

EDTMP and possible effects on calcium physiology in humans

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Calcium is the most abundant mineral in the human body and, in its dissolved, ionized form, is an essential cofactor of many vital enzymes. The average adult body contains a total of about 1 kg of calcium, 99% of which is in the skeleton in the form of calcium phosphate salts (hydroxyapatite). The amount of free, dissolved calcium in the body is approximately 10g, of which approximately 13 mmol occurs in the extracellular fluid (ECF), approximately 9 mmol in the blood serum and the rest in the intracellular cytoplasm (Peacock et al 2010). In a laboratory rat with an average body weight of 300g, the amount of free Ca^{2+} is approximately 0.16 mmol (Godinho et al, 2002). Over a period of 24 hours, about half of the extra-cellular calcium can be exchanged with or replaced by calcium ions that are released from the bone (Marshall JW 1995).

When there is an increased need for serum calcium (e.g. after blood loss), osteoclast activation by the parathyroid hormone PTH enables a short-term release of calcium from the bone matrix (Walter F, 2003). PTH can counteract reduced Ca^{2+} serum levels not only by stimulating calcium release from bone, but also by reducing calcium secretion by the kidneys. A side effect of a PTH-induced reduction in renal Ca^{2+} secretion is an increased phosphate excretion via the kidneys.

Reduction of Ca^{2+} serum levels in vivo by EDTMP

Phosphonates are able to bind free calcium ions through chelation. The 4 phosphonate groups of EDTMP in combination with the 2 nitrogen atoms can bind an average of 2.5 free Ca^{2+} ions. With a conditional stability constant of the EDTMP-Ca complex of 5, it can be assumed that these Ca^{2+} ions cannot be released again under physiological conditions, but are excreted via the kidneys or intestines. An oral intake of 0.5mmol EDTMP (218 mg) and its 100% absorption into the blood serum/ECF could result in a short-term, 10% reduction in the normal serum level of calcium ("hypocalcemia"). However, due to the Ca^{2+} regulation that takes place in the healthy organism, this also means that after the intake of EDTMP and Ca^{2+} binding there would actually be a temporary increase in PTH, which would last until the primary loss of dissolved calcium through release from the Bone matrix is balanced again. Such a temporary drop in free calcium in the serum and subsequent PTH-mediated mobilization of bound calcium from the bone is actually a physiologically completely normal and common process that occurs especially after blood donation, blood loss

during menstruation, and increased calcium requirements during pregnancy / breastfeeding or temporary lack of calcium in the diet. However, the situation is completely different if persistent hypocalcemia occurs as a result of repeated administration of a chelator such as EDTMP over a longer period of time.

Clinical symptoms of hypocalcemia

Only in the case of a pathological, long-lasting and uncompensated decrease in the calcium (and phosphate) level in the blood serum (in humans, mainly due to diseases of the parathyroid gland), symptoms of a primary hypocalcemia such as muscle cramps, numbness, cardiac arrhythmias arise (Minisola et al 2015).

A special form of hypocalcemia in humans is pseudohypoparathyroidism (OMIM 103580 and ICD-10 E20.1), in which the parathyroid gland produces the hormone PTH normally, but the PTH receptors required for its effect on bone cells have a genetic defect. The characteristic appearance of this disease is osteodystrophy (Albright's hereditary osteodystrophy), with frequent formation of benign bone growths outside the skeleton (including in the skin and skull) (Cortes 2006, Diercks 1996). Such osteodystrophies, especially multiple ectopic bone growths (osteoma), can easily be viewed as a true malignant tumor, although they are benign and therefore not considered cancer.

Local interaction of EDTMP with bone

In addition to the systemic effect of EDTMP on calcium metabolism throughout the organism, the substance could also have a local effect on new bone formation (similar to bis-phosphonates used therapeutically). A specifically high affinity of EDTMP for the bone matrix is exploited in therapy with Lexidronam (EDTMP-Sm153) directed against bone metastases. Bis-phosphonates, which accumulate on the bone surface, inhibit the activity of the osteoclasts there and thus have a positive effect on the overall balance of bone remodeling (through reduced bone loss).

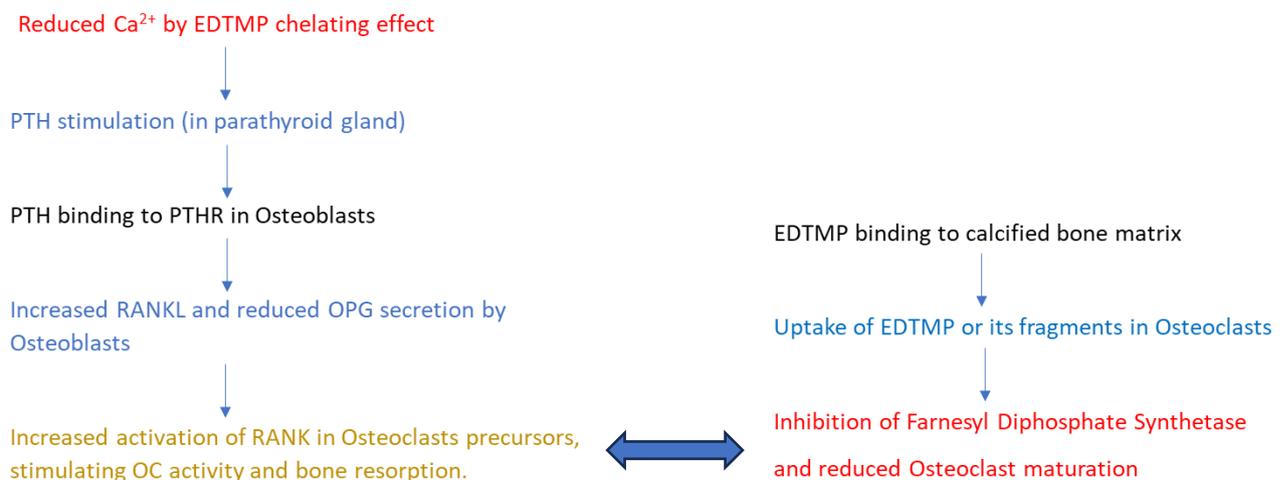
Under healthy conditions, bone homeostasis is controlled by a finely tuned mutual regulation of bone loss and bone formation. Paracrine signals from the RANKL-OPG-RANK axis are exchanged between osteoblasts and osteoclasts. If orally administered bisphosphonates are absorbed by osteoclasts, they inhibit the enzyme farnesyl diphosphate synthetase and thus block the maturation of osteoclasts or drive them to cell death. The cavities (lacunae) that are typical for bone loss are no longer formed, which means that the following steps of bone remodeling do not occur. However,

under certain circumstances, particularly in areas with very rapid bone turnover, a so-called drug-induced osteonecrosis of the jaw (MRONJ) can occur. However, the observed osteonecrosis is a sign of nonspecific bone destruction rather than increased physiological bone resorption.

It is conceivable that as a result of osteoclast activity disturbed by high doses of EDTMP, the reduced rate of bone resorption leads to a reactive response from the neighboring osteoblasts, as a result of which benign hyperplasia occurs in and outside the skeleton (osteomas). It is known from Paget's disease (ICD-10, M88.--) that chronic hyperactivity of lifelong bone remodeling can actually lead to osteosarcoma. Although in rodents such as rats, unlike humans, there is no lifelong bone remodeling and remodeling of the skeleton, at least during the course of skeletal growth immature bones are also broken down by osteoclasts and then replaced by osteoblasts with mature bone matrix. For this reason, the mechanisms of osteoclast inhibition triggered by phosphonates and their possible consequences for altered growth regulation of bone-forming osteoblasts should also take place in a laboratory rat.

Consequences of a simultaneous systemic and local effect of EDTMP

The described two mechanisms of EDTMP action (Ca^{2+} reduction in the serum and binding to the bone matrix) would lead to opposing signals at the level of the osteoclasts. While reduced serum calcium concentration must certainly result in osteoclast activation, a bis-phosphonate-like effect could inactivate these activated osteoclasts (see figure below).



Possible role of endogenous rat retroviruses in tumorigenesis

In the mouse *Mus musculus*, which is evolutionarily and genetically closely related to the rat, endogenous retroviruses play a major role in the development of leukemia (MuLV), but also solid tumors such as mammary tumors (MMTV) and soft tissue sarcomas (FBR, RSV). These endogenous retroviruses either carry oncogenes themselves or, in the course of malignant transformation, lead to the trans-activation of proto-oncogenes of the host cells or to the inactivation of its tumor suppressor genes (Anderson and Robbins 1976, Ozanne et al 1980). They are thought to be responsible for the relatively high rate of spontaneous leukemia, mammary tumors and sarcomas in mice, but can also be reactivated by genotoxic stress and thus play an amplifying role in the induction of cancer by exogenous noxious substances.

Endogenous retroviruses (RERVs) have also recently been found in the genome of many laboratory rat strains, where they have the ability to retrotranspose and thus contribute to genome instability (Wang et al 2010). The RSV virus, derived from the endogenous mouse leukemia-causing retrovirus MuLV, can cause soft tissue sarcomas quite effectively after injection into the rat (Kimball et al 1980). In humans, however, this mechanism of genome modification by reactivated, endogenous retroviruses has been shown to play no role in the course of tumorigenesis. For this reason, it is questionable whether observations in the rat model can simply be transferred to humans without more detailed investigations into the underlying mechanism of malignant transformation.

Conclusions for EDTMP medical effects after incorporation

From the knowledge about these complex, physiological processes of the provision, regulation and mobilization of calcium ions in the body and their importance for all cellular processes in various organs and tissues, it is possible to answer the question of realistic EDTMP exposure of humans on the one hand and to evaluate the relevance of the experimental studies on laboratory animals.

1.: In order for EDTMP to trigger chronic hypocalcemia, possibly with symptoms of osteodystrophy or (benign) osteomas, doses of approximately 200 mg of pure EDTMP would have to be consumed repeatedly at least daily over a longer period of time. But even if EDTMP is abused (e.g. teenage dares such as swallowing detergent tabs) or if small children accidentally swallow it, acute symptoms of poisoning could occur, as with countless other industrial or natural products, but most certainly not actual malignant tumors ("Cancer")

2.: The bone anomalies found in the experimental studies with Sprague rats (Ref A1, A2) most likely do not represent osteosarcomas, but rather osteomas or osteodystrophies. Due to the extremely

high daily dosage of up to 330 mg EDTMP per kg body weight (i.e. approx . 100 mg per animal) for more than a year, chronic hypocalcemia with symptoms similar to pseudohypoparathyroidism is unavoidable. However, due to the lack of a scientific publication disclosing the diagnostic criteria for both studies, the conclusion of an actual malignant risk of EDTMP remains purely speculative.

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