DISCOVERY OF NATURAL ANTI-DIABETIC DRUG CANDIDATES FROM ARCTIC MARINE ORGANISMS

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The Barents Sea and its seabed contain a diversity of vertebrates, invertebrate and algae species that have so far been utilized by humans mainly as a source of protein and marine lipids. In the last decades, however, researchers have become interested in secondary metabolites from these species, especially from invertebrates. Some of these metabolites demonstrate biological activity that can be utilised as drugs, and a few have advanced into clinical trails and already entered the drug marked. New drugs are needed, however; to replace existing imperfect drugs and to treat emerging life style diseases, such as obesity and its associated disorders (type 2 diabetes mellitus (T2DM), cancer and cardiovascular diseases) that have reached epidemic proportions worldwide.

The aetiology of T2DM is intricate and multifaceted, but insulin deficiency and insulin resistance with resulting hyperglycaemia are the most common symptoms to treat. Additionally, obesity is a predisposing factor for T2DM, which are often associated with a low-grade inflammatory state in adipose tissue. Eating is essential to life, and its episodic nature requires physiological adaptations to avoid excess or insufficiency of circulating fuels, especially glucose and lipids. Our modern lifestyle with an increasing imbalance between energy intake and energy expenditure, often resulting in obesity, is a challenge to this fine-tuned energy adaptation. Chronic disruption of the energy balance causes plasma glucose imbalance, hypertrophy and hyperplasia of adipocytes causing metabolic disorders such as T2DM. A number of potential drug targets have been identified and investigated with respect to treatment. Developed and released drugs have revealed moderate efficiency and many have shown low specificity with adverse effects. The focus of our research activities is on the discovery of bioactive constituents of marine organisms that can be developed into drugs to treat T2DM. The following targets are included in the current screening plan, using both cell based and isolated target assays: 1) The enzyme protein tyrosine phosphatase 1B (PTB-1B), 2) Insulin-stimulated glucose uptake, and 3) Peroxisome proliferator-activated receptors (PPARs) regulating the expression of genes involved in the control of lipid metabolism, glucose homeostasis and inflammatory processes

The protein tyrosine phospatases (PTPs) is an enzyme family that includes about 100 proteins which catalyze dephosphorylation of phosphotyrosine residues in protein substrates. Phosphotyrosine is a central element in cell signalling, and PTP activity is essential for both cellular homeostasis and for appropriate responses to extracellular signals. PTP-1B antagonizes insulin signalling by reducing the activation state of the insulin receptor kinase, thereby inhibiting post-receptor signalling in insulin-responsive tissue. The enzyme has generated a great deal of interest as a potential drug target, and PTP-1B null mice do not accumulate fat when placed on high-fat diet in contrast to their wild-type littermates. Unfortunately, due to the ~80% homology in the catalytic domain of the PTP superfamily, identification of inhibitors that are specific for PTP-1B has so far been proven difficult. Furthermore, the progress towards developing an efficient PTP-1B antagonist for therapeutic use has also been hampered by low bioavailability of inhibitor tested *in vivo*. Our biochemical screening regime, which also includes a counter-screening assay, has so far given us a few active fractions for further testing.

We screen for compounds which can potentiate insulin-stimulated glucose uptake using cell lines (differentiated into adipocyte-like cells) and primary adipocytes isolated from epididymal fat pads of rats. Our aim is to find compounds that interfere with the insulin signalling pathway, promoting translocation of glucose transport molecules (GLUT4) to the cell surface and increased cellular uptake of radioactive glucose (end-point parameter). GLUT4 is expressed only in muscle and fat cells, the major tissues in the body that respond to insulin. Any drug suitable for treatment of T2DM should probably display some potentiating of insulin action together with anti-inflammatory activity.

PPARs are major regulators of glucose and lipid metabolism. Furthermore, PPARs are also involved in the regulation of inflammation and angiogenesis. PPARs were originally named for their ability to induce hepatic peroxisome proliferation in mice in response to xenobiotic stimuli. The expression of three PPAR isoforms, alpha, beta/delta, and gamma, has been described. They share 60%-80% homology in their ligand-binding and DNA-binding domains. Nuclear receptors, to which PPARs belong, are promising targets for T2DM treatment strategies because they act as transcription factors and may produce selective alterations in downstream gene expression. PPAR agonists are used therapeutically in patients with T2DM, but unfortunately, PPAR agonists can have long-term adverse effects, such as increased body weight, fluid retention and increased risk of heart failure. The goal of our screening strategy is to find PPAR agonists that are more selective and have less adverse affects compared to the present PPAR drugs.

Taken together, new drugs to treat T2DM should be more selective with fever adverse effects. Screening strategies and initial results will be presented.

MECHANISM OF ACTION OF ANTIMICROBIAL PEPTIDES ISOLATED FROM INVERTEBRATES

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Antimicrobial peptides (AMPs) use several different strategies to inhibit bacterial growth. While many AMPs are responsible for membrane pore formation and cell lysis, other AMPs manage to permeabilize the membrane without membrane disruption. The aim of our studies is to characterize membrane non-disruptive AMPs, which act by targeting intracellular molecules and thereby inhibit processes vital for bacterial survival. Arasin 1 is a 37 amino acid long proline-rich antimicrobial peptide isolated from the spider crab, *Hyas araneus*. We report the localization of the pharmacophore of arasin 1 to be the proline/arginine-rich NH_2 terminus, whereas the C-terminal cysteine containing part does not seem to have any antimicrobial property. A kinetic killing study of *Escherichia coli* by using a synthetic peptide made of the first 23 NH_2 terminus amino acids, named arasin 1(1–23), revealed that this peptide acts as a bacteriostatic agent. The study implies that arasin 1(1–23) has a different mode of action than membrane lytic peptides like cecropin P1. An *in vivo* bacterial membrane integrity assay, using an *E. coli* strain expressing luciferase, showed that arasin 1(1–23) did not render the cells leaky, indicating that arasin 1(1–23) has intracellular target molecules and inhibits bacterial growth without lysing the cells. Transmission electron microscopy in combination with immunogold staining showed intracellular localization of arasin 1, which could be due to arasin 1 targeting intracellular molecules. Strategies involving peptide tagging together with chromatographic separation and mass spectroscopy identification of intracellular peptide targets will also be presented.