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Novel Drug Delivery of Calcitonin

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ABSTRACT

The essay is a short overview of the chemistry, pharmacology, pharmacokinetics, clinical uses and the delivery aspects of calcitonin. It will deal with different routes of delivery of calcitonin with the focus lying primarily on the intra-nasal delivery and oral delivery of the hormone. Calcitonin was initially administered as a subcutaneous/ intra-muscular injection and to overcome certain problems associated with these delivery routes such as patient compliance, inflammation at the site of injection, hypersensitivity reactions and stability of the formulation other routes of delivery are being explored and the most successful route of administration so far is the intra-nasal route. The essay will also give brief information of the nasal spray available in the market currently and the pump-system used for the intra-nasal delivery. The oral route of administration is also being dealt with in some detail as it is one of the most promising routes for delivery of calcitonin and other such peptide molecules and has a bright future. *In vitro* and *in vivo* research towards making a successful oral delivery formulation is being carried out and this essay gives a brief overview of some important areas studied so far.

Keywords: Calcitonin, Hormone, drug delivery.

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INTRODUCTION

Calcitonin is a polypeptide hormone consisting of 32 amino acids. It is secreted by the parafollicular cells (C-cells) of the thyroid gland and is responsible for maintaining calcium homeostasis in the body. Calcitonin acts along with parathyroid hormone and Vitamin D in maintaining calcium levels within a narrow range. In non-mammalian vertebrates it is present in the ultimobrachial bodies which are separate organs from the thyroid gland. [1]

The molecular weight of the hormone is 3431.9 Da. [2]

Chemistry:

Calcitonin has a single disulfide bond between cysteine residues 1 and 7. In all species, 8 of the 32 amino acids remain constant, including the disulfide bridge. The residues in the middle portion vary and are said to determine the potency of the hormone. Salmon calcitonin differs from the human hormone by 13 amino acid residues and is more potent than human calcitonin. Due to the increased potency and slower clearance from the circulation salmon calcitonin is preferred for therapeutic use. [1] The sequence of amino acids of salmon calcitonin is given below

Figure 1: Amino acid sequence for Salmon calcitonin

(RxList -The Internet Drug Index. Miacalcin® (salmon calcitonin) nasal spray.[3]

Mechanism of action:

Calcium is a necessary mineral that plays a vital role in a range of body functions such as muscle contraction, fusion and release of storage vesicles, blood coagulation and supports in formation and remodeling of the human skeleton. Its ionized form, Ca^{2+}, plays a pivotal role as a component of current flow across excitable membranes. [1]

The content of calcium in healthy adult males and females is approximately 1300 and 1000 g respectively. Of this more than 99% is present in the bones and teeth. Ca^{2+} is the main extracellular divalent cation. The levels of extracellular calcium even though small have to be maintained in a very narrow range. In adults, the normal serum calcium concentration ranges from 8.5- 10.4 mg/dL. This includes three different forms- ionized calcium (Ca2+, around 50%),

protein bound (40%) and complexed (10%). Parathyroid hormone and calcitriol (1, 25-dihydroxy vitamin D) play a vital role in calcium homeostasis along with calcitonin. [1]

When plasma calcium concentration is high calcitonin secretion increases and decreases when the plasma concentration is low. The concentrations of calcitonin circulating in the blood are low (less than 15 and 10pg/mL for males and females respectively). The circulating $t_{1/2}$ of calcitonin is very low (approximately 10 minutes). [1] Calcitonin acts via the calcitonin receptors (CTR). CTR is a member of the PTH/secretin subfamily of GPCRs. [1]

Calcitonin maintains blood calcium levels in the following ways:-

- It decreases the resorption of calcium from the kidney tubules and enhances its secretion in the urine
- It decreases the absorption of calcium from the intestine
- It inhibits osteoclast activity and hence bone resorption [4]

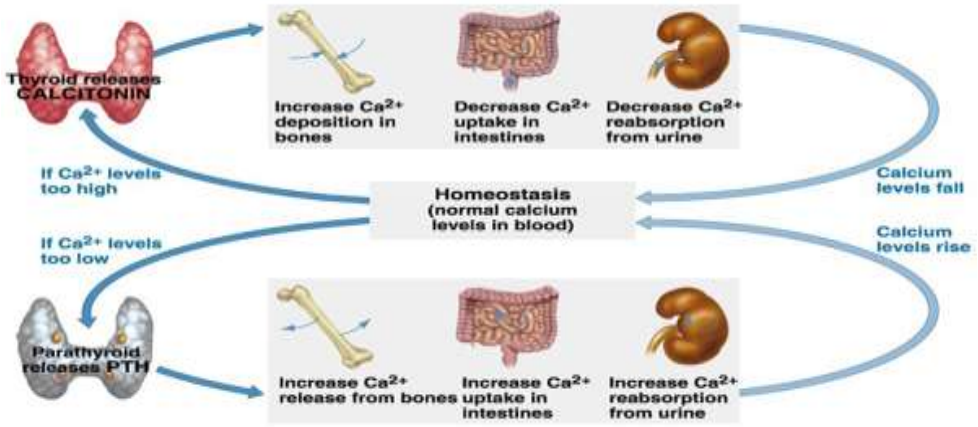

Figure 2: Mechanism Pathway of maintenance of blood calcium levels [4]

In the bone, calcitonin inhibits bone resorption by inhibiting the activity of osteoclasts. Calcitonin receptors present on the osteoclasts suggests the influence of calcitonin on their activity.[5]

There is a marked transient inhibition of bone resorption with a single injection of calcitonin and on prolonged administration there is a slow and persistent decrease in the process. This decrease in the rate of bone resorption has been attributed to the decrease in the number of osteoclasts. *In vitro* studies have been performed to gain insight on the effect of calcitonin on osteoclasts. The results of these studies show that calcitonin is responsible for the loss of function of osteoclasts and their ruffled borders which are the main site of bone resorption. Some bone forming activity, by acting on the osteoblasts, has also been reported. [5]

CLINICAL USES:

Osteoporosis

This disease is associated with fractures (both traumatic and spontaneous) which are the major cause for morbidity and mortality as well. Yet, this disease is under diagnosed, underestimated and undertreated.[6] Osteoporosis is characterized by low bone mass per unit volume of bony tissue. The main physiological conditions that lead to osteoporosis are (1) decreased rate of bone formation, (2) increased rate of bone resorption, (3) increased secretion of calcium in the urine, (4) decreased absorption of calcium in the gastrointestinal tract. [6]

Signs and symptoms:

early stages of osteoporosis are non-symptomatic. Backache is one major symptom. This disease usually causes fractures which are painful, deforming and sometimes even fatal. [6]

The recommended dose for post-menopausal women with osteoporosis is 200 I.U. per day given intranasally, alternating nostrils daily. [5]

Paget's Disease

This disease along with a number of other genetic disorders is classified as osteoscleroses. It is chronic and progresses slowly. It involves osteolytic and osteoblastic phases where first there is osteoporosis circumscripta cranii followed by the osteoblastic phase with abnormal and patchy bone formation. [6]

Symptoms:

It is often detected by an X-ray or bone scan of the affected area. Pain is the most common symptom. An elevated level of alkaline phosphatase acts an indicator. Bony overgrowths may appear. Neurological involvement may also be present. Progression of disease leads to calcium overload in the kidney causing hypercalciuria. [6] Calcitonin acts pharmacologically by inhibiting osteoclastic bone resorption. Calcitonin-salmon is available in injectable and nasal-spray forms, but only the injectable form is approved for treatment of Paget's disease by the U.S. Food and Drug Administration.

Hypercalcaemia

It is a disease where the calcium levels in the blood are elevated. Primary hyperthyroidism and malignant diseases are the main causes of this disease. Other causes include adrenal gland failure, kidney failure etc.

Symptoms:

Constipation, vomiting, anorexia, nausea, bone pain, lethargy, headache and polyuria. [7] For emergency treatment of hypercalcaemia in adults the recommended dose is 5-10 units/kg

body weight daily of injectable calcitonin in 0.9% saline by IV infusion over 6 hours. [8]

DELIVERY ASPECTS:

Parenteral route:

This route ensures highest bioavailability but the patient compliance is poor. Circulating antibodies in 30-50% patients are another drawback of this route. The antibodies are not usually clinically important but can prove to be in some patients.[9]

The side effects of the injectable formulations are certain allergy-type reactions such as bronchospasm, swelling of tongue or throat and anaphylactic shock. [5]

Pharmacokinetics: -

Absolute bioavailability of salmon calcitonin is 66% after subcutaneous (sc) and 71% after intramuscular (im) administration. Peak plasma concentrations after sc administration are reached in 23 minutes

Terminal half-lives for sc and im administration are 58 minutes and 59-64 minutes respectively. Apparent volume of distribution is 0.15-0.3 kg/L Plasma concentrations of 0.1-0.4 ng/mL are achieved with sc injection of 200 I.U. of salmon calcitonin.[9]

NOVEL DELIVERY ROUTES:

Intra-nasal delivery:

The nasal route of delivery is the much preferred route as it ensures better patient compliance. The advantages of this route are easy access, fast absorption and onset of action primarily due to the large surface area, porous endothelial membrane and high vascularization of the nasal mucosa, avoidance of enzymes and acid present in the GIT and prevention of first-pass metabolism. But the low bioavailability poses a barrier to the efficient delivery of drugs. The drug has to be highly potent to be administered via the intranasal route as frequent administration is not patient friendly. Also, the mucociliary clearance rate is very high and has to be taken into consideration as also is the degradation of the drug by the enzymes present in the mucus. [10]

In a study conducted to observe the effect of salmon calcitonin given intranasally on calcium and bone metabolism in postmenopausal women it was seen that a dose of 100 I.U. of salmon calcitonin administered intranasally affected the calcium metabolism and prevented bone loss only in the spine. There was no prevention of bone loss in the forearms or the total skeleton. From the study the authors deduced that since bioavailability of salmon calcitonin after intranasal administration was low (as observed in a separate study performed on the same preparation of salmon calcitonin) the dose had to be increased in order to prevent bone loss in regions other than the spine. [1]

Pharmacokinetics: -

Following intranasal administration onset of action is seen in 30 minutes. Bioavailability of salmon calcitonin delivered intranasally is 3% of the IM injection. No accumulation is reported with intranasal salmon calcitonin given once every 10 hours for 15 days [9]

MIACALCIN

It is available as a solution for injection as well as a solution for intranasal delivery of synthetically prepared calcitonin with the same linear sequence as that found in salmon calcitonin. [5]

Figure 3: Kaisar Permanente Miacalcin 200 unit/ actuation Nasal spray. [12]

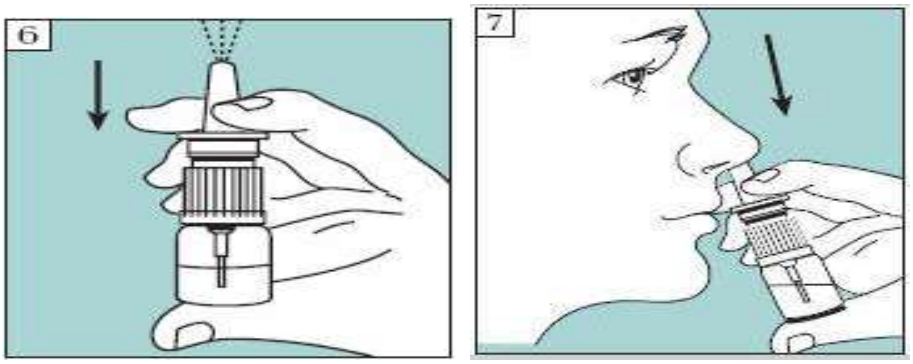

Figure 4: Nasal drug delivery using the actuation Nasal spray.[5]

It is available in a 3.7ml fill glass bottle, an amount sufficient for 30 doses. Each ml contains 2200 I.U. of salmon calcitonin. The inactive ingredients in the formulation are sodium chloride, benzalkonium chloride, hydrochloric acid (to adjust the pH) and purified water.

Miacalcin is primarily indicated for use in women with post-menopausal osteoporosis. It is used in patients who refuse or are intolerant to estrogen or in patients in whom estrogen is contraindicated. Miacalcin nasal spray is recommended with simultaneous administration of calcium supplements.

Priming of the pump provided with the medication is necessary to administer the right dose. It should not be carried out before every daily dose. The pump delivers 0.09ml of solution after

priming in every dose. The adverse reactions noted after the chronic treatment with the intranasal spray include rhinitis, back pain, bleeding nose and headache. [5]

Oral delivery:

Oral delivery is an upcoming delivery route as regards delivery of peptides. By administering peptides via this route a lot of problems associated with the current delivery routes can be overcome. The feasibility of this route, in terms of patient compliance, is a driving force for the research being carried out to develop a successful oral formulation.[13]

The primary obstacle in delivering peptide molecules by the oral route is their degradation by the enzymes present in the gastrointestinal tract. Proteolytic enzymes of the GI tract cleave the peptides before absorption. Low intestinal permeability is another barrier of major concern. Due to these barriers biologics fail to show therapeutic activity. [14]

Werle and Takeuchi synthesized chitosan-aprotinin coated multi-lamellar vesicles (MLVs) containing calcitonin and studied certain properties of these liposomes such as trypsin inhibition, mucoadhesive properties, characteristics of the liposomes and also performed *in vivo* studies in rats. They made a chitosan-aprotinin (enzyme inhibitor) conjugate and coated previously prepared calcitonin-loaded liposomes with it. The trypsin inhibition studies showed that the aprotinin present on the liposomes inhibited trypsin. The mucoadhesive studies showed mucoadhesion in the rat intestine. Blood calcium levels in rats were lowered as seen in the *in vivo* studies. In case of calcitonin solution alone and chitosan-aprotinin calcitonin-loaded MLVs, the lowest blood calcium levels were observed in around 30 minutes whereas that in case of chitosan-coated calcitonin-loaded MLVs, without the aprotinin, it was seen after 4 hours of administration. Low blood calcium levels were seen even after 24hours with chitosan-aprotinin-coated calcitonin-loaded MLVs whereas with the MLVs without aprotinin blood calcium levels went back to normal after 24 hours. Hence, chitosan-aprotinin conjugate prolonged the therapeutic action and also enhanced the pharmacological activity. [13]

Preparation of w/o microemulsions of calcitonin is one step towards improving intestinal absorption and in turn bioavailability of hydrophilic peptides given by routes other than the conventional parenteral route. In one such study microemulsions with different proportions of water phase, oil phase and surfactants were prepared and evaluated on the basis of physical characteristics such as particle size distribution, stability changes on dilution and the content of the salmon calcitonin loaded in the water phase. They were then administered to rats intraduodenally and the hypocalcaemic effects were studied. It was seen that the incorporation of Carbopol in the microemulsions, containing phosphate buffered saline as the aqueous phase and

medium chain triglyceride as the oil phase, for absorption enhancement, showed a decrease in the blood calcium levels and the pharmacological effects were maintained even after 24 hours. [15] Another approach to improve intestinal absorption is preparation of proliposomes containing bile salts as permeation enhancers. Studies carried out showed that proliposomes containing salmon calcitonin and sodium taurodeoxycholate improved bioavailability as compared to controls, in rats, when given intraduodenally. The reason for this increased bioavailability and enhanced therapeutic effect was speculated to be the formation of ion complexes between the anionic bile salt and cationic hormone. The improved entrapment efficiency in the liposomes was also attributed to the ion-pair forming capability. Hence, these proliposomes can prove to be a successful approach for delivery of salmon calcitonin orally in the future. [14]

CONCLUSION

Drug delivery aspects of various biopharmaceuticals still remains a challenge for scientists across the globe. Long chained Amino acids like calcitonin have various dimensions of drug delivery including both bioavailability challenges as well as physicochemical challenges. Although the most favoured oral route of drug delivery, seems to be promising, but for a system with optimum quality, efficacy, safety and stability it still has its limitations. Hence nasal route along with the actuator dispensing system not only overcomes the complex injections formulation aspect but also proves its bioequivalence.

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