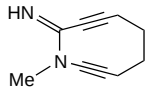
incorporated into a cyclodecaene ring was the best candidate for a new enediyne anticancer drug.



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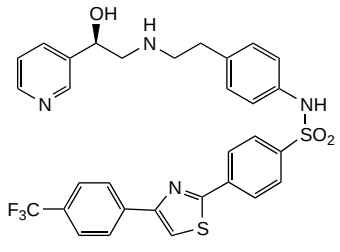
Orally bioavailable β_3-adrenergic receptor agonist as an anti-obesity agent

Obesity is a growing problem in the western world and is closely linked to type II diabetes, coronary heart disease and hypertension. Increasing energy expenditure is one approach to controlling percentage body fat and it has been observed that stimulation of β_3-adrenergic receptors (β_3 AR) located on adipocytes lead to lipolysis and upregulation of uncoupling protein UCP-1, producing an enhancement of metabolic rate.

A group at Merck (Rahway NJ, USA) has improved upon the pharmacokinetic properties of their previously disclosed series of pyridylethanolamine derivatives as potent and selective β_3-AR agonists[7]. Compound **(vii)** has been chosen for clinical evaluation. It is a full agonist with a potency of 35 nM and exhibits an oral bioavailability of 38% and 17% in dogs and rats, respectively. It has a prolonged half-life ($t_{1/2}$ >8 h) in all species tested, displays poor CNS penetration and a good therapeutic index for hyperglycerolaemia over cardiovascular effects.



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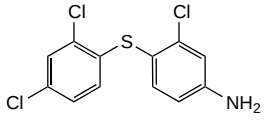
Novel *p*-arylthio cinnamides as inhibitors of the early phase of inflammation

The recruitment of leukocytes to an area of tissue damage or infection is a key event in the inflammatory process. Interactions between proteins on the cell surface of the endothelium and leukocyte lead to cell adhesion and, ultimately, migration of the leukocyte to the inflamed tissue. One of these interactions is between the integrin, the leukocyte function associated antigen-1 (LFA-1) and the intercellular adhesion molecule ICAM-1. An agent that blocks this association could modulate the early steps in the immune response.

A group at Abbott (Abbott Park IL, USA) identified the screening hit **(viii)** with an IC_{50} of 1.7 μM (Ref. 8). Careful pharmacophoric analysis of other screening hits identified an adjacent binding site. By choosing an appropriate linker, both binding pockets were accessed, facilitating a substantial increase in potency and eventually giving rise to compound **(ix)**. This molecule inhibits LFA-1–ICAM-1 binding with an IC_{50} of 44 nM and is also potent in an LFA-1 mediated cell adhesion assay with an IC_{50} of 35 nM. Opimization of the pharmacokinetic properties of this series will help to validate this target.



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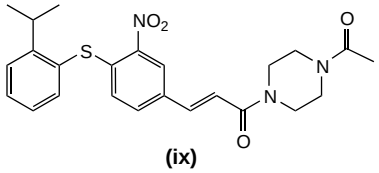

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Drug targeting

Antisense radiopharmaceuticals – agents for imaging gene expression

Gene expression *in vivo* can be imaged either indirectly or directly. In the indirect approach, a small-molecule radiopharmaceutical is administered and trapped in a target organ owing to the enzymatic activity of a specific gene product. The indirect approaches to imaging gene expression *in vivo* require pre-existing knowledge of the function of the gene. However, the vast majority of disease-causing genes that are being identified with disease-specific genomics programs, are genes of unknown function. This is illustrated by the Brain Tumor Cancer Genome Anatomy Project (BT-CGAP) database (http://www2.ncbi.nlm.nih.gov/CGAP/hTGI/). Review of this database shows that a total of 13 985 expressed genes have been detected in human brain cancer. Of these, 1095 genes are unique to human brain cancer, and of these 1095 unique genes, only 10 are genes of known function. Therefore, >99% of the expressed genes that are unique to human brain cancer are genes of unknown function! Such information suggests that indirect imaging of pathological gene

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expression in humans will not be feasible for a long time or, at least, until the function of the gene is identified. Until then, it would seem the only way that the expression of pathological genes can be confirmed non-invasively, is with the development of antisense radiopharmaceuticals that target a specific pathological gene sequence.

Scientists have imaged gene expression in the laboratory *in vitro* for years using antisense technology based on the complementary hybridization of a labeled nucleic acid with a target nucleic acid. The identification of target mRNA molecules *in vitro* can be achieved with antisense radiopharmaceuticals as short as 10 nucleotides. However, there are many barriers to the use of nucleic acid molecules *in vivo*. The classical phosphodiester oligodeoxynucleotides (ODN) are rapidly degraded *in vivo* by both exonucleases and endonucleases. Phosphorothioate (PS)-ODNs are metabolically stable but are avidly bound non-specifically by plasma and cellular proteins. PS-ODNs also activate RNase H, which cleaves the target mRNA molecule after duplex formation with the PS-ODN. Degradation of the target mRNA is not desired in a diagnostic modality. Peptide nucleic acids (PNA) represent a third class of antisense agents. These molecules are metabolically stable, are not significantly bound by proteins and do not activate RNase H. However, PNAs cross cell membranes poorly and cannot access mRNA molecules in the cytoplasm of target cells following intravenous injection of the PNA radiopharmaceutical. Therefore, the molecular formulation of the antisense radiopharmaceutical must be modified to enable transport of these molecules across membrane barriers *in vivo*. This is a classic example of the need to merge large molecule drug discovery with drug targeting technology in the overall drug development process.

Strategies to image gene expression

There have been two recent strategies reported that attempt to image gene expression in the brain with antisense radiopharmaceuticals that have been adapted to different drug targeting technologies. Paradoxically, the brain is the organ that should present most difficulties in imaging gene expression with antisense drugs because the brain is protected by the blood–brain barrier (BBB). Therefore, targeting of drugs to intracellular spaces in brain cells is a 'two-barrier' drug targeting problem. The antisense agent must undergo transcytosis through the brain capillary endothelial cell, which forms the BBB *in vivo*, and then endocytosis into the target cell in the brain. In one study[1], the expression of the gene encoding for glial fibrillary acidic protein (GFAP) was imaged in experimental brain tumors *in vivo* with a 25-mer PS-ODN. The PS-ODN contained a 5′ amino group that was labeled with [^{11}C] for imaging with positron emission tomography. The PS-ODN also contained a cholesterol moiety at the 3′ terminus to facilitate transport of the PS-ODN across cellular barriers. The targeting of antisense radiopharmaceuticals to brain intracellular compartments by adding a cholesterol adduct is a strategy that remains to be fully validated for drug targeting *in vivo*. The problem with this 'lipidization' approach is that the addition of a cholesterol moiety to the PS-ODN eliminates the solubility of the compound in aqueous solution. It then becomes necessary to solubilize the antisense agent in organic solvents. The [^{11}C] PS-ODN was solubilized in dichloromethane, which is neurotoxic, and then injected intravenously in the animal *in vivo*.

In another recent approach to imaging gene expression in the brain, rat glioma cells were permanently transfected with the gene encoding luciferase, and experimental brain tumors were formed *in vivo* following the implantation of these cells in the brain[2]. A PNA that was antisense around the methionine initiation codon of the luciferase mRNA was designed with a carboxyl terminal tyrosine to enable radiolabeling with [^{125}I] and with an amino terminal biotin residue to enabling conjugation to a BBB drug targeting system. The latter comprised a conjugate of streptavidin (SA) and a monoclonal antibody (MAb) against the transferrin receptor (TfR). This MAb undergoes receptor-mediated transcytosis across the BBB via the brain capillary endothelial TfR. The anti-TfR MAb drug targeting system also enabled transport of the PNA antisense radiopharmaceutical into the tumor cell owing to abundant expression of the TfR on the tumor cell membrane. When the unconjugated [^{125}I] anti-luciferase PNA was injected intravenously in rats, no imaging of the luciferase gene expression in the brain tumor was observed *in vivo*. Owing to negligible transport of the PNA across either the BBB or the tumor cell membrane, the unconjugated PNA could not access the target mRNA *in vivo*. However, when the PNA antisense radiopharmaceutical was conjugated to the brain drug targeting system, luciferase gene expression in the brain tumor was imaged *in vivo* in all rats studied.

The future

There is much more developmental work needed with antisense radiopharmaceuticals and *in vivo* imaging of gene expression before this technology can be practically implemented in a clinical setting. The imaging of 'any gene in any person' will be increasingly required in the future. As the vast majority of the targets will be genes of unknown function, it is difficult to envision the imaging of gene expression in humans *in vivo* without the use of antisense radiopharmaceuticals.

Antisense radiopharmaceuticals, like protein therapeutics, are 'large molecule' drugs. These large molecule pharmaceuticals do not, in general, cross cell membranes and certainly do not cross biological barriers such as the BBB. Therefore, it is unlikely that these drugs will be developed as functional pharmaceuticals *in vivo* without the application

of drug targeting technology. The problem is that drug targeting science in the year 2001 is at an embryonic stage and is rarely mentioned in the context of modern pharmaceutical development. Certainly, drug delivery is part of the pharmaceutical fabric, but drug delivery relates to controlled release of drugs and emanates from the material sciences. Drug targeting involves the movement of drugs through biological membranes, generally via endogenous transport systems, and originates in transport biology. The future application of genomics will lead to the discovery of thousands of new protein drug candidates[3]. However, future development of protein drugs will be limited, in many cases, by the ability to target these large molecule drugs to spaces deep within target cells. The use of antisense radiopharmaceuticals for the imaging of gene expression in humans *in vivo* is just one of many examples of future development of large molecule pharmaceuticals that will not be possible without the availability of effective drug targeting technology.

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Drug delivery

Adjuvancy enhancement by modulated release from microspheres

Compared with traditional vaccines based on heat-killed viruses, vaccines based on subunits, recombinant proteins or synthetic peptides are less immunogenic. The latter types of vaccines will require better adjuvants and/or delivery systems to induce an optimal response. Muramyl dipeptide (MDP) is an important immunostimulating compound under investigation as an adjuvant in human vaccines. It is the immunostimulating compound present in Complete Freund's Adjuvant (CFA), and maintains the adjuvant activity without the severe adverse effects of CFA. Unfortunately, when MDP is administered as a bolus, it is pyrogenic. There have been efforts to develop sustained release dosage forms of MDP to lower the dosage and reduce the adverse effects but the effect of these continuous release dosage forms on the immune response of adjuvants has not yet been assessed in animal models. Threonyl-MDP, which is an analog of MDP synthesized by replacing the alanine amino acid in MDP with threonine, is also under investigation as a possible adjuvant. Threonyl-MDP is more potent and less pyrogenic compared with MDP itself.

Puri *et al.* have recently reported an *in vivo* investigation of sustained release dosage forms of MDP and threonyl-MDP, and the resulting impact on adjuvant effect[1]. Ovalbumin microspheres (OVA-MSs) loaded with MDP were prepared from ovalbumin (OVA), glutaraldehyde and MDP by an emulsion technique. OVA-MSs possessing various degrees of crosslinking and matrix densities were prepared in order to study the effects of these properties on the resulting *in vitro* release rates of MDP. The *in vitro* MDP release rate exhibited a triphasic profile consisting of an initial burst, a sustained release phase and a terminal rapid release phase. The total amount of MDP released was inversely proportional to the degree of crosslinking and the matrix density of the OVA-MSs.

The *in vivo* effects of MDP- (and threonyl-MDP) loaded OVA-MSs were then studied. Mice were immunized intradermally in various treatment groups to investigate the factors affecting an antibody response to OVA. The factors investigated included the crosslinking and matrix density of OVA-MSs. Mice given a single immunization of MDP-loaded OVA-MSs exhibited an enhanced OVA antibody response for three months. An inverse relationship between the *in vitro* release rate of MDP from OVA-MSs and an *in vivo* antibody response was observed. Threonyl-MDP-loaded OVA-MSs produced better results than those loaded with MDP. Immunization with threonyl-MDP-loaded OVA-MSs led to a significantly increased induction of antibody response compared with MDP-loaded OVA-MSs. The sustained release of MDP also enabled a reduction in its dosage. The MDP dose in this study was 25% of that of previous studies and still resulted in a high antibody response for three months after a single immunization. This is the first reported observation of enhanced *in vivo* antibody response in the presence of continuous, controlled release of an adjuvant. Microsphere-modulated delivery of adjuvants might have applications in future vaccines.

Controlled release of nerve growth factor

The neurotrophin family is a group of growth factors that stimulate nerve growth and regeneration. Included in the family are β-nerve growth factor (β-NGF), brain-derived neurotrophic factor, and neurotrophin-3. *In vivo* experiments, in which β-NGF was added to nerve guide tubes, have resulted in increased nerve regeneration but it has proven difficult in these experiments to maintain growth factor release over the rather long duration of nerve regeneration. A delivery system that would provide prolonged release of active growth factor over the entire duration of wound healing would be very desirable. Heparin-containing delivery systems have been used to immobilize high affinity heparin-binding growth factors and protect them from degradation. However, the

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