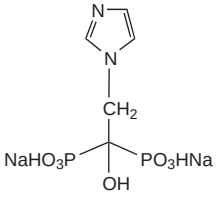
**Monitor Editor:** Matthew Thorne
m.thorne@elsevier.com

# monitor


## MOLECULES

### Calcium phosphate biomaterials for the delivery of anti-osteoporotic drugs

Postmenopausal osteoporosis, osteolytic tumor and Paget's disease are common metabolic bone disorders associated with a disruption in the balance of bone turnover as a result of increased generation and upregulated activity of osteoclasts. This upregulated osteoclastic activity leads to loosening of the substrate for coupled bone formation and resorption, which reduces bone strength and increases fracture risk [1]. During the past three decades, bisphosphonates (BPs) have been developed for the treatment of various metabolic bone diseases because of their potent inhibitory effect on osteoclastic bone resorption [2]. BPs are synthetic carbon-substituted analogues of pyrophosphates that bind tightly to calcium phosphate (CaP) crystals. Because of their powerful chelating properties towards cations, BPs form polymeric aggregates with calcium in biological systems. As a result, these compounds have a high affinity for mineralized bone matrix and are retained in the bone for many years, a property which contributes to their pharmacological effects on bone tissue. The most potent nitrogen-containing BPs, such as alendronate, risedronate and zoledronate **(i)**, act by inhibiting enzymes of the mevalonate pathway in cholesterol synthesis [3], thereby inducing osteoclastic cell death through apoptotic processes.




**(i)**

CaP ceramics [hydroxyapatite, β-tricalcium phosphate, calcium-deficient apatite (CDA) and biphasic CaP] are widely used as bone-graft substitutes in various orthopedic applications [4]. These ceramics are biocompatible (i.e. do not induce adverse effects to local tissues, immunogenicity or systemic toxicity), osteoconductive (i.e. act as a scaffold for new bone formation) and resorbable, (i.e. degradable through chemical and cellular processes). These biomaterials, already contemplated as potential carriers for bone therapeutic agents [5,6], are only available as solids (blocks or granules), which are of limited value when the surgical sites are not easily accessible or when it is preferable to perform microinvasive surgery. Thus, a new kind of injectable bone substitute (IBS), comprising a suspension of CaP granules in a cellulosic hydrogel, has been developed. The resulting injectable and ready-to-use composite has been successfully used in preclinical animal studies for orthopedic and periodontal applications [7].

Considering IBS and the biological properties of BP, Josse *et al.* [9] have developed a novel therapeutic approach designed for the treatment of osteoporotic fractures. The aim was to synthesize zoledronate-loaded CaP biomaterials that could be injected into potential osteoporosis-induced fracture sites. From scanning electron microscopy and $^{31}P$ solid-state nuclear magnetic resonance experiments, the presence of various types of zoledronate association modes was evident, depending on the nature of the CaP support. With CDA, a strong adsorption of zoledronate takes place onto the surface of the CaP carrier, probably driven by a $PO_3$–$PO_4$ exchange process. In this case, zoledronate can thereafter be released at very low concentrations, enabling evaluation of the biological activity of zoledronate-loaded materials using *in vitro* bone resorption assays. Zoledronate-loaded CDA was found to decrease the number and activity of osteoclastic cells. Indeed, in a pit resorption assay, osteoclastic resorption activity was markedly reduced. In addition, zoledronate-loaded CDA exhibited a dose-dependent inhibitory effect on osteoclastic activity similar to that observed with zoledronate solutions. These results clearly indicate that CDA is a suitable carrier for zoledronate, providing a bioactive drug delivery system whose release kinetics is compatible with the inhibition of bone resorption. This type of intraosseous delivery system for zoledronate would allow: (i) an increase in the efficiency of BPs by being locally released; and (ii) a significant decrease of the adverse effects associated with BP systemic treatments. Further biological and physicochemical experiments are under investigation with the aim of characterizing the association of various BPs to a series of CaP, along with the drug release profiles of the resulting biomaterials. To provide interesting predictive data before human clinical trials, preclinical experiments based on osteoporotic models are now in progress to determine the efficiency of zoledronate-loaded IBS in the mainly affected bone sites (femoral necks and vertebral bodies).

Until now, current osteoporosis treatments only partially fulfill the needs of a patient,




1359-6446/04/$ – see front matter  PII: S1359-6446(04)03318-5

thereby generating strong expectations. There is a need to develop new therapeutic strategies for osteoporotic patients that would not only reduce bone loss, but would also allow recovery of their bone integrity while preserving their comfort and safety.

With respect to the ageing of population, osteoporosis and related fractures are a growing matter of concern for public health. Innovative approaches for the treatment of osteoporosis could therefore be of major interest not only for medical reasons, but also from a social and economical viewpoint.

**Acknowledgments**

This work was partially supported by the French Ministry of Research (ACI Technologies pour la Santé), the C.N.R.S. (Programme Matériaux Nouveaux – Fonctionnalités Nouvelles) and the Fondation de l'avenir pour la recherche médicale appliqué. Partial support from the Région Pays de la Loire (CPER Biomatériaux – S3) is also acknowledged. We thank Novartis Pharma Research (Basel) for a generous gift of zoledronate. We also acknowledge P. Pilet, G. Grimandi, C. Faucheux, S. Josse, S. Laib, D. Massiot, B. Alonso, M.D. Arshad, G. Montavon, O. Gauthier, P. Weiss and G. Daculsi.

1 Rodan, G.A. and Martin, T.J. (2000) Therapeutic approaches to bone diseases. *Science* 289, 1508–1514
2 Fleisch, H. (2002) Development of bisphosphonates. *Breast Cancer Res.* 4 , 30–34
3 Fisher, J.E. *et al.* (2000) *In vivo* effects of bisphosphonates on the osteoclast mevalonate pathway. *Endocrinology* 141, 4793–4796
4 Daculsi, G. *et al.* (1997) Adaptive crystal formation in normal and pathological calcifications in synthetic calcium phosphate and related biomaterials. *Int. Rev. Cytol.* 172, 129–191
5 Guicheux, J. *et al.* (1998) Human growth hormone locally released in bone sites by calcium-phosphate biomaterial stimulates ceramic bone substitution without systemic effects: a rabbit study. *J. Bone Miner. Res.* 13, 739–748
6 Magne, D. *et al.* (2002) Calcium phosphate ceramics as carrier for bone therapeutic agents. *Drug Discov. Today* 7, 928–931
7 Gauthier, O. *et al.* (2003) Noninvasive bone replacement with a new injectable calcium phosphate biomaterial. *J. Biomed. Mater. Res.* 66A, 47–54
8 Bujoli, B. *et al.* INSERM-CNRS-University, Composé phosphocalcique modifié, composition injectable le contenant. WO03074098
9 Josse, S. *et al.* (2004) An innovative strategy for local treatment of bone resorption and prevention of osteoporotic fractures. *Adv. Mater.* 16, 1423–1427

***Jérôme Guicheux***
***Assem Soueidan***
***Jean-Michel Bouler***
***Bruno Bujoli***
***and Pascal Janvier***
*Jerome.Guicheux@nantes.inserm.fr*


## NEUROSCIENCE

### Knockdown not knockout

The discovery of candidate genes for neuropsychiatric disorders from high-throughput screens has necessitated the need for a rapid, consistent method for characterizing their function in model systems. Traditionally, this has been done using knockout or transgenic mice, but this approach is both time-consuming, technically demanding and complicated by genetic redundancy and developmental effects. The advent of RNAi technology (whereby cytoplasmic mRNAs that contain homologous sequences to a double-stranded RNA trigger are selectively destroyed) has negated many of these difficulties, but, using viral vectors, knockdown effects in the brain have, thus far, been limited to specific regions surrounding the infusion site. To ascertain the role of ubiquitously expressed genes in the brain that might affect multiple neuronal circuits, it would be desirable to have an RNAi protocol that elicited brain-wide knockdown.

Thakker and colleagues have devised and validated such a protocol in mice [1]. Briefly, through daily infusion of a double-stranded short interfering RNA (siRNA) of just 21 nucleotides into the dorsal third ventricle, the authors achieved widespread, specific knockdown of a ubiquitously expressed EGFP transgene; this knockdown effect was most pronounced adjacent to the infusion site but extended as far as the prefrontal cortex. After two weeks, knockdown was observed in the vast majority of brain regions. To confirm the efficacy of their approach the authors attempted to reduce expression of the endogenous *DAT* gene (encoding the dopamine transporter) in the ventral midbrain (i.e. far caudal to the infusion site). *DAT* mRNA levels were reduced by ~33% in this region, while protein levels were reduced by ~49%. At the functional level, this attenuation of expression gave rise to a hyperlocomotor response (quantitatively equivalent to that

## CARDIOVASCULAR BIOLOGY

### Anchoring proteins are multitasking

To target the action of second messenger such as cAMP, protein kinase A (PKA) and protein kinase C are specifically compartmentalized by one of the A-kinase anchoring proteins (AKAPs). This mode of regulation, used by ion channels and G-protein-coupled receptors for example, ensures that PKA is exposed to isolated cAMP gradients, which allows for efficient catalytic activation and accurate substrate selection.

Kurokawa *et al.* [2] studied the effects of Yotiao, an isoform of AKAP, on the cardiac potassium channel (IKs) and reported that Yotiao fulfills two roles in the modulation of cardiac action potential. First, in response to stimulation by the sympathetic nervous system, PKA phosphorylates KCNQ1, the α subunit of the IKs potassium channel, thereby modifying its response. Second, the authors established that Yotiao recognizes a specific motif located at the C-terminal domain of KCNQ1.

Interaction between the C-terminal domain of KCNQ1 and Yotiao modifies channel function. Moreover, this effect is only seen when the channel has been phosphorylated or if the serine involved in the phosphorylation is mutated in a negatively charged residue. This study shows that anchoring proteins such as AKAPs could have several roles. They not only recruit all the elements necessary to build specific protein complexes, they can also have a significant influence on their function.

2 Kurokawa, J. *et al.* (2004) Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16374–16378

***Muriel Laine***
*mul2001@med.cornell.edu*



  PII: S1359-6446(04)03326-4 