Journal of Biomedicine and Biotechnology • 2004:2 (2004) 93–98 • PII. S1110724304307060 • http://jbb.hindawi.com

REVIEW ARTICLE

Novel Marine Compounds: Anticancer or Genotoxic?

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Received 27 July 2003; revised 4 December 2003; accepted 4 December 2003

In the past several decades, marine organisms have generously gifted to the pharmaceutical industries numerous naturally bioactive compounds with antiviral, antibacterial, antimalarial, anti-inflammatory, antioxidant, and anticancer potentials. But till date only few anticancer drugs (cytarabine, vidarabine) have been commercially developed from marine compounds while several others are currently in different clinical trials. Majority of these compounds were tested in the tumor xenograft models, however, lack of anticancer potential data in the chemical- and/or oncogene-induced pre-initiation animal carcinogenesis models might have cost some of the marine anticancer compounds an early exit from the clinical trials. This review critically discusses importance of preclinical evaluation, failure of human clinical trials with certain potential anticancer agents, the screening tests used, and choice of biomarkers.

INTRODUCTION

Marine organisms provided numerous novel compounds with sensational multiple pharmacological properties [1, 2, 3, 4, 5, 6, 7]. During the past 20 years, thousands of novel compounds and their metabolites with diverse biological activities ranging from antiviral to anticancer have been isolated from various marine sources [3, 4, 5, 6]. The marine pharmacy currently holds more than 35 000 marine-derived biological samples, with approximately 150 compounds to be cytotoxic against the tumor cells [3]. Recent reviews further suggest that approximately 35 compounds have a known mechanism(s) of action for their antitumor effect while 124 marine compounds yet to be studied for their detailed mechanism of antitumor activity [2, 4, 7]. Out of 35 antitumor compounds, at least a dozen of them are currently in various phases of human clinical trials for treatment of different cancers [4]. Some of the prominent anticancer compounds in clinical trials include ecteinascidin (Yondelis), bryostatin-1, squalamine, aplidin, dolastatin-10, ILX651, and KRN7000 (α-galactosylceramide) (Table 1) [4].

PRECLINICAL EVALUATIONS AND CLINICAL TRIALS

Most of the compounds were tested in vitro by high-throughput cost-effective screening assays using exclusively cancer cell lines derived from human and rodent sources [2, 7]. Based on the in vitro antitumor activity, several of these compounds were tested for their therapeutic efficacy in the tumor xenograft models in animals. However, toxicity studies in animal models are limited to only few anticancer compounds (Table 1) [8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. Review of the published literature on the genotoxicity and anticancer potentials of these compounds in vivo revealed that very few compounds have gone through the preclinical evaluations in the chemical- or oncogene-induced pre-initiation animal carcinogenesis models [4, 8, 18]. Interestingly, cytarabine (Cytostar-U), isolated from the Caribbean sponge (*Cryptotheca crypta*) and currently being used in routine treatment of patients with leukemia and lymphoma [8], seems to be one of the very few marine anticancer drugs studied in long-term pre-initation cancer model in F344 rats for its anticancer efficacy [19]. It showed anticancer potential by inhibiting the promoting effect of mitomycin C in N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)-induced bladder carcinogenesis. Recent studies further showed that another marine compound, KRN7000, was found to be effective against methylcholanthrene (MC)-induced fibrosarcomas, mammary carcinomas in Her-2/neu transgenic mice, and spontaneous sarcomas in $p53^{-/-}$ mice [20], however, it was ineffective against BBN-induced bladder carcinogenesis in F344 rats [21]. These results indicate that marine anticancer compounds should be screened in different pre-initiation animal cancer models against several target sites to ensure the optimum efficacy prior to the human clinical trials.

Despite the facts, there were several marine compounds with anticancer potential observed in the in vitro tests and tumor xenograft models entered into the human clinical trials without being thoroughly investigated in the preclinical animal models for their anticancer potentials using diverse biological endpoint biomarkers [22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32]. Ultimately, some of them were prematurely withdrawn from the clinical trials due to life-threatening toxic side effects in the patients.

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Table 1. Antitumor marine natural compounds in clinical trials with preclinical toxicity evaluation in animals.

Compound	Phase of clinical trails	Animal model	Toxicities
Bryostatin-1	II	Mice	In mice, toxicities include lethargy, unsteadiness, haematuria, and myelosuppression [8].
Dolastatin-10	II	Mice, rats, dogs	Maximum tolerated doses of 1350 $\mu g/m^2$ (mice), 450 $\mu g/m^2$ (rats), and ~ 400 $\mu g/m^2$ (dogs); Myelotoxicity was the most severe dose-limiting effect [9].
LU103793[a]	II	N/A	Toxic effects in hematopoietic, lymphoid systems, gastrointestinal tract, and heart [8].
Discodermolide[a]	I	Mice	Immunosuppresive [10].
Yondelis	II/III	Mice, rats, monkeys	Reversible hepatobiliary toxicity in monkeys [11]. Hematotoxic at MTD 600 $\mu g/m^2$ in mice [12]. Hepatotoxic in rats [13].
Squalamine	II	Monkeys	Systemic squalamine injection inhibited the development of iris neovascularization and caused partial regression of new vessels in a primate model [14]
Kahalalide F	I	Rats	MTD is 1800 $\mu g/m^2$; no-adverse-effect dose is 480 $\mu g/m^2$/day; nephrotoxic and neurotoxic [15].
Aplidin	II	Mice	More toxic effects with prolonged exposure [8].
KRN7000[b]	I	N/A	N/A [4, 8]
Cryptophycin-52	II	N/A	N/A [8]
Epothilone B	II	N/A	N/A [16]
LAF389[c]	I	N/A	N/A [4, 16]
ILX651[a]	I	N/A	N/A [4]
HTI286	I	N/A	N/A [4, 17]

N/A = not available.

[a] Dolastatin-15 analogue.

[b] Agelasphin analouge (alpha-galactosylceramide).

[c] Bengamide B analouge.

One of the most promising marine compounds made to the human clinical trials was didemnin B, a cyclic peptide isolated from the Caribbean tunicate *Tridemnum solidum* with antiviral and immunosuppressive activity [22]. It was one of the very first marine compounds entered into the human clinical trials conducted simultaneously by several groups for treatment of various cancers [6, 23]. It has shown ineffectiveness to moderate anticancer response in different target sites and always invariably accompanied with high toxicity to the patients [24, 25, 26, 27, 28, 29]. However, ultimately due to its extreme toxicity, it was withdrawn from the phase II clinical trials [1, 4, 6]. Recent literature search on the MEDLINE for didemnin B resulted in 102 publications in the peer-reviewed journals without finding a single study on the preclinical anticancer evaluation of didemnin B in pre-initiation animal carcinogenesis models. However, the agent demonstrated antitumor activity against a variety of tumor xenograft models [6, 8]. Animal toxicities in CD2F1 mice, F344 rats, and beagle dogs were also reported in lymphoid system, gastrointestinal tract, liver, and kidney [8]. Further, dehydrodidemnin B or aplidin, an oxidation analog of didemnin B, isolated from Mediterranean tunicate *Aplidium albicans* with presumably more potent anticancer potential is also being developed for its phase I clinical trials in Europe [3, 30]. However, without proper preclinical evaluation for this compound, it may also meet the same fate as didemnin B.

Similarly, girolline and jaspamide, isolated from the sponge *Pseudoaxyssa cantharella* [31] and the Indo-Pacific sponge *Jaspis splendus* [32], respectively, were also withdrawn from the clinical trials due to their extremely toxic side effects. Girolline resulted in hypertension problems in the patients while jaspamide was withdrawn gracefully from the preclinical evaluation stage because it was too toxic [5]. Literature search on both of these compounds resulted in 115 published reports for jaspamide but none were found for girolline. Out of 115 published articles for jaspamide, no report on its toxicity or anticancer potential in the rodent models was found.

Yondelis, a promising anticancer compound, is currently in phase II and phase III clinical trials and has

been approved as an Orphan Drug [4]. However, substantial hepato- and hemato-toxicities of Yondelis in rats, mice, and monkeys could actually limit its potential use in the human cancer treatment (Table 1) [11, 12, 13]. However, recent study showed that high dose of dexamethasone offered complete protection against the hepatotoxicity in rats by yondelis [13]. Another anticancer compound, LU103793, a dolastatin 15 analogue, has failed to show activity in patients with melanoma and breast cancer in phase II trials, however, trials are ongoing in ovarian, prostate, and colon cancer patients [33, 34]. Besides their therapeutic efficacy studies in tumor xenograft models, these compounds along with many others have not been evaluated for their anticancer potentials in the chemical/oncogene-induced animal carcinogenesis models.

IMPORTANCE OF BIOMARKERS

Genotoxic and anticancer profiles for the majority of the marine compounds currently in the clinical trials are scarcely available. A few reports appeared regarding the genotoxicity of marine compounds in vitro. Most of them employed relatively nonspecific and superficial assays (eg, Ames Salmonella, SOS induction, micronuclei, sister chromatid exchange, and mutatox) for measuring genotoxic potential [35, 36]. Further, the majority of the articles published so far have considered the cytotoxicity, apoptosis, and/or cell proliferation assays as benchmark screening tools/markers for assessing the anticancer potential of a compound. However, the majority of biomarkers currently in use have not been rigorously validated [37]. Thus far, there is no one universal biomarker for malignant neoplastic diseases. It is therefore possible that more than one biomarker, for example, DNA adducts, gene expression, and DNA repair activity may need to be considered for assuring the anticancer potential of a compound.

Central dogma of carcinogenesis involves multiple steps of initiation, promotion, and progression which simultaneously also provides numerous opportunities for chemoprevention and/or therapeutic strategies. One of the widely accepted notions is that the covalent DNA adduct formation represents a prerequisite step in the process of cancer initiation. Further, DNA adducts represent the net balance between the metabolism, detoxification, and to certain extent the DNA repair and therefore have been considered as an excellent intermediary biological marker to study the genotoxic and anticancer potentials of compounds [38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53].

The applicability of DNA adducts as early biomarkers in carcinogenesis and cancer prevention studies is supported by several facts: (i) carcinogen-DNA adduct formation, their presence and levels in animals and humans correlate with carcinogen exposure (reviewed in [39, 40]); (ii) the presence of DNA adducts in humans has been associated with an increased risk for cancer development [41]; (iii) the adduct forming capacity, mutagenic and carcinogenic potentials correlate for many carcinogens [42, 43, 44]; (iv) the inhibition of carcinogen-induced DNA adducts both in vitro and in vivo generally classifies a compound to be a probable anticancer [45, 46, 47, 48, 49, 50]; and (v) inhibition of DNA adducts by chemopreventive agents has been correlated with decreased tumorigenesis in animals [51, 52, 53].

Despite the fact that several marine anticancer compounds are considered to be DNA-interactive, causing from reductive DNA cleavage [54] to DNA adduct formation [55], not much attention has been given to the DNA adduct as early biomarker in the development of marine anticancer drugs. In our continuous effort to search for new potential anticancer compounds, we have screened few marine compounds for their genotoxic and chemopreventive potentials using benzo[*a*]pyrene (BP)-derived DNA adducts as endpoint biomarker in MCF-7 cells as described by Smith et al [47]. These new marine compounds, for example, manzamine A, sarcophine, curcudiol, curcuphenol, aaptamine, and verongiaquinol were reported in the literature with antimalarial, antiviral, and antitumor activities [56, 57, 58, 59, 60, 61, 62, 63, 64]. Manzamine A and sarcophine were reported to have anticancer activity [56, 59, 64]. Sarcophine, a furanocembrane diterpene, isolated from the Red Sea soft coral *Sarcophyton glaucum*, was found to serve as an effective inhibitor of JB6 cell transformation [59]. A substantial increase (up to 400%) was noticed in the BP diol epoxide-DNA adduct formation in the MCF-7 cells by nearly all the compounds (Arif, Kunhi, Siddiqui, El-Sayed, Orabi, Al-Hazzani, Al-Ahdal, Al-Khodairy, manuscript in preparation). Interestingly, sarcophine (100 μM) and manzamine A (50 μM), the probable anticancer compounds, were of the significant inducers of BP-DNA adduct formation. These marine compounds themselves did not actually form any lipophilic DNA adducts, however, they modulated the DNA adduct formation by BP suggesting that they could enhance the DNA adduction in the animal and/or human tissues which could be preexposed to different carcinogens via diet and environment. Though the mechanism(s) of increased BP-DNA adduct formation by these compounds is not known, it could be due to modulation in the metabolic activation (phase I enzymes), detoxification (phase II enzymes) and/or DNA repair. Based on the preliminary data, sarcophine and manzamine A may be considered as probable genotoxic rather than anticancer compounds and they should be cross-examined using additional biomarkers prior to be tested in the animal models and human clinical trials. This being the first observation with the marine compounds using DNA adducts as intermediary biomarker further stresses the importance of DNA adducts in assuring the anticancer potential of new compounds. Further studies on DNA repair and related genes with marine compounds are in progress in our laboratory.

CONCLUSIONS

In the past several decades, thousands of marine compounds with tremendous pharmacological activities have been isolated and more than a dozen of them are in different stages of human clinical trials against various diseases. However, several of the known marine compounds were also withdrawn prematurely from clinical trials because of their extremely toxic side effects. Most of these anticancer marine compounds currently in clinical trials utilize their therapeutic potentials were tested in short-term animal studies using human tumor xenografts, however, only two compounds (Cytarabine and KRN7000) were tested in chemical- and/or oncogene-induced pre-initiation animal carcinogenesis models for their anticancer potentials.

In summary, it is the time to carefully and thoroughly screen any marine compound for anticancer and genotoxic potentials using variety of biomarkers in animal carcinogenesis models prior to their entry into the clinical trials, otherwise, there may be no surprise that they could also meet similar fate as didemnin B and others with substantial setback to the marine drug development programs.

ACKNOWLEDGMENTS

The authors would like to thank the Research Center Administration for their continuous support and encouragement. We also would like to thank Drs. K. A. El-Sayed (USA) and K. Y. Orabi (Kuwait) for their collaborations on anticancer potential studies with the selected marine compounds.

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