Potential of calcitonin as a novel cotreatment for arthritis

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After its discovery in the middle of the last century, calcitonin was quickly recognized as an extremely powerful inhibitor of osteoclast activity. Such a potent action prompted its use in Paget's disease, and determined its status as an elective anti-osteoporotic therapeutic. Over the years, however, its use has steadily declined, often because of costs and tachyphylaxis in calcitonin-induced responses, paralleled by the emergence of (cheaper) biphosphonates that now have wide diffusion and application. Modern technologies enable cheaper production costs for calcitonin; in addition, prednisolone and other glucocorticoids might impede the instauration of tachyphylaxis, such that calcitonin would retain its therapeutic efficacy. Therefore, prompted by recent experimental data generated with a model of rheumatoid arthritis, where calcitonin displayed synergistic effect when given as a cotreatment with prednisolone, as well as by clinical data showing efficacy of this hormone in osteoarthritis, we here propose a revamped use for calcitonin, as a cotreatment for different forms of arthritides.

Calcitonin (CT) is a 32-amino acid peptide hormone produced by the C cells of the thyroid and secreted in response to hypercalcemia. Its major effect is inhibition of the resorptive activity of osteoclasts, with a potential action also on their recruitment. In humans, serum CT rises during pregnancy and lactation in order to limit skeletal loss during this period of calcium stress [1]. High levels of serum CT are also associated with medullary thyroid cancer and the measurement of this hormone has become a classical marker for this form of cancer [1,2].

In view of its potent bone-modifying properties, CT has been widely used for the treatment of bone metabolic disorders, including osteoporosis, Paget's disease and hypercalcemia associated with malignancy [1,2]. Despite its efficacy in reducing bone loss, there is a limitation to the therapeutic application of CT due to receptor desensitization and downregulation following continuous treatment, hence exposure to the ligand [2]. This fact, associated with its uneasy administration route (by injection or nasal spray), has led to the prevalence of bisphosphonate therapy over CT in the management of the conditions mentioned above.

Bone metabolism & rheumatoid arthritis

In the past few years, a key regulator of bone metabolism has been identified in the receptor activator of nuclear factor (NF)-κB-ligand (RANKL) a member of the TNF ligand superfamily of cytokines that binds to receptor activator of NF–κB (RANK) expressed on osteoclasts. RANKL is required for osteoclast differentiation and augments osteoclast's activity and survival. Osteoprotegerin (OPG), a member of the TNF-α receptor family that acts as a decoy receptor for RANKL, strongly inhibits bone resorption. In fact, when OPG binds to RANKL it prevents its interaction with the transduction receptor RANK [3,4].

Rheumatoid arthritis (RA), a condition characterized by progressive synovial inflammation and joint destruction, is also characterized by enhanced bone resorption. Patients with RA have lower bone mineral density and are at risk of pathological fractures. Bone erosion in RA is caused by osteoclast activation triggered by the production of RANKL by synovial fibroblasts and T lymphocytes, and it is therefore susceptible to OPG inhibition. However, OPG has no major anti-inflammatory effects on synovitis or pannus [5]. The implications that bone erosion might have in RA could also be aggravated in the context of clinical management; in fact, an elective treatment for RA is glucocorticoid (GC) therapy.

Glucocorticoids in rheumatoid arthritis

GCs are potent immunosuppressive and anti-inflammatory agents widely used in all forms of chronic inflammation, although their long-term use is limited by the induction of secondary osteoporosis. GC action on bone cells has been associated with an increase in parathormone release, leading to higher bone resorption and a decreased number of bone-forming cells [6,7]; furthermore, the effect of GCs on osteoblasts has been linked to RANKL upregulation and OPG downregulation [8]. More recently, a direct action

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of GC on osteoclast cytoskeletal re-arrangements resulting in a suppression of the whole bone remodeling process, by preventing optimal microfilament formation, has also been reported [9]. Prolonged treatment with GCs in rheumatoid arthritis [10] is associated with secondary osteoporosis [11], which may result from a multiplicity of effects: beside their proapoptotic effect on bone-forming cells, GCs also increase bone resorption via augmented parathormone release; more recently, GCs have been shown to upregulate RANKL and decrease OPG levels, as well as directly affect the osteoclast activation process. Therefore, the beneficial anti-inflammatory action of GCs can be impaired by an exacerbating effect on bone erosion.

Calcitonin in arthritis

It is of interest that GCs have been shown to restore CT-receptor expression and reduce its degradation in osteoclasts, hence preventing its down-regulation [12], while, in more macroscopic analyses, CT is effective in preventing GC-induced spine fractures [13]. Of interest, sparse clinical analyses indicate that CT might produce analgesic properties in patients suffering from painful osteolytic metastases and vertebral crush fractures [2]. This excellent review also summarizes the scant evidence in the literature suggesting that calcitonin might have anti-inflammatory activity in specific animal models of inflammation, which is possibly related to its analgesic effect in patients affected by rheumatoid arthritis [2].

More recently, a therapeutic role of this hormone in the prevention and treatment of degenerative joint diseases, such as osteoarthritis, has been highlighted [14–16]. Osteoarthritis is the most common joint disease and a major cause of disability in people over 50 years of age. The hallmark of this disease is progressive degeneration of articular cartilage accompanied by alterations of the structure of the subchondral bone. Administration of oral salmon CT (0.5–1 mg) to osteoarthritis patients for 84 days produced a significant reduction in the levels of matrix metalloproteinase (MMP)-3 and hyaluronan as well as C-terminal telopeptides of collagen type II [16]. In *in vitro* experiments, CT reduced progression of cartilage damage by attenuating MMP-3 expression and activity in articular chondrocytes; complementary to this, CT stimulated proliferation, matrix synthesis and maturation of chondrocytes [15].

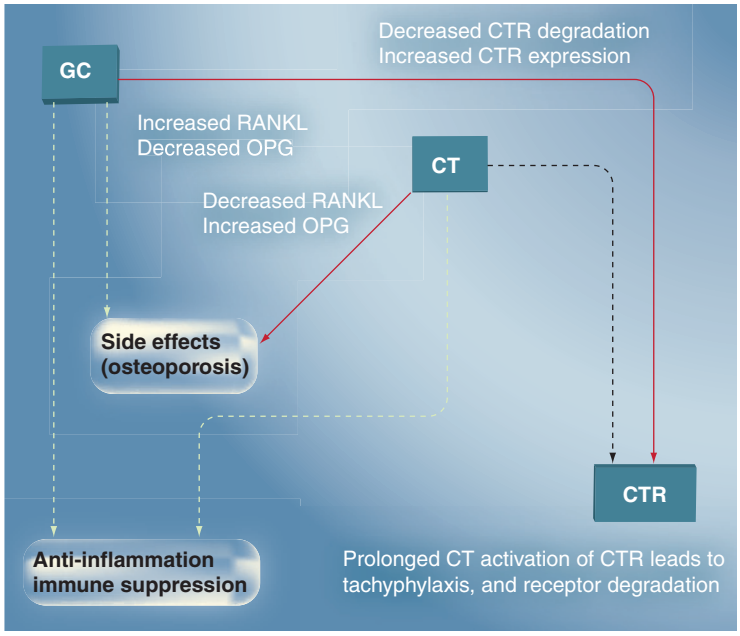
We have recently demonstrated that CT is able to increase OPG and decrease RANKL expression in the osteosarcoma cell line U2OS: this novel effect might underline/contribute to the known antiresorptive activities of this hormone [17]. In addition, we have unveiled a novel macroscopic joint-protective effect of CT evident in combination with GC treatment. Of interest to us, the protective effect observed with CT on joint structure was not reported following treatment with OPG instead [17]. Below we will comment and review these two novel aspects of CT biology.

Glucocorticoids & calcitonin in experimental arthritis

Our work began from the notion that the multiple actions on CT bone remodeling could arise from complex and, at least partially, indirect modulation of different target cells. CT might produce an anabolic effect on osteoblasts *in vitro*, at least as determined with immortalized cell lines [18,19]; the presence of the CT receptor has been reported in the murine osteosarcoma cell line UMR-106–06. We recently demonstrated that the osteoblast-like cell line U2OS expressed both message and protein for the CT receptor. In addition, the receptor was functional since it produced increases in cAMP [1], which could be measured following addition of low CT concentrations. Since osteoblasts also express the GC receptor, we then tested whether the two treatments could have opposite modulatory effects on the RANKL/OPG system. Indeed, addition of prednisolone to U2OS cells augmented RANKL synthesis, whereas the expression of OPG decreased. Comparison of these effects with those produced by CT, in the same experimental settings, revealed mirrored results: CT addition to U2OS cells downregulated RANKL and increased OPG expression [17]. Such effects on osteoblast responsiveness by CT would have been considered heretical a few years ago, however there is recent evidence in the literature that CT might have a wider spectrum of target cells: CT displays antiapoptotic effects on osteocytic cells as well as mature osteoblasts [18]. This hormone stimulates proliferation, calcium uptake and appearance of alkaline phosphatase activity (a distinct marker of osteoblast differentiation) in osteoblast-like cells [19]. It has also been recently reported to produce anabolic effects on chondrocytes [14–16].

Changes in crucial elements of the RANKL/OPG system suggested to us to determine CT effect on bone resorption in the presence of GCs, finding that picomolar concentrations of CT abolished the stimulating effect of prednisolone on bone resorption. Therefore, we concluded that

Figure 1. Schematic link between CT and GC in arthritis and inflammation: indication for a new therapeutic circuit.



Addition of CT to the therapeutic regimen would enable reduction of the dose of GC as well as prevent its disruptive actions on bone. CT would inhibit metalloprotease-3 release, and would alter the RANKL/OPG balance in favor of the latter. However, prolonged treatment with CT produces rapid tachyphylaxis as a consequence of loss of the CTR. Conversely, the GC (prednisolone in our experimental settings) would exert profound anti-inflammatory and immunosuppressive effects (via multiple mechanisms, the discussion of which is outside the scope of this review); their therapeutic benefit is limited by profound alteration of bone metabolism, switching the RANKL/OPG balance in favor of the ligand, hence producing secondary osteoporosis. We propose that the GC + CT cotreatment would have multiple beneficial effects: it will potentiate the anti-inflammatory properties of the GC (the mechanism[s] behind this are still obscure); it will prevent osteoporosis and bone loss: CT will successfully counteract GC and will prevent osteoclast activation; it will assure prolongation of CT efficacy, because the GC would prevent CTR loss, by blocking degradation and augmenting expression. The end point of these multiple phenomena will be better GC efficacy (evident at a lower dose) and absence of secondary osteoporosis. The latter aspect would be particularly relevant to chronic inflammatory diseases, including osteoarthritis and rheumatoid arthritis, where net bone degradation occurs. Altogether, our hypothesis (and underpinning model shown herein) might underline the re-evaluation of CT as a novel strategy for producing anti-inflammatory and joint protective actions.
Dashed lines: activation and/or potentiation; Solid lines: inhibition and/or prevention.
CT: Calcitonin; CTR: CT receptor; GC: Glucocorticoid; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor-κB-ligand.

opposite modulation of OPG and RANKL expression might be functional [17]. This analysis also prompted testing of the effects of CT in association with a GC, in a more complex situation, such as the one that is characteristic of RA.

Using a model of RA in the rat (collagen-induced arthritis), we determined that a therapeutic regimen with CT, while ineffective by itself on disease progression and intensity of the disease manifestations, markedly improved the anti-arthritic properties of a sub-therapeutic dose of prednisolone [17]. This dramatic synergism apparently increased the efficacy of prednisolone by a factor of five. In addition, treatment with CT preserved bone morphology.

We wish to propose that the synergism between CT and GC application could lie, at least partially, in the protective effect that the latter would have on CT-receptor expression, hence preventing its downregulation. This is consequent to reduced mRNA stability and receptor binding activity. As an example, treatment of human osteoclasts with GC increases transcription of CT-receptor gene expression. This preservation of CT-receptor expression may then allow CT to display anti-inflammatory activities that would synergize with those produced by the GC. **Figure 1** illustrates, in a schematic fashion, this relationship between CT and GC, and how it could be applicable to the experimental and clinical management of rheumatoid arthritis and other arthritides.

Conclusion

To summarize, we have stressed here the opportunity that revamping an 'old horse', hence reconsidering an old drug such as CT, might provide, producing novel and safer applications for the clinical management of rheumatoid arthritis. We hypothesize that the coadministration of CT with potent anti-inflammatory GCs would have at least a dual beneficial outcome: on one hand, it will enable the protection of the affected joint by the aggressive and bone-disruptive potential of GC therapy; on the other hand, in view of the synergistic anti-inflammatory properties of CT in experimental settings, it would allow dose reduction of the GC selected, hence again allowing a better control of the side effects associated with prolonged GC therapy.

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