

25 Years of Salmon Calcitonin: From Synthesis to Therapeutic Use

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In the 25 years since it was first identified and synthesized, salmon calcitonin has been the subject of constant R & D activity on all “fronts”—chemical, biological, pharmacological, pharmaceutical, and clinical. As a result, it is currently established as the most potent of all the different varieties of the calcitonin hormone and as a standard treatment for several major diseases of bone and bone metabolism. The recent development of an effective intranasal formulation of the drug has further increased its usefulness by overcoming the patient compliance obstacle of the injectable forms. The story of calcitonin’s discovery is related by the discoverer himself, and the other aspects of its biography are briefly reviewed by authors whose involvement dates back almost as long. One of the hormone’s most exciting features is undoubtedly its potential as one of the safest solutions currently available to the growing problem of postmenopausal osteoporosis in the increasingly aging populations of both developed and developing countries.

Salmon calcitonin was first synthesized at Sandoz in Basel, Switzerland in 1969 and in the intervening 25 years has established a place in the treatment of Paget’s disease of bone, hypercalcemia of malignancy and algoneurodystrophy, and in the management of postmenopausal osteoporosis. It is now the most widely used of the four species of calcitonin—salmon, porcine, human, eel and an analog of eel calcitonin—that have been developed for clinical use, as a result of its higher intrinsic potency and its greater analgesic effect. This therapeutic advantage of salmon calcitonin was enhanced by the introduction, in 1987, of a nasal spray, which is already available in more than 70 countries worldwide. This review, which draws on Copp’s [1] recent account of the discovery, development, and clinical uses of calcitonin as well as on Azria’s [2] monograph on calcitonin physiology and pharmacology, highlights the properties of salmon calcitonin that underlie its established and potential future clinical applications.

History

Calcitonin was discovered in 1961 by Copp et al. [3, 4], based on the evidence that perfusion of the thyroid and parathyroid glands of dogs with blood containing a high calcium concentration released a hormone that lowered blood calcium. This discovery was confirmed by Kumar et al. [5] in 1963, and in the same year Hirsch et al. [6] showed that the source in the rat was the thyroid and thus suggested the alternative name, thyrocalcitonin. In 1967, Pearse and Carvalho [7] showed that the calcitonin-producing C cells of the rat thyroid were derived from the ultimobranchial body of the embryo and ultimately from the neural crest. Inspired by these observations, Copp et al. [8] demonstrated calcito-

nin in the ultimobranchial glands of chicken and dogfish, and later found it in the ultimobranchials of salmon.

In the summer of 1968, Copp organized the collection of 100 kg of ultimobranchials from half a million salmon in the packing plant of the Canadian Fishing Company in Vancouver. In a remarkable example of scientific collaboration, the glands were bulk processed by the Armour Pharmaceutical Company in Kankakee, Illinois, pure salmon calcitonin was isolated by O’Dor et al. [9] in Vancouver, the aminoacid sequence was determined by Niall et al. [10] in Boston, and the peptide was synthesized by Guttmann et al. [11] in the Sandoz Pharmaceutical Company in Basel, Switzerland—all in the space of 4 months.

Physiology and Pharmacology

Calcitonin has an ancient lineage, having been identified in unicellular animals such as *Escherichia coli* and *Candida albicans* as well as in mammals, birds, amphibians, and fish. The physiological role of calcitonin in such a wide range of species is generally unknown. In man there is no known disease that results from hypersecretion or hyposecretion of calcitonin. Hypersecretion occurs in medullary thyroid carcinoma and serum levels provide a useful tumor marker. However, the high levels of calcitonin are not associated with any significant bone changes.

The evidence from which the physiological role of calcitonin has been deduced comes from studies of the effects of porcine, human and salmon calcitonin in laboratory animals, in mammalian tissues and cells in culture, and from clinical studies. Most of the experimental studies of calcitonin, especially those carried out in the context of potential therapeutic application, have been carried out with salmon calcitonin because of its relatively high potency and its availability as a synthetic peptide of low toxicity.

Calcitonin’s principal role in man and other mammals is thought to be the short-term fine tuning of calcium homeostasis. In conjunction with parathyroid hormone and 1,25 hydroxycholecalciferol (1,25(OH)₂D₃), it is thought to protect the skeleton against “calcium stress,” e.g., during growth, pregnancy, lactation, and, briefly though repeatedly, after meals. Calcitonin may also be involved in controlling the movement of other ions, such as phosphate and magnesium, as part of the process of maintaining ionic equilibrium. The hormone exerts its effects, directly or indirectly, at many levels—in bone, the kidney, calcium homeostasis, the gastrointestinal tract, the central nervous system, and in the endocrine system.

In healthy adults the blood-calcium-lowering effect of salmon calcitonin is only slight and levels do not fall below the lower normal limit. When the rate of bone remodeling is high, however, as in patients with Paget’s disease, blood calcium falls significantly, although without giving rise to hypocalcemic manifestations. Salmon calcitonin also pro-

okes an acute hypophosphatemic response as a result of slower bone resorption, and a hyperphosphaturic effect. It lowers high blood concentrations of bone GLA protein and, in long-term use, of serum alkaline phosphatase as well as of the bone breakdown markers urinary hydroxyproline, pyridinoline, and deoxypyridinoline.

Acutely, calcitonin appears to have a direct role in normal renal function, being involved to some extent in electrolyte and water excretion and thus in calcium homeostasis. At high doses it raises blood levels of $1,25(\text{OH})_2\text{D}_3$ (through stimulation of renal 1α -hydroxylase) and cyclic AMP. Calcitonin reduces osteoclast activity *in vitro* [12], and this appears to be the mechanism underlying its inhibitory effect on bone resorption *in vivo*. Both *in vitro* and *in vivo* data suggest that, acutely, salmon calcitonin might have a direct or indirect "anabolic osteoblastic effect" on bone [13]. This means that it could improve bone quality [14] and reduce the incidence of bone fracture [15].

Many pharmacological effects of calcitonin, especially salmon calcitonin, have been identified from experimental work. These effects include antiinflammatory activity, an antihistaminic effect, interaction with prostaglandins, an antistress action, and analgesia and gastrointestinal effects. Calcitonin's principal effect on gastrointestinal function is to inhibit gastric and pancreatic secretory activity. This suggests that it might have potential in the prevention, and possibly treatment, of peptic ulcer. The peptide has found clinical application in the treatment of acute pancreatitis, relieving pain and exerting a normalizing effect on serum amylase production. Acutely, calcitonin increases the secretion of water, sodium, potassium, chloride, and other ions in the gut, and this might account for the diarrhea that may occur with high doses.

Salmon calcitonin exerts an analgesic effect, relieving both bone pain (due to tumor metastases, Paget's disease, or osteoporosis) and nonbone pain (e.g., acute pancreatitis and posthysterectomy pain). The mechanism of pain relief is not known but is under investigation, and a number of hypotheses have been advanced. They include an increase in circulating β -endorphin, inhibition of PGE_2 synthesis, interference with calcium flux, involvement of the cholinergic or serotonergic systems, a direct action on central nervous system (CNS) receptors, and a neuromodulator effect, although several of these probably reflect different aspects of the same mechanism [2].

Both the calcitonin peptide (or an analog) and calcitonin receptors are found in areas of the central nervous system involved in the control of pain perception, appetite, prolactin secretion, and lactation, and both have also been found in the hypothalamus, suggesting that calcitonin may have a neuromodulator function.

Defining Potency

The bioactivity of the calcitonins was initially measured on the basis of hypocalcemic effect in rats. In those early days it was rapidly established that the potency of salmon calcitonin in "units"/mg is some 40–50 times greater than that of the mammalian calcitonins. Ampouled preparations designed to serve as internationally available stable reference standards were prepared in the late 60s/early 70s as soon as each peptide became available in sufficient quantity. These reference ampoules were subsequently maintained and distributed on behalf of the World Health Organization (WHO) by the National Institute for Biological Standards and Control (NIBSC) in the UK. Continuity of the "International

Unit" for each species of calcitonin is still maintained today and the International Standard for salmon calcitonin (ampoules containing highly purified synthetic peptide donated to WHO by Sandoz) defines the potency of clinical doses of salmon calcitonin [16].

Routes of Administration

Calcitonins have to be given parenterally because they are hydrolyzed, and thus inactivated, in the gastrointestinal tract. Traditionally, calcitonin has been administered by subcutaneous and intramuscular injection or, less often, by intravenous injection. However, routes of administration that avoid the discomfort of repeated injections and the reactions that may follow are obviously to be preferred, and the recently developed nasal spray formulation of salmon calcitonin is particularly convenient. Other noninvasive dosage forms are also being investigated.

Therapeutic Use

Therapeutically, salmon calcitonin has proved most effective in Paget's disease of bone [17, 18] and the hypercalcemic condition associated with bone malignancy [19]. It has also had some application in other bone-related diseases such as osteogenesis imperfecta [20], reflex sympathetic dystrophy [21], and rheumatoid arthritis [22], and nonbone-related uses in which it has been investigated include nonskeletal pain [23], peptic ulcer [24], acute pancreatitis [25], and occlusive arterial disease [26].

Currently, salmon calcitonin is being explored and used in the prevention [27, 28] and treatment [29] of postmenopausal osteoporosis and in acute postovariectomy osteoporosis [30]. In fact, in August 1995, just over 25 years after the original synthesis, the intranasal formulation received FDA approval in the USA as a treatment for postmenopausal osteoporosis in women in whom estrogen therapy is not advisable. Salmon calcitonin has also been shown to be effective in senile [15, 31], immobilization-induced [32], and steroid-induced [33, 34] forms of osteoporosis. It may even reduce the fracture rate (vertebral and nonvertebral), as a number of studies have shown [15, 35, 36]. It has been reported to exert an analgesic effect in acute vertebral fracture syndrome [37, 38].

Clinical findings with salmon calcitonin nasal spray confirm earlier results with the injectable form, notably, a sustained increase in trabecular bone without adverse effects on cortical bone—and even a slight increase in some studies. Bone balance remains positive, even with intermittent treatment. Moreover, it has been reported that the higher the rate of bone turnover, the greater the response to salmon calcitonin, enabling the dose to be adjusted accordingly [39].

Thus, salmon calcitonin, particularly the intranasal form, is gaining increasing recognition as a practical, safe, effective, well-tolerated (both locally and systemically), and high-compliance drug for the management of diseases of bone metabolism.

Side Effects and Safety

Side effects of parenteral salmon calcitonin are mostly mild and include gastrointestinal symptoms (nausea, vomiting, abdominal pain, diarrhea, metallic taste), facial flushing, and

paresthesias [40]. Local pain and erythema can follow intramuscular injection of the hormone [40]. Nasal discomfort, nausea, and facial flushing can occur with use of the nasal spray but are usually mild and transient [41]. The very low toxicity of calcitonin makes it especially suitable for long-term treatment.

Resistance

Long-term use of salmon calcitonin can lead to antibody-mediated resistance and/or escape phenomenon, with specific antibody formation in up to 70% of pagetic patients, for example [42]. However, even high antibody titers are not necessarily accompanied by loss of efficacy [43, 44]. It is equally true that resistance can occur in the absence of antibodies [17, 45], indicating that the whole problem is highly complex and in need of more systematic investigation. Until such time, the only reliable solution is to try to avoid the problem, and this is best done by using an intermittent treatment regimen from the outset. If done in accordance with the schedules described in the literature, this does not seem to affect efficacy [28, 31], whether assessed on the basis of bone biomarker levels or, assuming they are both accurate and relevant, of bone mass measurements.

At the same time, it should not be forgotten that there are many reports of continuous treatment for as long as 2 years or more maintaining its effectiveness [15], in spite of the known incidence of antibody formation of 50–65%.

Conclusion

Although calcitonin has an established place in the treatment of Paget's disease of bone and certain disorders of calcium homeostasis, its most exciting clinical application is in the management of postmenopausal and senile osteoporosis. This disorder affects a vast number of women and, with the increasingly aging population, imposes a heavy burden on health care systems. Although other therapies for osteoporosis and other disorders of bone turnover (e.g., bisphosphonates, fluoride) are in use, many questions remain to be answered concerning their side effects and long-term safety, whereas salmon calcitonin is of proven safety. The introduction of salmon calcitonin nasal spray is a significant therapeutic advance, and the development of other noninjectable preparations will further increase the acceptability of calcitonin to patients and physicians alike.

M. Azria
R & D Division
Sandoz Pharma Ltd.
Basel, Switzerland

D. H. Copp
Department of Physiology
Faculty of Medicine
University of British Columbia
Canada

J. M. Zanelli
Division of Endocrinology
National Institute for Biological Standards and Control
United Kingdom

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