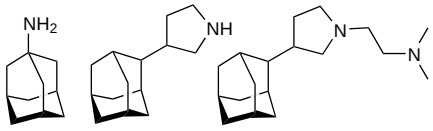
3 Kati, W.M. *et al.* (2001) Novel α- and β-amino acid inhibitors of influenza virus neuraminidase. *Antimicrob. Agents Chemother.* 45, 2563–2570

New adamantyl-based anti-influenza A agents

Amantadine **(v)** has long been known as an antiviral agent active against influenza A and is believed to act as a blocker of the M2 ion-channel of influenza A (Ref. 4). Despite the effectiveness of this compound it is only active against the A strain of the virus, not the B strain, and resistance mutations arise quickly during treatment. Research efforts to develop improved versions of this drug are under way in several laboratories.

Cyclic pyrrolidine-based adamantanes, such as **(vi)** and **(vii)**, have recently been reported[5]. These compounds were found to be active in preventing virus-induced cytopathogenicity in influenza A infected MDBK (Madin–Darby bovine kidney) cells. It is worth noting that dialkylaminoethyl substitution of amantadine yields inactive compounds but that the analogous substitution in the pyrrolidine series is tolerated.



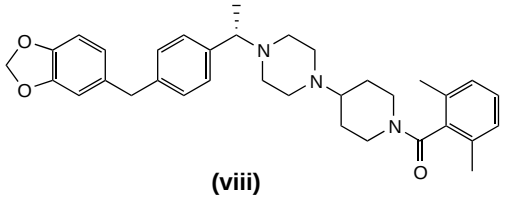
(v) (vi) (vii)

4 Hay, A.J. *et al.* (1985) The molecular basis of the specific anti-influenza action of amantadine. *EMBO J.* 4, 3021–3024

5 Stamatiou, G. *et al.* (2001) Novel 3-(2-adamantyl)pyrrolidines with potent activity against influenza A virus-identification of aminoadamantane derivatives bearing two pharmacophoric groups. *Bioorg. Med. Chem. Lett.* 11, 2137–2142

CCR5 antagonists as HIV-1 inhibitors

Viral entry is an emerging target in the fight against HIV infection. Recently, it was shown that viral binding to the chemokine receptors CD4 and CCR5 was required for entry by macrophage (M)-tropic strains of the virus[6]. Fortunately, it was found that HIV-binding to the CCR5 co-receptor, and thus viral entry, could be inhibited by the endogenous ligands for this receptor, RANTES (regulation upon-activation, normal T-cell expressed and secreted) and the macrophage inflammatory proteins, MIP-1α and MIP-1β. These observations suggested that small-molecule antagonists of the CCR5 receptor might be useful as anti-HIV agents.



(viii)

Recently, an example of this approach was disclosed by Tagat and coworkers[7] from the laboratories of Schering-Plough (Kenilworth, NJ, USA). For example, compound **(viii)** was found to inhibit the binding of RANTES to the CCR5 receptor *in vitro* (K_i = 31 nM). In cell culture it inhibited HIV-1 entry with an IC_{50} value of 1.7 nM. Furthermore, when tested against a primary HIV-1 isolate (US-1), compound **(viii)** inhibited viral replication in peripheral blood mononuclear cells with a mean IC_{50} value of 8 nM.

6 Littman, D.R. (1998) Chemokine receptors: keys to AIDS pathogenesis? *Cell* 93, 677–680

7 Tagat, J.R. *et al.* (2001) Piperazine-based CCR5 antagonists as HIV-1 inhibitors I: 2(*S*)-methyl piperazine as a key pharmacophore element. *Bioorg. Med. Chem. Lett.* 11, 2143–2146

Michael A. Walker
Bristol-Myers Squibb
Pharmaceutical Research Institute
Wallingford, CT 06492, USA
tel: +1 203 677 6686
fax: +1 203 677 7702
e-mail: walkerma@bms.com

Drug delivery and drug targeting

Drug targeting using thermally responsive polymers and local hyperthermia

There is an ongoing need for drug delivery methods that are aimed at targeting cancer chemotherapeutics to tumors. Many cytotoxic chemotherapeutics are equally as toxic to healthy tissues, so specific targeting to tumor cells is a great advantage. Attaching these drugs to soluble polymeric drug carriers improves drug pharmacokinetics, and leads to increased accumulation of the drug at the tumor site over free drug because of passive targeting, an effect that is referred to as the enhanced permeability and retention (EPR) effect. Although this can be an advantage, polymeric carriers do not target a specific site. The tumor cytotoxicity of chemotherapy or radiotherapy is enhanced synergistically by the application of hyperthermia[1,2]. Hyperthermia preferentially increases the permeability of tumor vasculature over normal vasculature, which can further enhance preferential delivery to tumors. Thermal targeting of polymeric drug carriers could offer synergistic advantages over either technique used alone.

Synergism studies

Meyer and coworkers have recently reported the use of two thermally responsive polymeric drug carriers to target tumors[3]. Their working hypothesis was that polymeric drug carriers that undergo a lower critical solution temperature (LCST) phase transition could be designed so they remain in solution *in vivo* after systemic injection, until they reach a tumor that is locally heated above the LCST. The temperature of the LCST was chosen at 40°C, because this is higher than the physiological body temperature (37°C) but lower than 42°C, a temperature that is regularly used for hyperthermia treatments in cancer patients. Using rhodamine as a model compound, they examined the effects that an LCST transition had on

  PII: S1359-6446(01)02039-6

the accumulation of polymer-bound drug in tumors that were locally heated.

Two different polymer systems, which exhibit an LCST, were tested; poly-(*N*-isopropylacrylamide-co-acrylamide; pNIPAAm) and elastin-like polypeptides (ELP). The physical properties of pNIPAAm, including its LCST, can be varied by changing the ratios of the monomers. ELPs are biopolymers of the pentapeptide repeat Val-Pro-Gly-Xaa-Gly, where Xaa, the 'guest residue', is any amino acid except proline. The physical properties of ELPs can be varied by changing the nature and ratio of the various guest residues used. Each of these two polymers has distinct advantages and disadvantages. Because ELPs are polypeptides, they are genetically encodable, which provides excellent control over their physicochemical properties. By contrast, the physicochemical properties of pNIPAAm polymers cannot be controlled as readily, but these synthetic polymers are much simpler to produce in large quantities.

Polymer synthesis

Two different polymers of each type were synthesized. An ELP sequence with guest residues Val, Gly and Ala in a 5:3:2 molar ratio was synthesized, with an LCST of 42°C (ELP1). A second, control sequence was synthesized, in which the same guest residues were used in a molar ratio of 1:7:8, with an LCST of 67°C (ELP2). Similarly, two pNIPAAM polymers were synthesized; one from an 84:16 molar ratio of *N*-isopropylamide to acrylamide, with an LCST of 41°C (pNIPAAm1), and a second (to be used as a control) from a 68:32 molar ratio of *N*-isopropylamide to acrylamide, with an LCST of 50°C (pNIPAAm2). Each polymer was labeled with a rhodamine dye to facilitate visualization by *in vivo* fluorescence video microscopy.

Human ovarian tumors were implanted in dorsal skin-fold window chambers in nude mice. The window chambers were either kept at 34°C, the average subcutaneous temperature, or at 42°C, a temperature that is safely achievable without inducing deleterious effects in healthy tissue, but is above the LCST of the test polymers. A videomicroscope was used to focus on a region of the tumor with clearly visible microvasculature. Rhodamine-labeled polymer was injected through the tail vein of the mice and six groups of five animals each were studied: ELP1 at 42°C, ELP2 at 42°C, ELP1 at 34°C, pNIPAAm1 at 42°C, pNIPAAm2 at 42°C and pNIPAAm1 at 34°C. Video images were recorded continuously for 40 s after injection and then for 10 s every 2 minutes for 60 minutes. Image analysis of total fluorescence intensity in the window allowed quantitative comparison because measured fluorescence intensity is linearly related to fluorophore concentration.

The accumulation of the carriers in the tumors was quantified by digital analysis of the whole window fluorescence intensity. Because they exhibited different pharmacokinetics, separate time points were selected for the ELP and pNIPAAm carriers to illustrate the enhancement of tumor accumulation that can be achieved by each system. At 44 minutes post-injection, the total window intensity increased by 250% for heated ELP1 relative to its initial window intensity, indicating that significant accumulation of the carrier had occurred over time. After 26 minutes, pNIPAAm1 had similarly increased by 180% relative to its initial window intensity. In cases where the tumors were not heated, or the polymer was not thermally responsive to the hyperthermia applied, the effect was not as dramatic. Total accumulation for the thermally responsive carriers was twofold greater than that for the heated and unheated control groups. Visual inspection of the videomicroscopy results suggests that the increase in total window intensity in heated tumors of the thermally responsive carriers was caused by both increased extravasation of the thermally responsive polymer and the accumulation of aggregated carrier in the vasculature.

Synergistic advantages of LCST polymers

There are several advantages to this approach using thermally responsive polymers. One unique advantage of these polymers over other thermally sensitive carriers, such as temperature-sensitive liposomes, is that accumulation of the drug in the target tissue occurs through the LCST transition of the carrier rather than through triggered release of the drug. Therefore, a concentration gradient is not required to drive accumulation of the drug into the tumor; thermally responsive polymers will continue to accumulate because of aggregation in the heated tumor, even when their blood concentration is less than that in the tumor. A second advantage is that this strategy is not specific to a particular cell or tissue, so any organ can be targeted as long as an appropriate source of hyperthermia can be applied to it. Finally, the use of hyperthermia as a technique is facilitated both by the abnormal physiology of tumors and the availability of hyperthermia applicators for clinical use. Compared with normal tissues, the vasculature of tumors cannot perfuse heat adequately so, as the tumor is heated, vasodilation does not occur and the tumor temperature continues to rise. Several different hyperthermia applicators are already in use clinically.

This is the first study to investigate the feasibility of using soluble, thermally responsive polymer-conjugates for targeted delivery to solid tumors, in conjunction with hyperthermia of tumors. It is also important to note that these studies were carried out in an *in vivo* model. However, the authors point out that several more steps must be taken before this strategy could be used in the clinic. First, the rhodamine dye facilitated visualization but the strategy must be extended to actual chemotherapeutic drugs. Some of these drugs, particularly if their mode of action requires uptake

by tumor cells, must be conjugated to the polymeric carriers in such a way that they are released upon accumulation in the tumor. Also, the polymer system itself is not yet optimized, although a twofold increase in accumulation over non-thermally responsive controls is a good starting point. In time, this approach could prove to be a good strategy for targeting cytotoxic drugs to tumors.

1 Engin, K. *et al.* (1995) Thermoradiotherapy in the management of superficial malignant tumors. *Clin. Cancer Res.* 1, 1139–1145
2 Dewhirst, M.W. *et al.* (1997) Hyperthermic treatment of malignant diseases: current status and a view toward the future. *Semin. Oncol.* 24, 616–625
3 Meyer, D.E. *et al.* (2001) Drug targeting using thermally responsive polymers and local hyperthermia. *J. Control. Release* 74, 213–224

John Weidner
Scientist, Parallel Synthesis
Medicinal Chemistry
Emisphere Technologies
765 Old Saw Mill River Rd
Tarrytown, NY 10591, USA
tel: +1 914 785 4792
fax: +1 914 593 8250
e-mail: Jweidner@emisphere.com

Therapeutic Targets

Osteoprotegerin: a new therapeutic agent for the treatment of bone disease

Physiological bone remodelling depends on an equilibrium between two cellular activities, resorption and apposition. Osteoclasts are the major agents of the resorption process, whereas apposition results mainly from bone-forming osteoblast metabolism. These cellular activities are strongly orchestrated by a large and complicated cytokine network, which has a role in bone development and the maintenance of bone integrity. Osteoclasts and osteoblasts are then interconnected, the first controlling the activation of the second[1,2]. Because osteoclasts are highly specialized elements required for bone resorption, it is not surprising that they are commonly observed at the osteolysis foci, which are associated with systemic bone diseases, for example, hyperparathyroidism and Paget disease. Furthermore, they localize at primary (giant cell tumours, chondrosarcoma) or secondary (bone metastasis) osteolytic tumours and at the membrane around loose joint implants[3]. From these data, the bone resorption can be considered as a consequence of a disturbance in the mechanisms that govern the formation, activation and function of these cells, such as the communication between osteoclasts and osteoblasts.

RANK–RANKL–OPG: a novel signalling pathway

Recent discoveries have elucidated a key signalling pathway between stromal cells and osteoclasts. A novel soluble protein, osteoprotegerin (OPG), which inhibits osteoclastogenesis *in vitro* and *in vivo,* has been cloned[4]. In response to this discovery, an osteoprotegerin-ligand, RANKL (receptor activator of NF-κB-ligand), expressed on the stromal cell membrane, and which binds OPG and stimulates osteoclast differentiation, activation and survival, has also been cloned[5]. Stromal cells also expressed a soluble form of RANKL, explaining that its effects on osteoclasts are maintained in the absence of cell contacts between osteoclasts and stromal cells. Finally, RANK, the third protagonist, is localized at the surface of the osteoclastic lineage and is the appropriate receptor for the OPG-ligand[6]. Among the protagonists of this triad, OPG acts as a decoy receptor (antagonist) and inhibits the binding between RANKL and RANK. Given that, RANK is localized at the surface of the osteoclastic lineage, mature osteoblasts and marrow stromal cells express RANKL, and OPG is ubiquitously produced by a variety of cell types including stromal cells and osteoblasts; therefore, common paracrine pathways could be suggested in the regulation of bone metabolism. Thus, the RANK–RANKL–OPG triad has created a new molecular and cellular dimension for the osteoclastic lineage. If RANK–RANKL–OPG are involved in the physiology of osteoclasts, they are clearly implicated in pathological bone disorders, thus the clinical use of the natural inhibitor OPG can be envisaged.

The role of OPG

Studies have investigated the role of OPG by generating OPG-deficient mice. These $OPG^{-/-}$ mice exhibit a decrease in total bone density, which is characterized by severe bone porosity and a high incidence of fractures similar to post-menopausal osteoporosis[7]; this can be reversed by OPG administration, which inhibits endosteal osteoclasts[8]. The role of RANK–RANKL–OPG has also been investigated in post-menopausal women with osteoporosis. Serum OPG concentrations were increased significantly in post-menopausal women with low bone mass and a high rate of bone turnover[9]. In addition, short-term administration of glucocorticoids significantly suppresses serum OPG, which might participate in the development of glucocorticoid-induced osteoporosis[10]. However, these points were recently discussed by Seck and coworkers who failed to observe the expected changes in the expression of OPG and RANKL in human bone samples at menopause[11].

Gene therapy approach

Despite this controversy, a mouse ovariectomy model of oestrogen deficiency was employed to investigate gene therapy with OPG as a means of preventing osteoporosis[12]. Mice subjected to ovariectomy surgery, followed by immediate adenoviral gene transfer, had significantly higher bone volume with reduced osteoclast numbers. This study demonstrates that a single adenoviral gene transfer can provide sustained delivery of OPG useful in the treatment of osteoporosis. The potential effects of OPG on bone tumours, mainly in hypercalcaemia associated with tumour

  PII: S1359-6446(01)02037-2