Background

In 2005, under the Marine RTDI Measure of the NDP 2000-2006, the Marine Institute funded a Biodiscovery Proof of Concept project-collaborative network that linked individual 'clusters' within a serial project. The ultimate objective of the work programme was to identify what materials from the marine environment can be exploited to develop vaccines and anti-viral drugs that can play a part in addressing diseases that cause major quality of life issues in humans.

In order to progress the project to the next stage the groups wish to focus on the purification of the active isolates and the elucidation of the chemical structure(s) of the active component(s) through the use of the full range of modern spectroscopic and analytical techniques. More extensive bioactivity screening can then commence and the chemistry can be expanded to the preparation of potentially more active analogues of the lead compounds discovered. The process becomes iterative thereafter with the structure(s) of the active compound(s) becoming targets for synthetic chemistry to prepare more substantial quantities of natural product(s) and closely related structures. This need could be addressed through funding for 3 PhD Scholarships. Under the respective direction of

1. Professor Oliver Dolly, Dr. John Sack International Centre for Neurotherapeutics, DCU
2. Professor Oliver Dolly, Dr. Christine, Loscher, School of Biotechnology, DCU
3. Professor Patrick Guiry, Centre for Synthesis and Chemical Biology, UCD

1. Exploiting marine biodiversity to develop drugs for normalising neural communication in disease: therapeutics targeting neuronal channels

Supervisors: Dr. J. Sack and Prof. J. O. Dolly(DCU)

Overview of this proposed research:

There is an unmet need for therapeutic agents to normalise aberrant communication in the nervous system. The International Centre for Neurotherapeutics (ICNT) focuses on developing novel drugs to correct the transfer of neural signals in disease states. Electrical signalling in neurons is mediated by a super-family of genes encoding ion channel proteins. When this fundamental process becomes compromised by disease, tuning of ion channels provides a means to ameliorate the symptoms. Current therapies for epilepsy, arrhythmia, anxiety, insomnia, diabetes and neuropathic pain include pharmaceuticals that target ion channels.

Our focus is on voltage-activated K^+ channels (Kvs), especially Kv1 which comprises the largest sub-family, due to their functional importance and because we possess unique experience having successfully identified, localised, purified and structural characterised these neuronal membrane proteins. Such channels are tetrameric combinations of α subunits (Kv1.1-1.6) encoded by 6 different genes; importantly, one of these subunits (Kv1.1) is involved in a variety of human diseases including genetic channelopathies (e.g. Episodic ataxia I), some forms of epilepsy and multiple sclerosis (Lehmann-Horn and Jurkat-Rott, 1999; Manganas et al., 2001). In experimental models of the letter, inhibition of Kv1 channels in axons with 4-aminopyridine corrects the inefficient conduction of nerve impulses (Smith et al., 2000); whilst this highlights the prospect for therapeutic intervention, its potency is too low for clinical application. Thus, to find better inhibitors of Kv1 channels, the biodiversity of marine natural products is to be exploited.

Outline of Ph.D. project

Compounds capable of inhibiting recombinantly-recreated Kv1 channels, exclusively available at ICNT, will be assessed via automated screening methodologies found to be

most effective in the Marine Biodiscovery Programme Proof-of-Concept phase. These involve a combination of determining inhibition of rubdium (Rb^+) efflux from cells expressing the authentic oligomeric subtypes of K^+ channels, and quantifying changes induced on membrane potential (Sokolov et. al.. 2007). Also, influence of bio-active marine compounds on electrical excitability of living neurons, via other channels, will be surveyed. By these means, extracts from two species of Irish algae (*Bonnemasonia hamifera* and *Heterosiphonia japonica*) have been identified which inhibit brain K^+ channels. Further characterisation of these activities requires chemical separations of active compounds from the extracts and elucidation of their structures, facets to be carried out by Prof. P. Guiry (UCD). Specificities of the resultant K^+ channel inhibitors will be confirmed by patch-clamp recordings in cultured neurons, and their mechanism of action elucidated. As a family of potent and selective Kv1 channel inhibitors occur in species of Irish sea anemones (*Anemonia sulcata* and *Actinia equina*), these should provide another good source of additional compounds with therapeutic properties (Honma and Shiomi, 2006). Emphasis is to be placed on finding molecules that selectively affect individual K^+ channel concatamers as this would allow tuning of a particular subtype at a given location, thereby, achieving the desired therapeutic benefit and avoiding unwanted side-effects. The best candidate drugs will be further improved by combining their structural information, obtained by the chemistry partners, with the atomic resolution structure available for Kv1 channels (Long et al., 2005) to model their interaction. Binding sites thus identified will be validated by mutagenesis of pertinent amino acids in the channel, followed by assay of the drug potencies. In the longer term, novel high-affinity compounds with desirable subtype specificity will be structurally modified to optimize their drug-like qualities. Drugs deemed to hold sufficient therapeutic potential will then be further developed with Pharmaceutical partners.
The inter-disciplinary nature of this project will ensure that the graduate obtains first-rate training in a range of advanced cellular and molecular technologies relevant to the Biopharmaceutical Industry.

2. Characterisation of anti-inflammatory potential of marine extracts

Supervisors: Dr. C. Loscher (School of Biotechnology) and Prof. J. O. Dolly(DCU)

Introduction

Inflammatory diseases, such as inflammatory bowel disease (IBD), account for significant ill health and morbidity worldwide. The incidence of IBD in Europe is increasing and suggests that, in many parts, as many as 1 in 100 people will suffer one or more often prolonged episodes of IBD in their lifetime. IBD is a complex disease, controlled by multiple risk factors including genetic and environmental. IBD and several other inflammatory diseases are typically associated with a dysregulated type 1 T cell response (Th1) and a lack of a sufficient T regulatory response (Treg). Activated dendritic cells (DC) are responsible for the generation of these responses. Novel strategies that re-balance the excessive Th1 response and enhance Treg responses may prevent and treat such inflammatory diseases. Inflammation is also now known to be a key factor in a number of other illnesses including Alzheimer's disease, atherosclerosis and type 2 diabetes mellitus; therefore, therapies that suppress the inflammatory responses could reduce the risk of developing a range of diseases.

Project outline

In the Marine Biodiscovery Proof of Concept Programme, preliminary evidence was obtained that marine extracts prepared from *Bonnemesonia hamifra, Heterosiphonia japonica, Cliona clata, Alcyonium digitata and Membranipora membranacea* have the ability to decrease the secretion of pro-inflammatory cytokines from murine macrophages and to increase the release of anti-inflammatory cytokines. This indicates that they possess potent anti-inflammatory potential. Given the importance of DC in generating these responses, this project will investigate the effects of fractions purified from these marine extracts, in collaboration with Prof. P. Guiry at UCD, on the activation of DC by examining their effects on cytokine production and cell surface marker expression. Furthermore, it will examine the consequences of these actions on the generation of subsequent T helper cell responses, in particular on Th1 and Treg responses. For marine extracts that yield positive results *in vitro*, the graduate will examine them in an *in vivo* murine model of inflammation (LPS shock). These studies will pinpoint the compounds in marine extracts exhibiting the greatest potential for attenuating inflammatory response and provide evidence of the mechanisms involved.

3. Purification of the active isolates and the elucidation of the chemical structure.

Supervisor: Prof. Patrick Guiry(UCD)

This area of the collaborative research programme is inherently linked to and dependant on the results of the screening activities of the other groups. The work entails the purification of the active isolates and the elucidation of the chemical structure(s) of the active component(s) through the use of the full range of modern spectroscopic and analytical techniques. More extensive bioactivity screening can then commence and the chemistry can be expanded to the preparation of potentially more active analogues of the lead compounds discovered. The process becomes iterative thereafter with the structure(s) of the active compound(s) becoming targets for synthetic chemistry to prepare more substantial quantities of natural product(s) and closely related structures. The training of a PhD researcher in this field will begin to address the critical gap in expertise in Ireland in the field of natural products chemistry, and in particular in marine natural products chemistry.