identification of biologically active natural products from marine sponges usually requires the collection of a substantial wet mass (>250 g) of each sponge. As a consequence of their filter-feeding life style, sponges consist predominantly of seawater, so that their organic biomass is often less than 25% of the total wet mass, depending on species. The preliminary cytotoxic data from the five sponges collected off Marion Island suggest that the *Halichondria cf. paniacea* specimens induced the greatest mortality of oesophageal cancer cells. Extracts of this sponge would therefore be worth fractionating to isolate and identify the secondary metabolites responsible for the cytotoxicity. Unfortunately, the paucity of biomass (c. 200 g) of *Halichondria cf. paniacea* collected in 2004 prevented any further investigation of the natural products involved. Further sponge material will be sought during the next research cruise to the island in 2005.

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