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Isolation, analytical measurements, and cell line studies of the iron–bryostatin-1 complex

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ABSTRACT

Bryostatin-1 is a marine natural product that has demonstrated medicinal activity in pre-clinical and clinical trials for the treatment of cancer, Alzheimer's disease, effects of stroke, and HIV. In this study, iron–bryostatin-1 was obtained using a pharmaceutical aquaculture technique developed by our lab that cultivates marine bacteria for marine natural product extraction. Analytical measurements ^{1}H and ^{13}C NMR, mass spectrometry, and flame atomic absorption were utilized to confirm the presence of an iron–bryostatin-1 complex. The iron–bryostatin-1 complex produced was then tested against the National Cancer Institute's 60 cell line panel. Adding iron to bryostatin-1 lowered the anti-cancer efficacy of the compound.

In 1968, nearly three hundred marine specimens were collected from the Gulf Coast of Florida and screened for medicinal activity by the National Cancer Institute (NCI) and its collaborators. From this collection, extracts obtained from the sessile, filter feeding bryozoan *Bugula neritina* demonstrated medicinal activity. In the 1980's Cherry Herald, a scientist working with George Pettit at Arizona State University, isolated the first milligram quantities of the bryozoan extract, bryostatin-1, from a five hundred kilogram collection of *B. neritina*. The compound exhibited significant activity against murine P388 lymphocytic leukemia, and its structure was determined by crystallographic and spectroscopic techniques (see Fig. 1).[1,2] After the first structural determination of bryostatin-1, at least nineteen additional structural variations of bryostatin were identified from the bryozoan.[3] Davidson et al.[4] provided evidence that bryostatin is biosynthesized from a bacterial symbiont of *B. neritina* rather than produced by the bryozoan itself. It was demonstrated that the proteobacterial symbiont of the bryozoan, *Candidatus Endobugula sertula*, is capable of producing the compound, and that a reduction of the symbiont bacteria is associated with decreased levels of bryostatin from the host. The study also suggested cloning the genes responsible for production of bryostatin and replicating them in host bacteria as an alternate source of the compound.[4] Since its initial demonstration of in vitro anti-cancer activity, bryostatin compounds have undergone studies not only as a cancer treatment, but as a potential therapeutic agent for Alzheimer's disease, effects of stroke and HIV as well. Demand of the macrolide for clinical evaluation remains constant, however techniques for obtaining bryostatins have hindered further development of its therapeutic uses.

Bryostatins serve a key role in regulating the cell cycle by inactivating or activating certain cyclin-dependent kinases, such as CDK2 and Protein Kinase C (PKC), that are responsible for cell differentiation and signal transduction. PKCs have a role in cell growth and death with a number of different types of the protein differentiated based on functionality.[5,6] At sub-nanomolar inhibitory concentrations, the marine based compound binds to the C-terminus region of the PKC active site which ultimately results in auto-phosphorylation, protein translocation, down-regulation, and ubiquitination.[5] Short term exposure to bryostatin-1 can induce PKC activation and auto-phosphorylation, while prolonged exposure can induce PKC inhibition by depleting the cell. Bryostatin can induce apoptosis by affecting activity of cell cycle regulatory proteins. It has been suggested that medicinal

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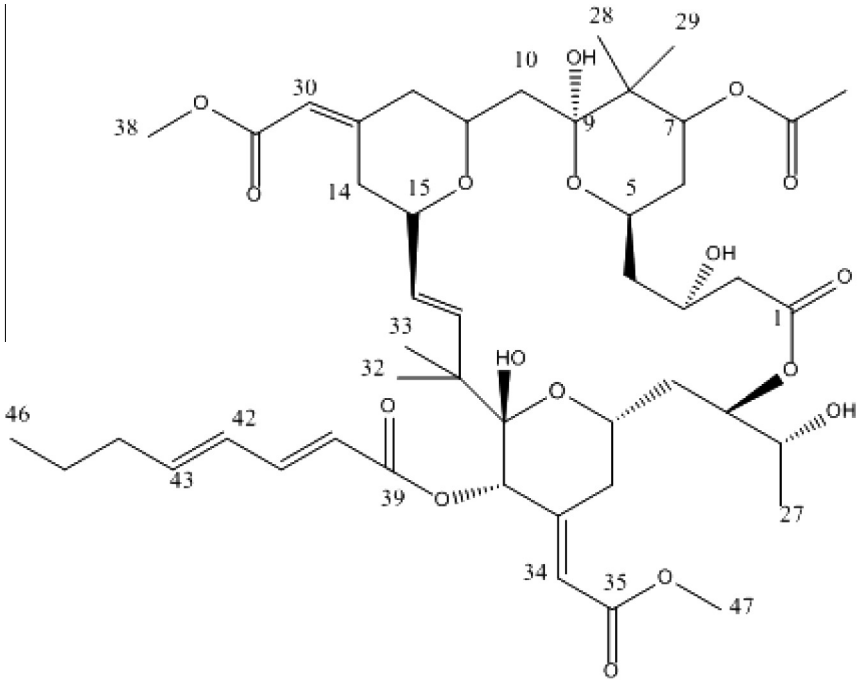

Figure 1. The two dimensional structure of bryostatin-1. ($C_{47}H_{68}O_{17}$; 905 g/mol). The bryophan ring, present in all bryostatin molecules, can bind a host of cations including Na^{+} and Fe^{3+}.

properties of the compound may not be exclusively attributed to PKC binding.[6]

Pre-clinical data indicating that bryostatin-1 improves efficacy of chemotherapy, hinders cancer cell proliferation, induces differentiation, and stimulates apoptosis has resulted in bryostatin-1 being evaluated in phase I and phase II clinical trials as a treatment for melanoma, non-Hodgkin's lymphoma, soft tissue sarcoma, ovarian carcinoma, and other forms of cancer. The findings of these studies and others suggest combination therapy of bryostatin-1 and other agents, including paclitaxel and cytarabine, to be more encouraging for future use of the drug once associated toxicities can be ameliorated.[5]

A phase II clinical trial demonstrated that combination therapy of cisplatin and bryostatin-1 resulted with modest responses in patients with persistent or recurrent ovarian cancer. However, the grade three and four toxicities associated were detrimental to the therapy, determining that this therapy should not be tested further unless significantly altered.[7] Results from a phase II clinical trial of bryostatin-1 treating non-Hodgkin's lymphoma patients demonstrated that the therapy was non-efficacious and too toxic, with myalgia and phlebitis being the primary side effects. Explanation for the lack of efficacy was unclear, however Blackhall et al.[8] argued pre-clinical data of bryostatin-1 synergism with cytotoxic agents and cytokines warrant further evaluation of combination therapies with bryostatin-1.[8] A phase II clinical trial combining bryostain-1 and interleukin-2 to treat renal cell carcinoma resulted with common grade three toxicities and only a 3.2% response rate among patients, rendering no further need for trials using this regimen.[9] A phase II clinical study by Madhusudan et al.[10] found that bryostatin-1 alone was not effective in treating advanced renal cancer, but it was suggested that combination therapy with bryostatin-1 may be a better option to assess.[10] Combining bryostatin-1 with pacliatxel increased response rates compared to paclitaxel alone in treating advanced gastric or gastroesophageal junction adenocarcinoma (i.e., 29% response rate compared to 17% response rate). However, over half of patients experienced myalgia. The data warrant further studies if bryostatin-1 induced myalgia can be controlled.[11]

Bryostatin-1 has also been studied for the treatment of conditions associated with Alzheimer's disease (AD) through a variety of proposed mechanisms. Several animal models show that PKC activation is involved in normal memory processes but is defective in AD. In vivo data has shown that bryostatin-1 could be a potential AD treatment via modulation of the alpha secretase pathway, which is induced by PKC activation, and to be preventative for toxic fragment accumulation effects. Bryostatin-1 was also associated with higher survival rates and longevity in murine models.[12] Another study demonstrated that the loss of neuronal synapses, a characteristic of AD, can be prevented by up-regulation and prevention of PKC α and PKC ε suppression by bryostatin-1 in murine models.[13] A recent study demonstrated that bryostatin-1 had significant impacts on a patient with advanced, early onset AD including improvements in speech, ability to focus, and muscular control.[14]

Pre-clinical data has suggested that bryostatin-1 could be effective in preventing and treating side effects of stroke. In vivo results demonstrated that bryostatin-1 improved deficiencies in synaptogenesis, neurotropic activity, and spatial learning and memory after ischemic/hypoxic cerebral conditions were induced in aged mice. Data indicate that the drug utilizes certain PKC isozymes to hinder pathophysiological molecular cascades and apoptosis which are activated by cerebral ischemia and hypoxia.[15,16] Administering bryostatin-1 after acute ischemic strokes has demonstrated improved survival rates and functioning by reducing damage and preventing cerebral tissue from further damage in murine models.[17]

Administration of bryostatin-1 alongside antiretroviral therapy has shown to be a potential method to help eradicate HIV. Bryostatin-1 induces activation of latent reservoirs of the virus, which represent a hindrance in treatment and elimination of HIV. In vitro evaluation of bryostatin-1 demonstrated its ability to inhibit acute HIV-1 infection while inducing latent infections in monocytic and lymphocytic cells by PKC activation. Furthermore, the drug was not toxic towards cells and it did not activate T-cells.[18] Analogues of bryostatin developed by DeChristopher et al.[19] were equally or more efficacious than bryostatin in in vitro models, and at dosages that were up to 1000 fold lower than the HIV drug prostratin.[19]