Anticancer compounds from Seaweed

Catherine Murphy*, Sarah Hotchkiss, Stephanie McKeown, Stephen McClean
Introduction

• Much evidence of the benefits of seaweed consumption
• Animal studies and human studies aimed at understanding the lower risk of certain cancers in countries with high seaweed intakes
• This makes seaweed an interesting target for the extraction of natural products
• Natural products have been studied as chemotherapeutic agents for many years
• Marine organisms good candidates for drug discovery
• Source of novel compounds not found in terrestrial plants
## Marine Drugs

### Table 1  Anti-cancer agents from marine sources which are currently in clinical development

<table>
<thead>
<tr>
<th>Chemical name and synonyms</th>
<th>Class of molecule</th>
<th>Isolated from</th>
<th>Stage of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplidine</td>
<td>Depsipeptide</td>
<td><em>Aplidium albicans</em></td>
<td>Phase II clinical trial</td>
</tr>
<tr>
<td>Dehydrodideiminin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plitidepsin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bryostatin-1</td>
<td>Macrocyclic lactone</td>
<td><em>Bugula neritina</em></td>
<td>Phase II clinical trials</td>
</tr>
<tr>
<td>Cryptophycins</td>
<td>Depsipeptide</td>
<td><em>Nostoc sp.</em>, <em>Dysidea arenaria</em></td>
<td>Phase II clinical trials on cryptophycin 52 discontinued in 2002, phase II trials of analogues in progress</td>
</tr>
<tr>
<td>Didemnin B</td>
<td>Cyclic depsipeptide</td>
<td><em>Didemnidae</em></td>
<td>Phase II clinical trials</td>
</tr>
<tr>
<td>Dolastatins</td>
<td>Peptide</td>
<td><em>Dolabella auricularia</em></td>
<td>Phase II clinical trials</td>
</tr>
<tr>
<td>Trabectedin and Yondelis</td>
<td>Isoquinoline alkaloid</td>
<td><em>Ecteinascidia turbinata</em></td>
<td>Approved for sarcoma and ovarian cancer, other clinical trials ongoing (Phase II/III)</td>
</tr>
<tr>
<td>Halichondrin B</td>
<td>Polyether macrolide derivative</td>
<td>Found in a variety of marine sponges</td>
<td>Phase II clinical trials</td>
</tr>
<tr>
<td>Kahalalide F</td>
<td>Depsipeptide</td>
<td><em>Elysia rubescens</em></td>
<td>Phase I/phase II clinical trials</td>
</tr>
</tbody>
</table>


### Table 2  Drugs in current use originally derived from marine sources

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Brand name</th>
<th>Chemical class</th>
<th>Use</th>
<th>Isolated from</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>Cytosar-U®, Depocyt®</td>
<td>Nucleoside</td>
<td>Anti-cancer</td>
<td><em>Cryptotheca crypta</em></td>
<td>Sponge</td>
</tr>
<tr>
<td>Vidarabine</td>
<td>Vira-A®</td>
<td>Nucleoside</td>
<td>Anti-viral</td>
<td><em>Tethya crypta</em></td>
<td>Sponge</td>
</tr>
<tr>
<td>Trabectedin</td>
<td>Yondelis®</td>
<td>Isoquinoline</td>
<td>Anti-cancer</td>
<td><em>Ecteinascidia turbinata</em></td>
<td>Sea squirt</td>
</tr>
<tr>
<td>Ziconotide</td>
<td>Prial®</td>
<td>Peptide</td>
<td>Pain reliever</td>
<td><em>Conus magus</em></td>
<td>Snail</td>
</tr>
</tbody>
</table>
Introduction

Problems

• Synergistic or antagonistic effects in a crude mixture
• Adequate supply / industrially feasible?
• Variation in seasonal / spatial distribution of the bioactive of interest

Some solutions

• Environmentally sound, sustainable harvesting
• Mariculture / Aquaculture
• Find (semi)synthetic route to drug
Anticancer vs cytoprotective

Anti-cancer drugs

• Cytotoxic - kills cancer cells
• Used as a chemotherapeutic agent

Cytoprotective

• Protects cells
• Reduce DNA damage

May be a matter of dose

• Low dose may prevent oxidative damage
Overview

Aim: to determine the cytotoxicity of several crude seaweed extracts rich in polyphenols

Seven species of seaweed
- Four Brown, Two red, One green

Several extraction methods
- Water, Enzyme assisted, Solvent
Methods

Polyphenol content was quantified by Folin-Ciocalteu method

- Standard method for total polyphenol determination
- Reacts with phenols and other reducing substances. Interferences have been shown to account for less than 5% response
- Reacts to give a mixture of blue complexes which absorb between 725 and 750 nm
- The absorbance is proportional to the amount of polyphenols in the extract
- Phloroglucinol or gallic acid can be used as standards;
- Phloroglucinol for phlorotannins, gallic acid for red and green seaweed polyphenols
Cytotoxicity against MCF-7 (breast cancer) cells was tested

- Viable cell counts measured by trypan blue staining
- Excluded by live cells, but permeates dead cells
- Viable cells appear clear under a microscope and dead cells are dyed blue
- Docetaxel used as a control - an extract from the bark of the Pacific yew tree *Taxus brevifolia*. 
Water extracts

- Brown fucoids highest in polyphenol content
- 4.0-6.0 % for brown
- 0.3-1.5 % for red and green
- Moderate polyphenol content compared to commercial extracts

*Figure 1: Polyphenol content of water based extracts*
Water extracts

- All extracts had some cytotoxic effect
- In general, browns were better (50% or more of cancerous cells killed compared to 75% or less for red and green)

Figure 2: Cell viability after treatment with water based extracts
Enzyme assisted extracts

- Reports in the literature of using enzymes to enhance the extraction of bioactives*
- Cell wall can be degraded to make the intracellular contents more accessible for extraction
- Flavourzyme (protease) and Celluclast (carbohydrase) were used

Enzyme assisted extracts

- Celluclast extracts worked best (cell viability was reduced to 50-90%)
- Not as good as water extracts
- Polyphenol content (not shown here) was not as high as for water extracts with exception of *Palmaria palmata*
Solvent extracts

- Polyphenol contents were higher than water extracts (7-9%)
- Also good cytotoxicity

Figure 5 Viability of MCF-7 cells after 48 h treatment with solvent based extracts
Correlation between polyphenol content and cytotoxicity?

- No correlation seen
  \[ R^2 = 0.17 \]
  
- Polyphenols not responsible for cytotoxic effect?
Cytoprotective effect

- Antioxidants may slow or prevent the development of cancer
- Protect cells from the action of free radicals which may damage DNA
- In vitro evidence for this, evidence from human clinical trials is not so clear
- Vitamin C, E, lycopene, polyphenols
Methods

- DPPH method for radical scavenging potential
- Commonly used
- DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical
- Reduction of DPPH. Due to the presence of an odd electron it has a strong absorbance at 517 nm.
- Once this electron is paired (e.g., by the action of a free radical scavenging antioxidant/DPPH is reduced), the absorbance falls.
Results

- Extracts had good anti-radical effects
- A correlation between polyphenol content and anti-radical power was found ($R^2 = 0.86$)
- May be good cancer protective agents

Figure 7 Correlation between polyphenol content and anti-radical activity
Conclusions

• Good cytotoxic effect
• Cytotoxicity not related to polyphenol content
• Other compounds may be responsible: sulphated polysaccharides, carotenoids, other small molecules?
• Good antioxidant activity
• Maybe there is an application for high polyphenol extracts as cancer-protective agents
• Seaweed a good target for extraction of natural products
Future work

• Fractionate most promising extracts by semi-preparative HPLC and re-assay for cytotoxic effect
• Use tandem mass spectrometry as an aid towards the structural elucidation of compounds of interest
Acknowledgements

Komal Kaluskar and Alwynne Cleary who analysed samples for cytoxicity
Fore more information

See review article: