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Bisphosphonates and implants in the jaw bone

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Populärvetenskaplig sammanfattning

Insättning av metall-implantat för ersättning av förlorade kroppsdelar är ett vanligt förekommande behandlingsmetod inom odontologi och ortopedi. Lyckandefrekvensen för dessa behandlingar är direkt kopplade till implantatens stabilitet, som i sin tur beror på kringliggande benvävnad. När man sätter in en skruv av titan i käkbenet utlöses ett frakturläkningssvar som skapar nytt ben runt skruven. Frakturläkningssvaret innehåller både uppbyggnad och nedbrytning av ben. Genom att selektivt minska bennedbrytningen med ett läkemedel (bisfosfonat) kan man med bibehållen uppbyggnadskomponent få mer och starkare ben. Bisfosfonater används kliniskt bland annat för att hämma bennedbrytning hos patienter med benskörhet eller skelettmetastaser. Under de senaste åren har bisfosfonatbehandling för att förbättra implantatfixation testats i både djurförsök och kliniska studier, men inte i käkar. Detta kan bero på att det finns ett samband mellan användningen av bisfosfonat (speciellt intravenöst) och förekomst av ett tillstånd som kallas för ”osteonekros i käken”. Patofysiologin och behandlingen av detta tillstånd är kontroversiell.

Syftet med denna avhandling är att öka förståelsen om hur bisfosfonater förstärker benvävnaden runt ett implantat. Kan bisfosfonatbeläggning på metallytan förbättra fixeringen av implantat i käken? Kan man reproducera eller förhindra uppkomsten av osteonekros i käken i en djurmodell?

Totalt opererades 96 implantat i överkäken på 21 patienter, som alla fick ett implantat med bisfosfonat.

Resonansfrekvensmätning visade att de bisfosfonat beklädda tandimplantaten hade bättre stabilitet jämfört med kontrollimplantaten efter 6 månaders läkning.

Röntgenundersökning visade mindre benförlust kring bisfosfonatbeklädda implantat. Vi utvecklade tre djurmodeller för att studera osteonekros i käken. I ett experiment studerades effekten av lokal och systemisk bisfosfonatbehandling på käkbenet. Skruvar beklädda med ett potent bisfosfonat (zoledronat) orsakade bättre implantatinläkning, även under betingelser där systemisk bisfosfonat framkallar osteonekros i käken. Vi har också visat att osteonekros i käken inte uppkommer förrän benet exponerats, t ex genom tandborttagning. Slutligen kunde vi förebygga uppkomsten av detta tillstånd genom omedelbar täckning med slemhinna efter tandborttagning.

Slutsatsen är att lokalbehandling med bisfosfonat ger bättre fixering av implantat i käkarna. Detta kan leda till nya möjligheter för ortopedisk och dental implantatkirurgi. Patofysiologin av osteonekros i käken är relaterad till exponering av benvävnad och till läkemedel som förhindrar nedbrytning av benvävnad.

Abstract

Insertion of metal implants in bone is one of the commonest of all surgical procedures. The success of these operations is dependent on the fixation of the implants, which, in turn, depends on the strength of the bone that holds them. If the quality of the bone holding the implant could be improved locally, surgical procedures would become simpler and rehabilitation would become faster. Bisphosphonates are anti-resorptive drugs that act specifically on osteoclasts, thereby maintaining bone density and strength. Once released from the surface of a coated implant, bisphosphonates reduce osteoclast activity, thereby changing the balance of bone turnover in favor of bone formation, leading to a net gain in local bone density. During the last decades, the effects of bisphosphonate treatment on the stability of implants have been tested in several clinical and animal studies, but not in human jaws. This may be because it has been suggested that there is a link between the use of bisphosphonates (especially those given intravenously) and a condition called osteonecrosis of the jaw (ONJ). The pathophysiology and treatment of ONJ is controversial. The difficulty in treating ONJ has highlighted the importance of prevention.

The overall aim of the present thesis was to evaluate the effect of local and systemic use of bisphosphonates on bone tissue. Could a thin, bisphosphonate-eluting fibrinogen coating improve the fixation of metal implants in the human jaw? Would it be possible to reproduce ONJ and prevent the development of this condition in an animal model?

In two clinical studies, a total number of 96 implants were inserted in 21 patients. In a randomized trial with a paired design, one implant in each pair

was coated with a thin fibrinogen layer containing two bisphosphonates (pamidronate and ibandronate). The bisphosphonate-coated implants showed better stability as measured by resonance-frequency analysis. Radiographic intraoral films also showed less bone loss. Three animal models were developed. In a study comparing local and systemic effects of bisphosphonates, zoledronate-coated screws inserted in rats showed better fixation in spite of a drug treatment that is known to induce ONJ-like lesions when given systemically. In another rat model, ONJ-like lesions were reproducibly induced at sites of tooth extraction whereas there were no signs of bone cell death in uninjured sites. Finally, rat experiments showed that the development of ONJ-like lesions after tooth extraction could be prevented by early mucoperiosteal coverage.

In conclusion, a thin, bisphosphonate-eluting fibrinogen coating can improve the fixation of dental implants in human bone. This principle may lead to new possibilities in orthopaedic surgery and dentistry. The pathophysiology of ONJ is strongly linked to bone exposure in combination with drugs that reduce resorption.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Bisphosphonate coating might improve fixation of dental implants in the maxilla: A pilot study.
Abtahi J, Tengvall P, Aspenberg P
Int J Oral & Maxillofac Surg 2010 39(7): 673-7.

II. Bisphosphonate-coating improves the fixation of metal implants in human bone. A randomized trial of dental implants.
Abtahi J, Tengvall P, Aspenberg P
Bone 2012 50(5): 1148-51.

III. Bisphosphonate-induced osteonecrosis of the jaw in a rat model arises first after the bone has become exposed. No primary necrosis in unexposed bone.
Abtahi J, Agholme F, Sandberg O, Aspenberg P
J Oral Pathol Med 2012 41(6): 494-9.

IV. Effect of local versus systemic bisphosphonate on dental implant fixation in a model of ONJ.
Abtahi J, Agholme F, Sandberg O, Aspenberg P
Journal of Dental Research 2012 [Epub ahead of print].

V. Prevention of osteonecrosis of the jaw by mucoperiosteal coverage in a rat model.
Jahan Abtahi, Fredrik Agholme, Per Aspenberg
Int J Oral & Maxillofac Surg 2013, accepted, manuscript number: IJOMS-D-12-00927R1.

Abbreviations

ATP	Adenosine triphosphate
BIC	Bone-to-implant contact
BMP	Bone morphogenetic protein
BRONJ	Bisphosphonate-related osteonecrosis of the jaw
Cbfa 1	Core binding factor 1
CSF	Colony-stimulating factor
ISQ	Implant stability quotient
FPPS	Farnesyl diphosphate synthase
GTP	Guanosine triphosphate
HAC	Hydroxyapatite coating
OH	Hydroxyl group
ONJ	Osteonecrosis of the jaw
OPG	Osteoprotegerin
PTH	Parathyroid hormone
PTV	Periotest value
RANK-L	Receptor activator of NF-kappa B ligand
RFA	Resonance-frequency analysis
TGF-β	Transforming growth factor-β
TNF- α	Tumor necrosis factor-α

Introduction

During the last decades there have been problems with the condition called "bisphosphonate-related osteonecrosis of the jaw" (BRONJ). This condition is defined as an area of exposed bone in the maxillofacial region that does not heal within 8 weeks of identification by a healthcare provider, in a patient who currently receives or has been exposed to a bisphosphonate and has not had radiation therapy to the craniofacial region (from here on, I use the shorter acronym ONJ). The pathophysiology of ONJ is poorly understood and as maxillofacial surgeon, I have wondered why these lesions localize specifically in the jaws. It is remarkable that orthopedic surgeons and osteoporosis researchers consider bisphosphonates to be beneficial and useful in many areas, while dental practitioners reject these drugs. By working with an orthopedic research team, I had the opportunity to address the problem of the paradoxical effects of bisphosphonates. Previous animal studies by this team have shown that bisphosphonate coatings can improve the fixation of implants in bone. Clinically, this idea has been tested in orthopedics but not in dentistry. From the literature it can be deduced that high implant survival rates would be expected in jaws. However, dental implant surgery can be risky in bone of low density. We therefore decided to use bisphosphonates in the hope of improving the fixation of implants in the maxilla. Considering that ONJ was apparently non-existent ten years ago, the field has progressed through knowledge gained from case reports, population-based studies and emerging animal models. Still, there are preconceptions that need to be tested and important clues that need to be investigated in order to translate pathophysiology into improved patient care. In the clinic, I have also met cancer patients who have suffered from exposed bone in the jaw associated with intravenous bisphosphonate

therapy. Is it possible to treat or prevent the development of ONJ? I hope that my research will improve our knowledge oft this condition.

Bone metabolism

Bone tissue structure

The skeleton consists of specialized cells, mineralized and unmineralized connective tissue matrix, and spaces that include bone marrow cavities, vascular canals, canaliculi, and lacunae. At the nano-scale level, bone tissue is a composite material composed of an organic phase, consisting mainly of the protein-based material collagen, and a mineral phase, consisting primarily of hydroxyapatite (1, 2). Hydroxyapatite is present as plate-like crystals, 20-80 nm long and 2-5 nm thick, which are themselves composed of calcium and phosphate. The crystals are found in and around collagen fibers and give bone its compressive strength. The organic matrix determines the structure and mechanical properties of the bone. Of the organic matrix, approximately 90% is type-1 collagen. The remainder consists of non-collagenous matrix proteins, minor collagen types, proteoglycans, and lipids.

At the microscopic level, there are two types of bone tissue, woven bone and lamellar bone. Woven bone is considered to be immature, with collagen arranged randomly. At birth, it makes up all the bone in the body, and in later years it is found at sites of fracture healing or in response to extreme mechanical loading (3). Lamellar bone is the name given to bone that eventually replaces woven bone. By the age of 4 years, most of the skeleton is lamellar bone. Anatomically, both woven bone and lamellar bone can be organized into compartments as either cortical or trabecular bone (cancellous bone). An important difference between cortical bone and trabecular bone is in the way the bone matrix and cellular elements are arranged. Between 80% and 90% of cortical bone volume is mineral, but only 15-25% of trabecular

bone volume is mineral (4). The trabecular arrangement allows bone marrow, blood vessels, and connective tissues to be in contact with bone.

The main function of cortical bone is to give structure and protection. In cortical bone, lamellae are arranged concentrically around a central vascular channel (Haversian canal). This arrangement of cortical bone around a vessel is called an osteon. Osteons are usually aligned with the long axis of bone and are connected to each another by Volkmann's canal, which runs at right angles to the osteon. The outer surface of cortical bone, facing the soft tissue is covered by periosteum, while the inner surface that faces the bone marrow is covered by endosteum. Cells lining the endosteum are metabolically active and very involved in bone formation and resorption.

Bone cells

There are mainly four types of bone cells. These are the osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts. The **osteoblasts** are mononucleate bone-forming cells that are derived from the local osteoprogenitor cell line (mesenchymal cells) located in the deeper layer of periosteum and the bone marrow (5-7). These progenitors are capable of differentiating into other mesenchymal cell lineages such as chondrocytes, fibroblasts, myoblasts, and bone marrow stromal cells including adipocytes (8-12). Mature osteoblasts have an average lifespan of 1 month, after which they either undergo apoptosis, to be replaced by newly differentiated osteoblasts, or alternatively about one-third of them may be incorporated into deposited bone matrix as osteocytes (15).

Several growth factors and hormones regulate osteoblast differentiation. Growth factors are soluble proteins that act as signaling agents for cells and influence critical functions, such as cell division, matrix synthesis, and tissue differentiation, by receptor-ligand binding. Of these, bone morphogenetic proteins (BMPs) are the most potent inducers and stimulators of osteoblast differentiation (13, 14). These proteins not only stimulate osteoprogenitors to differentiate into mature osteoblasts but also induce non-osteogenic cells to differentiate into cells of the osteoblast lineage. Proliferation and differentiation of osteoblasts is also regulated by many transcription factors. Expression of the transcription factor core binding factor 1 (Cbfa 1) is an absolute requirement for osteoblast differentiation and bone formation (15). Similarly, the transcription factor ppar-c 2 can specify adipocyte differentiation (16), and sox-9 expression is required for chondrocyte differentiation (17).

Osteoblasts express receptors for various hormones including parathyroid hormone (PTH), glucocorticoids, 1α, 25-dihydroxyvitamin D3, and estrogen, which are involved in the regulation of osteoblast differentiation (18-20). Parathyroid hormone acts directly on the skeleton to promote calcium release from bone and on the kidney to enhance calcium reabsorption by binding to its receptor. The anabolic effect of PTH on bone tissue occurs by upregulation of expression of transcription factor *c-fos*, which is a key regulator of osteoblast and osteoclast differentiation (18). Inactivation of c-*fos* causes the bone-remodeling disease osteopetrosis, which is characterized by impaired osteoclastic bone resorption, resulting in a net increase in skeletal mass (21). In contrast, when c-*fos* is overexpressed in tissues, bone tumors develop that are typically chondroblastic osteosarcomas, containing large amounts of neoplastic bone with foci of cartilage (22, 23).

Glucocorticoids also play an important role in the normal regulation of bone remodeling (24). The precise role of glucocorticoids in bone formation is still poorly understood. In vivo studies have shown that continued exposure of bone to pharmacological doses of glucocorticoids excess results in osteoporosis (25, 26). This event is due to the effect of glucocorticoids on bone cells. Glucocorticoids increase the expression of receptor activator of NF-kappa B ligand (RANK-L) and reduce the expression of its decoy receptor, osteoprotegerin (OPG), in stromal and osteoblastic cells (27). They also enhance the expression of colony-stimulating factor (CSF)-1, which in the presence of RANK-L induces osteoclastogenesis (28). Furthermore, several *in vitro* studies have shown that glucocorticoids reduce the number of cells of the osteoblastic lineage and shift the differentiation of stromal cells towards the adipocytic lineage (29) Glucocorticoids also increase peri-lacunar osteocytic bone resorption to an extent that negatively influences bone material properties (30).

Osteoclasts are large, multinucleate cells derived from hematopoietic stem cells, and they are equipped with phagocytic-like mechanisms similar to those of circulating macrophages (31). Osteoclast literally means “bone eater”. Osteoclast differentiation has various characteristic features, such as multinucleation induced by the cell fusion of mononuclear osteoclasts to cover a larger area, synthesis of the vacuolar proton pump and acid to dissolve the bone mineral, the formation of ruffled borders to secrete protons and acid, and the formation of a sealing zone to prevent proton and acid leakage (31). Their proliferation and differentiation (osteoclastogenesis) depend on the presence of two different osteoblast expressed cytokines, macrophage colony-stimulating factor (M-CSF) and RANK-L (32, 33). The protein osteoprotegerin (OPG), which is secreted by osteoblasts, acts as a

decoy receptor that competes with RANK for RANK-L and thereby inhibits osteoclast differentiation (34, 35).

Osteoclasts have developed efficient and unique machinery to dissolve mineral and degrade bone matrix. To maximize bone resorption, osteoclasts expand their surface area by fusion to many mononucleated macrophages (36). After migration of the osteoclast to a resorption site, a specific membrane domain, the sealing zone, forms adjacent to the bone surface (37, 38). Inside the sealed region, extensive infoldings of the cell membrane form, the so-called "ruffled border" which increases the membrane surface (38). A cytoplasmic proton pump (H^+-ATPase) produces protons that generate a pH of 4-5 in the extracellular space adjacent to the bone surface (39). This event results degradation of the mineral component of bone, which is composed of hydroxyapatite (31). The organic matrix of the bone (collagen) is removed through enzymatic activity, by cathepsin K. This enzyme reaches the bone surface by exocytosis through the basolateral membrane of the osteoclast (40).

Osteocytes are found within individual lacunae in the mineralized bone matrix. The lifespan of osteocytes is higher than that of osteoblasts, which is an estimated 3 months in human bone (41) and 10–20 days in newly formed murine bone (42). The lifespan of osteocytes is probably largely determined by bone turnover and they may have a half-life of decades if the particular bone they reside in has a slow turnover rate (4).

The morphology of embedded osteocytes is dependent on the bone type. Indeed, osteocytes found in trabecular bone are more rounded than osteocytes from cortical bone (43). Each osteocyte communicates with its

neighbors and with the cells lining the surface of bone through long, slender cytoplasmatic processes (canalicular processes) that connect by means of gap junctions (44-46). Osteocytes are capable of detecting mechanical stimuli, which are mediated by loading-induced dynamic fluid flow in the canaliculi (47, 48). Osteocytes differentiation is under the influence of several bone markers such as alkaline phosphatase, bone sialoprotein, osteocalcin, and collagen type I (49). Once the osteoid mineralizes, osteocyte ultrastructure undergoes further changes including a reduction in endoplasmic reticulum and Golgi apparatus corresponding to a decrease in protein synthesis and secretion (50). At this stage, many of the previously expressed bone markers are downregulated in the osteocyte (50).

Osteocytes are cells that not only play a physiological role during their lifetime, but also achieve functions through their apoptosis. Osteocytes have been hypothesized to play a role in this targeted remodeling process (51, 52). Damage to the bone matrix, such as micro-cracks induces apoptotic death of osteocytes, which initiate signals for bone resorption by expression of osteoclast-stimulatory factors, such as RANKL and M-CSF (53, 54).

Osteoclast-osteoblast interplay

Throughout life, bone is constantly renewed through a two-stage process called remodeling (55, 56). This condition is a dynamic process that relies on the correct balance between bone resorption by osteoclasts and bone deposition by osteoblasts. In healthy adults, under normal circumstances, bone resorption is always followed by an equal degree of bone formation, a tightly balanced process referred to as coupling (57). The regulation of bone resorption involves a complicated set of hormonal and/or cytokine

interactions that initially stimulate osteoblasts, which then elaborate factors that signal osteoclasts to degrade bone (58, 59). Osteoblast differentiation is promoted by lipid-modified glycoproteins of wingless (Wnt), bone morphogenic proteins (BMPs), and several transcription factors (15, 60). Furthermore, Wnt signaling has been shown to reduce osteoblast and osteocyte apoptosis *in vivo* and to increase bone formation by stimulating differentiation and replication of osteoblasts (61).

Cells of the Osteoblast lineage produce the osteoclastogenic cytokines RANKL and M-CSF, which recognize their respective receptors RANK and c-fms on macrophages, prompting them to take on the osteoclast phenotype (32, 33). RANKL activity is negatively regulated in the circulation by osteoprotegerin (OPG), which competes with RANK as a soluble decoy receptor (34). Administration of RANKL to mice causes osteoporosis (35), whereas disruption of the RANKL gene in mice leads to severe osteopetrosis, impaired tooth eruption, and the absence of osteoclasts (60). Several hormones including calcitonin, parathyroid hormone, vitamin D, estrogen, interleukins, glucocorticoids and tumor necrosis factor-α (TNF- α), transforming growth factor-β (TGF-β), among others, regulate osteoclast and osteoblast function (18-20, 24, 62, 63) (Figure 1).

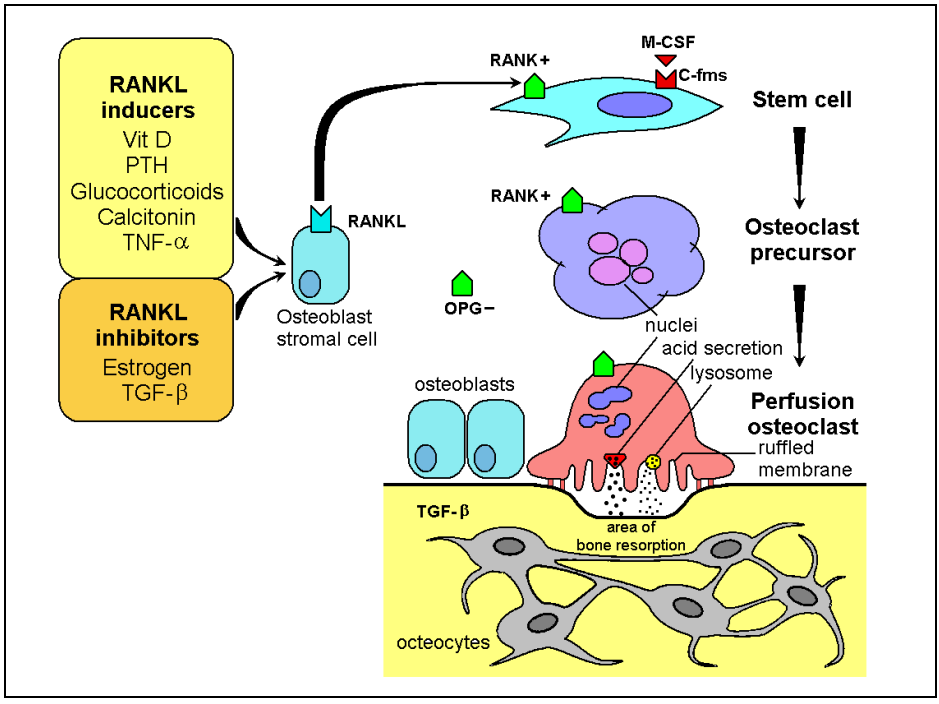

Figure 1. Differentiation and activation of osteoclasts. M-CSF and RANKL are essential for osteoclastogenesis. OPG can bind to RANKL and thereby inhibit osteoclast differentiation.

The coupling of bone resorption and formation suggests that autocrine and paracrine factors are produced and released within the local bone environment (64, 65). Transforming growth factor β (TGF-β) is a multifunctional cytokine with potent effects on bone metabolism (66, 67). Both osteoblasts and osteoclasts synthesize and secrete latent TGF-β (65). Resorption of bone by osteoclasts releases latent TGF-β from the organic matrix, where it potently stimulates osteoblastogenesis and at the same time inhibits RANKL expression by osteoblasts. (65).

Integration of titanium implants in bone tissue

The initial events

Insertion of metal implants in bone is one of the most common of all surgical procedures. Brånemark et al. first defined "osseointegration" in 1969 as a direct structural and functional contact (at the light-microscopic level) between living bone and implant.

The initial host response after implantation is characterized by an inflammatory reaction elicited mainly by the surgical trauma. Inflammatory cells, initially polymorphonuclear granulocytes and later monocytes, emigrate from post-capillary venules into the tissue surrounding the implant (69). At this stage, damage to the pre-existing bone in the implant- bone cavity is often a consequence of heating, located within 100 μm (70). Immediately after the surgical damage, the walls of bone are covered with blood, which initiates a clotting reaction. This is the first tissue to come into contact with the implant surface after insertion of the implant in the bone cavity (71). It has been shown that the implant-blood interface is composed of a fibrin film containing platelets and red blood cells, and they appear to respond differently to different implant surface topographies (71).

A strong correlation has been found in several studies between the adsorption of fibrinogen and surface adhesion of platelets (71-74). The enhanced aggregation may be due to the increased surface area of the micro-roughened surface, suggesting that this topography induces more agglomeration of red blood cells/platelets than machined surfaces (71).

A few days after implantation, osteoblasts produce collagen matrix directly on the early- formed lamina limitans layer on the implant surface (69-75). During the first week, mesenchymal cells and multinuclear giant cells are present in the area around the implant (75). Areas of newly formed bone (woven bone) can be seen at the endosteal surfaces towards the implant 1-2 weeks after implantation. Woven bone contains osteocytes, and the trabeculae are lined with osteoblasts. At this time, in areas that are responsible for primary mechanical stability, the bone tissue shows signs of ongoing bone remodeling, resorption, and apposition. After 4 weeks, the dense woven bone often combined with lamellar bone approaches the implant surface, to fill the threads. The remodeling process for rabbits and dogs starts after 4 weeks and is complete after 90 days (69, 75).

Bone-implant interface

The interface zone between bone and implant has been the subject of a vast number of recent publications (76-86). Many investigators have shown an interface zone consisting of connective tissue (76, 77). Initially, it was suggested that the goal for the surgeon should be a periodontal membrane around the implant (76). Several authors believe that direct contact between implant and bone is possible only if the implant is ceramic, and not if it is metal (78, 79). Furthermore, light microscopy of the interface zone has revealed intimate contact between newly formed bone and the oxidized surface of titanium implant (80). Early studies on bone-implant contact at the electron microscopic level showed a close relationship between implants and collagenous filaments from bone (81). Albrektsson and co-workers (82) compared interfacial arrangements around stainless steel implants with those seen around commercially pure titanium. The titanium implants, in contrast

to stainless steel ones, became directly anchored in bone without any cellular layer at the interface. Moreover, the authors found a thin (20-40 nm) layer of amorphous material consisting proteoglycans adjacent to the implant surface (83). Similar results have been found by others (84-86). Further ultrastructural studies of machined implants by Sennerby et al. (85, 86) showed a 100-nm- wide electron-dense line (lamina limitans) at the border between the mineralized bone and the non-calcified amorphous layer. This finding indicated that the stability of the implant is mainly mechanical.

The role of micromovement

In the literature, implant movement relative to the surrounding bone has been suggested to be a crucial parameter in the prognosis of implant osseointegration (87, 88). Early movement of the implant during the initial healing phase will lead to a preponderance of interfacial connective tissue (89-92). There appears to be a consensus that excessive micromotion impairs osseintegration (93, 94). However, many of these studies are not comparable due to the influence of other factors such as implant geometry, surface characteristics, and implant site. The threshold of micromotion, as experimentally evaluated in animals, is between 50 and 100 µm (95). In a cadaver study by Burke et al. (96) micromotion of knee and hip prostheses was measured using sensitive displacement transducers. Such movement has been shown to be in the range of 100-600 µm (97).

Søblle et al. (97) studied the influence of micromotion between bone and titanium implant with and without a hydroxyapatite coating (HA) in a dog model. They showed that micromotion of 150 µm inhibits bone ingrowth and results in development of a fibrous membrane. Moreover, 4 weeks after

implantation, a fibro-cartilaginous membrane was seen around unstable HA-coated implants, whereas around titanium implants, only fibrous connective tissue was found. Furthermore, continuous loading of initially unstable titanium implants resulted in the development of a permanent fibrous membrane, whereas HA-coating had the capacity to replace the motion-induced fibrous membrane with bone. These findings show that micromotion has a role in tissue differentiation (98).

In an experimental study by Akagawa et al. (99) dental implants were inserted in dog mandible by one-stage or two-stage procedures. The animals were fed with hard pellet food for 3 months. The submerged implants showed direct bone apposition and sparse fibrous, dense connective tissue. The unsubmerged implants also showed direct bone apposition. However, the apical part of the implant was frequently in contact with dense connective tissue. From these experimental studies, it appears that micromovements like those induced by early loading of dental implants should be avoided if the intention is osseointegration.

Trisi et al. (100) evaluated *in vitro* the correlation between the micromotion of cylindrical- screw implants and the insertion torque in fresh bovine bone of different densities *ex vivo*. They found that increasing the peak insertion torque reduced the micromotion between the implant and the bone. However, micromotion in soft bone is always high and immediate loading of implants in low-density entails a higher risk of loosening.

The role of surface topography

Surface Roughness

Titanium and its alloys are the materials most often used in implant manufacture because of their excellent biocompatibility, favorable mechanical properties, and well-documented beneficial results. When exposed to air, titanium immediately develops a stable oxide layer which forms the basis of its exceptional biocompatibility. In the last decade, most dental implant manufacturers have focused on implant surfaces to improve bone-to-implant contact (101). It is well known that modification of the implant surface (including topography and chemistry) alters the cellular and bone tissue responses (102, 103). Earlier studies (104, 81) have suggested that implant surface topography is the only parameter that significantly affects bone-to-implant contact (BIC). These findings were later confirmed by studies with animals, which indicated that a certain degree of surface roughness favored BIC, as assessed by the removal torque test (105-108). Albrektsson and Wennerberg (109) defined smooth surfaces to have an S_a value of < 0.5 μm; minimally rough surfaces were identified with an S_a of 0.5-1 μm, moderately rough surfaces with an S_a of 1-2 μm, and rough surfaces with an S_a of > 2 μm.

Another advantage of a roughened titanium surface is a shorter healing period and the option of using shorter implants, still with a good long-term prognosis because of the better bone anchorage (110). Therefore, many surface modifications of titanium implants have been developed to achieve better osseointegration (electron-polished, anodization, plasma-spraying grit-blasting or acid-etching).

One way to produce an increased surface roughness is to blast the surface. Wennerberg et al. (111) showed higher bone-to-implant contact and higher removal torque values for implants blasted with TiO (25 µm) rather than Al_2O_3 (75 µm) and machined implants. However, no differences were seen when implants were blasted with different materials ($A1_2O_3$ and TiO), but the same degree of roughness (25 µm) was used (112). In recent years, high-strength ceramics have become attractive as new materials for dental implants, due to their biocompatibility and higher fracture resilience (113). At the ultrastructural level, there was no difference between the tissue response of zircornium implants and that of titanium implants (114, 115). The TiOblast ™ surface is grit-blasted with titanium dioxide particles to achieve a moderately rough surface. Several studies (116-118) have shown promising clinical outcome after 5 years of loading of this surface. *In vivo* and experimental studies (119-121) have shown a high cell biocompatibility and better anchorage in bone when using titanium oxide-blasted implants rather than implants with a machine-prepared surface.

Surface Chemistry

Beyond surface topography, surface chemistry may provide important and possibly synergistic cues for bone formation at titanium implants. Recent studies advocated that surface chemistry is changed by many surface topographic modifications (122, 123).

Ellingsen et al. (124) placed 80 implants with and without a fluoride-modified surface in the tibia of 20 rabbits. Removal torque measurements were performed and biopsies were obtained after 1 and 3 months of healing. While no differences in removal torque were detected between the two types of implants after 1 month, the fluoride-modified (test) implants in the 3-

month healing group had significantly higher torque values than the control implants. Furthermore, examinations done in ground sections representing both 1 and 3 months of healing revealed that at fluoride-modified implants, there were larger proportions of BIC than at the control implants. Berglundh et al. (125) found that the amount of new bone that formed in the voids within the first 2 weeks of healing was larger at fluoride-modified (test) implants than at TiOblast (control) implants. Similar findings have been reported by Cooper et al. (126). Calcium phosphates, such as hydroxyapatite (HA), have been successfully used as bone replacement materials in the case of periodontal lesions, or as a pulp-capping agent (127, 128). Synthetic hydroxyapatite that is very similar to the inorganic component of bone has been found to be osteoinductive. It is capable of supporting ingrowth of osteoprogenitor cells into the graft or implant. Hydroxyapatite coatings on titanium alloy are used in dental and orthopedic procedures (129).

Surface Orientation

The orientation of the surface topography has been the subject of a number of studies (130-132). Ivanoff and co-workers (131) investigated microimplants in a human test model. They found that when blasted and turned implants with similar roughness were compared, the blasted implants had significantly more bone-to-implant contact than turned implants. Despite the fact that both implants had the same grade of roughness, the turned implants had a clear orientation of the topography with an orientation perpendicular to the long axis of the implant. This finding indicated that surface orientation may be as important as the degree of surface roughness. Moreover, Wennerberg et al (132) found no differences between the

horizontally grooved implants and the vertically grooved implants when measuring bone-to-implant contact.

Burgos et al. (133) showed that titanium implants with a turned or an oxidized surface are integrated in bone in different ways. As observed at the light-microscopic level, bone formation occurs directly on the moderately oxidized surface, while turned titanium surfaces are integrated by growth of bone from the adjacent bone marrow and bone tissue. The mechanisms behind the different integration patterns are not fully known, but surface topography most certainly plays an important role. Moreover, a positive effect of titanium surface roughness on osteoblast proliferation and gene expression associated with collagen biosynthesis has been discussed by several authors (134-136). The surface profile in the nanometer range plays an important role in the adsorption of proteins, adhesion of osteoblast cells, and thus, the rate of osseointegration (137). However, the optimal size of nonometer particles applied on implant surfaces is still unknown.

Implant stability measurements

It is clear that stability both at placement and during function is an important criterion for the success of dental implants. Primary implant stability is a mechanical phenomenon influenced by factors related to the implant (design and dimensions of the fixture), the patient (quality and quantity of bone), and the operator (surgical technique). Primary implant stability is highest just after implant placement, because of mechanical compression of the fixture on bone walls, and it decreases with time. Secondary stability is the progressive increase in stability related to biological events at the bone-implant interface such as new bone formation and remodeling (138). The

clinical definition of implant osseointegration considers the level of stable marginal bone and absence of mobility in the bone (139). The diagnosis is therefore based on radiographic and mechanical stability criteria.

Evaluation before and during implantation

Imaging studies

Different radiographic techniques (intraoral radiographs, panoramic radiographs, lateral cephalograms, and tomographic images) are commonly used for evaluation of jaw bone quality and volume when planning for implant treatment (140). The use of computerized tomography (CT) for the evaluation of the bone density of patients requiring implant therapy was introduced by Schwarz et al. (141) and this method has been used in several studies (142-146). The authors suggested that there are strong correlations between bone density as estimated by CT, the insertion torque, and resonance-frequency values at implant placement. These findings support the idea that the preoperative CT examination may be a helpful technique for predicting primary stability. Although CT examinations have advantages over conventional radiography techniques, CT exposes the patient to higher doses of radiation. The recently developed limited cone-beam CT involving a two-dimensional X-ray sensor has been reported to be useful in presurgical evaluation. Limited cone-beam CT provides high-resolution images and does not need the use of high doses of radiation (147, 148).

Drilling/cutting resistance

Currently the most popular method of bone quality assessment is that developed by Lekholm and Zarb, who introduced a scale of 1to 4, based on both the radiographic assessment and the sensation of resistance experienced by the surgeon when preparing the implant site (149). The grading refers to

individual experience and provides only a rough mean value for the entire jaw. This classification has therefore been questioned recently due to its poor objectivity and reproducibility (150, 151). A similar index of four different density classes, based on the tactile feeling during drilling and implant insertion was introduced by Mish and Friberg (152, 153). Norton et al (143) concluded that there is a need for an objective quantitative classification of bone quality, which can be applied preoperatively and is not operator-dependent. They proposed a new classification based on the Hounsfield units of the bone on the CT scan, and related it to the existing classification of Lekholm & Zarb (149). A Hounsfield unit represents a normalized index of x-ray attenuation based on a scale where air corresponds to -1000 units water at standard pressure and temperature to 0 (154). Klinge et al. (155) proposed an individualized healing period after implant placement based on an objective score of bone quality. Altogether, 15 bone biopsies from 12 patients were harvested at mandibular sites before insertion of an implant. However, this idea has not been developed for practical reasons. Furthermore, a more objective method has been described by Johansson and Strid (156), who measured the cutting resistance during implant insertion as a function of the electric current drawn by the handpiece. In a series of studies by Friberg et al. (157-159), a positive correlation was found between values of cutting resistance, bone density, and resonance-frequency measurements. However, it was not possible to use this instrument to identify implants that are at risk of failure already at implant placement (159). The majority of failures were seen in bone of medium-to-high density, while implants inserted in bone of poor density gave a better outcome, perhaps due to an adapted surgical protocol and an extended healing period.

Post-implantation evaluation

Invasive and non-invasive clinical tools are available for objective assessment of implant stability. *Invasive biomechanical tests* including removal torque measurements, pullout tests, and histological and histomorphometric evaluation can give valuable information about the fixation of the implant. However, these destructive methods can be used mainly in experimental studies.

Experimental studies

Biomechanical tests

The removal torque test is a simple method that has often been used to evaluate the tissue response to titanium and other materials in experimental studies (160-166). Johansson and co-workers (160) reported a gradually increasing implant removal torque and bone-to-implant contact within a 12-month period. The stability and resistance to shear forces of implants also appear to be dependent on the mechanical properties of the bone at the bone-implant interface. Eulenberger et al. (161) used small cortical screws of stainless steel and titanium to evaluate the stability of implants by measurement of removal torque in rabbit tibia. They found higher removal torque value for titanium implants after 12 weeks. In the rabbit study by Sennerby et al. (162), higher removal torque values were recorded in commercially pure titanium implants than in vitallium implants.

The importance of cortical bone fixation of implants has been discussed in several studies (26, 27). Ivanoff et al. (163) studied the influence of different implant diameters on removal torque after 12 weeks of healing in the rabbit

tibia. The authors also showed higher removal torque values in rabbit tibia when implants engaged two cortical layers rather than one (164).

Rasmusson et al. (165) studied the effect of barrier membranes on bone resorption and implant stability in onlay bone grafts. Disc-shaped bone grafts were harvested from the calvarium and placed with titanium implants in the tibia of rabbits. On one side (test), the bone graft/implant was covered with a membrane, while the contralateral side (with no membrane) served as a control. The results showed that postsurgical bone graft resorption was inhibited as long as the membrane was in place. However, after removal of the membrane at 8 weeks, the resorption rate was higher on the test side. No differences were found between the test and control sites after 24 weeks, as measured by removal torque. Furthermore, Rasmussen et al. (166) used a rabbit model to study the healing and stability of titanium implants in free bone grafts, placed simultaneously or after 8 weeks of healing and followed for 24 weeks. Removal torque tests after 24 weeks did not reveal any differences between the two procedures.

Clinical studies

Non-destructive conventional methods, such as clinical evaluation (through manipulation with forceps or judgment of percussion sound) are highly subjective and lack reliability. Other objective methods such as Periotest® (Bensheim, Germany) or the Dental Fine Tester® (Kyocera, Kyoto, Japan) have been used for monitoring of the stability of implants over the healing period. Their lack of resolution, however, and their poor sensitivity and susceptibility to operator variables has been criticized (167).

The Periotest system is an electronic instrument that was originally designed to quantify signs of stress resorption by the periodontal ligament surrounding the tooth, as a measure of mobility (168). The Periotest instrument comprises a hand piece containing a metal slug that is accelerated towards a tooth by an electromagnet. The duration of contact of the slug with the tooth is measured by an accelerometer. The software in the instrument is designed to relate contact time as a function of tooth mobility. The result is displayed digitally and audibly as Periotest values (PTVs) on a scale from -8 (low mobility) to 50 (high mobility). Inter-operator and inter-instrument variability has been studied extensively (169-171). Several studies (172, 173) have shown the sensitivity of this method for the variation in the occlusal-gingival position. Furthermore, it has also been found that Periotest values are dependent on the angulations of the hand piece. A change in position of 1 mm in striking height may produce a difference in Periotest values of between 1 and 2 (169, 174).

Osseointegrated implants placed in the mandible have shown systematically lower PTVs than those placed in the maxilla (175). Moreover, Salonen et al. (176) recorded Periotest values of four different implant systems after an average of 22.5 months after installation. 14 of 204 implants lost stability. The lost implants showed significantly higher Periotest values than stable implants, except for ITI implants in the maxilla. In a clinical study by Drago et al. (177), implant stability was evaluated by Periotest at abutment connection, 6 and 12 months after occlusal loading. Periotest values recorded at abutment connection and after 12 months of functional loading failed to predict loss of implant stability. The positive predictive value for these two occasions was 64%. Thus, the prognostic value of Periotest to detect loss of implant stability has been questioned.

In recent years, the Ostell™ device for resonance-frequency analysis (RFA) has been advocated to provide an objective measurement of primary implant stability and to monitor implant stability over the healing period.

Resonance-frequency analysis

The technique

Resonance-frequency analysis (RFA) is a non-invasive, objective method for evaluation of implant stability and has been validated through several *in vitro* and *in vivo* studies (178-180). Meredith et al. (181) measured the frequency response of the transducer attached to an implant fixture in an aluminium block using abutments of various lengths (0-5 mm). A strong correlation was found between the RFA value and the exposed height of the implant above the block, while the overall implant length was of no significance. Resonance frequency was determined by the stiffness of the bone–implant complex as demonstrated by performing repeated measurements of implants placed in self-curing resin. A significant increase in resonance frequency was found to be correlated with increase in stiffness.

The RFA technique used in this study (Osstell; Integration Diagnostics, Sävedalen, Sweden) is based on magnetic pulses (3,500 to 8,500 kHz) instead of electrical excitement. A transducer called a "SmartPeg" is attached to an implant or abutment and a measurement is made by holding a probe near to the peg. The peg is excited and the resonance frequency is expressed electromagnetically in implant stability quotient (ISQ) units, on a scale from 1 (lowest) to 100 (highest). An increase in ISQ of 1 unit appears to correspond to 50Hz in resonance.

Clinical and experimental studies

Early studies by Meredith et al. (182) showed in increase in implant stability from placement to abutment connection in 54 of 56 implants inserted in the maxilla, as measured by RF. Moreover, the authors found a significant correlation between effective implant length (abutment length and bone loss) and RF.

Friberg et al. (183) correlated cutting resistance (bone density) with primary stability for 61 maxillary implants placed in different densities of bone. Repeated measurements indicated that all implants reached similar ISQ values at abutment connection and after 1 year of loading irrespective of initial stability at the time of installation. Similar findings have also been reported by other authors (184-186). These results indicate that the stiffness of the implant-bone contact is low in soft bone and high in dense bone. Furthermore, the remodeling process of soft trabecular bone seems to result in increased stiffness of the peri-implant bone.

Moreover, the influence of implant diameter and location on RFA values has been studied by Östman et al. (187). Measurements of a total of 905 Brånemark dental implants in 267 consecutive patients showed higher ISQ values for wide-platform implants in comparison to regular/narrow-platform implants. Moreover, a lower stability was seen with increased implant length, which may be explained by the fact that long implants may have a reduced diameter in the coronal direction.

The resonance-frequency technique has shown higher implant stability in mandibular bone than in maxillary bone (185, 188). A correlation has been found between bone quality (Lekholm & Zarb) and ISQ values by some

authors (187, 188) but not by others (189). In a clinical study by Miyamoto et al. (190), a total of 225 implant stability measurements were made at the time of implant placement using a resonance frequency analyzer. Before surgery, cortical thickness was determined by using CT scans. The authors showed that dental implant stability at the time of surgery was weakly influenced by implant length, but strongly related to cortical bone thickness.

To monitor the outcome of implant installation and determine the prognostic value of RFA in predicting loss of implant stability, Huwiler et al. (191) assessed ISQ values at the time of implant installation and 1, 2, 3, 4, 5, 6, 8, and 12 weeks thereafter. ISQ values of 57–70 represented stable implants. One implant lost stability at 3 weeks. At this time, its ISQ value had dropped from 68 to 45. However, the latter value was determined after the clinical diagnosis of instability. In a longitudinal study by Glauser et al. (192), ISQ values of 72 stable implants were compared with those of nine implants that had lost stability during 1 year according to an immediate/early-loading protocol. The implants that failed during the course of the study showed significantly lower stability already after 1 month. The risk of loss of stability was 18%, if ISQ values were between 49 and 58. In contrast to these findings Bischof et al. (188) performed RFA measurements on immediate and delayed loaded implants during the 3 months of healing. The resonance-frequency analysis method did not reveal any differences between these groups.

It is well known that when performing longitudinal measurements of implant stability, the position of the transducer must be highly reproducible because the measured values are dependent on the orientation of the transducer. The role of direction-dependence of the Osstell^{TM} transducer was evaluated in a

parametrical finite element study (193). The data indicated that, when measuring perpendicularly to the long axis of the alveolar crest, the deviation must not exceed 30° from the ideal perpendicular position. In this case, the first resonance frequency is recorded. When measuring in the position parallel to the long axis of the alveolar crest, however, the deviation must not exceed 10°. In order to monitor the stability of an implant over time correctly, it seems important that the same transducer orientation is kept during the different measurements. In a prospective study (194), resonance-frequency measurements at different orientation were performed in a total of 55 implants in the maxillae of nine patients. The results showed that, when measuring the RFA perpendicular (buccopalatal) to the bony crest, the ISQ values may be up to approximately 10 units lower compared to paralel (mesiodistal) orientations. Similar results were found by Pattijn et al. (195). Moreover, Park et al. (196) found no differences when measuring RF from different directions.

The resonance frequency technique has also been used to measure implant stability in grafted bone (197-198). Recently, Rasmusson et al. (199) evaluated implant stability in particulate bone, onlay block bone, interpositional bone, and non-grafted maxillary bone using RFA. A total of 260 TiO_2-blasted implants were placed 5-6 months after bone grafting, and the abutments another 6 months later. The results showed that implants placed in non-grafted and grafted maxillary bone using a two-stage protocol had similar stability. Similar findings have been presented by others (200). Degidi et al. (201) showed higher RFA values for implants in a site previously treated with a sinus augmentation procedure than for implants in non-grafted maxillary bone.

Bisphosphonates

Structure and bioactivity of bisphosphonates

Bisphosphonates are anti-resorptive drugs that act specifically on osteoclasts, thereby maintaining bone density and strength (202). Bisphosphonates are used in many clinical settings, including prevention and treatment of primary and secondary osteoporosis, Paget's disease of bone, hypercalcemia, multiple myeloma and osteolysis associated with bone metastases of malignant tumors (203, 204). They may directly inhibit the bone-resorbing activity of osteoclasts by mechanisms that can lead to osteoclast apoptosis (205). Moreover, a study by Sahni et al. (206) suggested that part of the inhibitory action of bisphosphonates on the osteoclasts is mediated through an action on the osteoblasts. However, it is not yet known whether this plays any important role *in vivo*.

Bisphosphonates also directly promote the proliferation and differentiation of human osteoblast-like cells *in vitro* (207). It has been reported that these drugs cause a number of effects on other cells, including inhibition of cell proliferation (208) and causing a decrease in cell adhesion, in fibroblasts (209) and in macrophages (210, 211).

Bisphosphonates are synthetic pyrophosphate analogs with a P-C-P bond instead of the P-O-P bond of inorganic pyrophosphates, which are used as anti-tarter agents in toothpastes and as a bone-specific radionuclide in technetium 99m methylene diphosphonate (Tc 99m MDP) bone scans. Unlike pyrophosphates, bisphosphonates are resistant to breakdown by enzymatic hydrolysis, which explains their accumulation in the bone matrix

and their extremely long half-life (212). The P-C-P structure (Figure 2) allows a great number of possible variations, especially by changing the two lateral chains (R_1 and R_2) in the carbon atom. The two phosphate groups are essential for binding to bone mineral such as hydroxyapatite and together with the R_1 side chain they act as a "bone hook". A hydroxyl (OH) group or an amino group at the R_1 position increases the affinity for calcium and thus for bone mineral (213, 214).

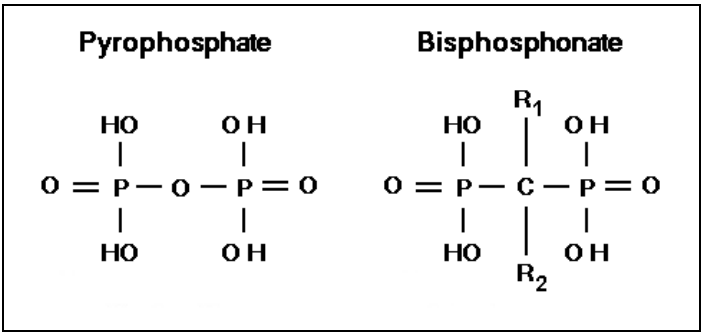

Figure 2. The chemical structure of pyrophosphate and bisphosphonate. R1 and R2 signify the side chains of bisphosphonate.

The structure and three-dimensional conformation of the R2 side chain determine the anti-resorptive potency and the enhanced binding to hydroxyapatite (213, 215). It has been shown that bisphosphonates containing a basic primary nitrogen atom in an alkyl chain are 10-100 times more potent at inhibiting bone resorption than earlier-generation bisphosphonates, such as clodronate, which lack this feature. Compounds that contain tertiary nitrogen, such as ibandronate and olpadronate, are even more potent at inhibiting bone resorption. Residronate and zoledronate are among the most potent of bisphosphonates, containing a nitrogen atom

within a heterocyclic ring; they are up to 10,000 times more potent than etidronate (216).

The non-nitrogen-containing bisphosphonates (etidronate, clodronate, and tiludronate) inhibit bone resorption by generating the cytotoxic, methylene-containing analogs of ATP that interfere with mitochondrial function and induce apoptosis of osteoclasts (217-219). In contrast, the nitrogen-containing bisphosphonates (alendronate, zoledronate, pamidronate, risedronate, and ibandronate) bind to and inhibit farnesyl pyrophosphate synthase (FPPS), a key enzyme of the mevalonate pathway, thereby preventing the prenylation and activation of small GTPases that are essential for the bone-resorbtion activity and survival of osteoclasts (220-222).

There is no evidence that orally or intravenously administered bisphosphonates are metabolized in animals or humans (223, 224). The gastrointestinal uptake of oral bisphosphonates is low, with a bioavailability of 0.7 % for alendronate (225) and 0.3% for pamidronate (226). The poor absorption of bisphosphonates can probably be attributed to their very poor lipophilicity, which prevents transcellular transport across the epithelial barriers. Consequently bisphosphonates must be absorbed by the paracellular route, which means passage through the pores of the tight junctions between the epithelial cells. Oral absorption of alendronate in rats is fourfold to fivefold higher in the fasted state than in the fed state (227). The same effect of food on the absorption of alendronate has also been observed in healthy volunteers (225).

Intravenous administration of a single dose of alendronate leads to rapid accumulation of this drug in bone tissue: 30% in 5 min and 60% in 1 hour.

At 5minutes after dosing, 63% of the dose is present in non-calcified tissues. This drops to about 1% 6-24 hours post-dose (227). The distribution of alendronate in bone is determined by blood flow and favors deposition at sites of the skeleton that is undergoing active resorption (227). Following administration of a single dose of ^{14}C-alendronate in rats, over 70% of the bone resorption surface was densely labeled in comparison with 2% of the bone formation surfaces (228). This preferential localization of alendronate (in areas of high bone turnover) could be due to exposure of hydroxyapatite at sites that are undergoing bone resorption and the accessibility of these bone surfaces to substances in the circulation

Following administration of ^{14}C-alendronate in rats a larger proportion of the dose is taken up by trabecular bone than by cortical bone, and in the latter at the metaphysis rather than the diaphysis (229, 230). Similar results were observed in dogs when etidronate was given intravenously (231). Furthermore, Lin et al. (230) showed that the uptake of alendronate is proportional to the intravenous dose up to 5 mg/kg IV, but at 10 mg/kg or higher, the concentration of drug in bone increases less than linearly

In recent years, it has been hypothesized that another target of bisphosphonates may be osteoblasts, which subsequently influence osteoclasts. The mitogenic effect of bisphosphonates on osteoblasts has been reported by several authors (232, 233). Furthermore, it has been shown that these drugs inhibited the expression of RANKL in a rat osteoblast cell line (234) and increase the expression of OPG in human osteoblastic cells (235), suggesting that the anti-resorptive effect of bisphosphonates is mediated by the influence of osteoblasts on RANKL signaling (234, 235). Moreover, it has been shown that bisphosphonates promote osteoblast

differentiation in cultures of osteoblast-like cell lines in a dose-dependent manner and inhibit the osteoblast apoptosis (236). However, the clinical relevance of this is unclear.

Local and systemic delivery, experimental studies

Extraction of teeth necessitated by factors such as developmental problems, trauma, severe periodontal disease, or unsolvable endodontic problems often causes reduction of the residual alveolar ridge height and width in the jaws. These reductions usually cause difficulties in prosthetic restoration, poor aesthetics and insufficient function. Many investigators have shown that tooth extraction stimulates osteoclastic activity with varying amounts of alveolar crest loss (237, 238). Systemic alendronate was found to be significantly effective in reducing bone loss associated with experimental periodontitis in monkeys and beagle dogs (239, 240). Yaffe et al. (241) found that local delivery of alendronate reduced alveolar bone resorption activated by mucoperiosteal surgery. In orthodontics, topical administration of amino bisphosphonate caused significant reduction of tooth movement in rats, when orthodontic force was applied (242, 243).

Several methods have been reported to increase the bone density around experimental porous implants, but to varying degrees (244-254). In animal models, several investigators have shown that surface-immobilized bisphosphonates improve the mechanical fixation of metal screws in terms of an increased pullout force and bone-to-implant contact (255-259). Yoshinari et al. (256) used plasma-sprayed HA-coated titanium dental implants that were immersed in pamidronate and implanted in mandibular bone of beagles. This study showed a 10% increase in bone contact area. Tengvall et al. (260) showed an increase (by 28%) of the pullout force of

steel screws inserted in rat femurs by using fibrinogen/pamidronate /ibandronate coating. Hydroxyapatite-coated implants releasing zoledronate induced an increase in pullout force by up to 42% compared to implants without zoledronate (257). However, at higher zoledronate concentrations (> 2.1 µg/implant) the pullout force decreased by 35%. The authors' hypothesized that this might be correlated to the lower bone mineral density close to the implant, due to a negative effect on osteoblast function.

Several authors who performed animal studies have also described the efficacy of bisphosphonates on mechanical fixation of implants in osteoporotic bone (258, 259, 261). However, there are many differences between the bone metabolism of small animals that of humans such as mineral density and healing capacity (262, 263). Even after different strategies such as ovariectomy and steroid application, the bone density is higher than in healthy human bone equivalents (264). Thus, it is questionable to extrapolate the *in vivo* results from small animals to specific clinical situations with osteoporotic patients.

Local and systemic delivery, clinical studies

Bisphosphonate have been given orally or systemically in order to improve fixation of orthopedic implants (265-267). A single infusion of 4 mg zoledronate showed promise in improving initial fixation of a cementless implant (265). In a randomized, double-blind trial of a hybrid-type total hip arthroplasty in patients with osteoarthritis, Wilkinson et al. (267) found that a single dose of 90 mg of pamidronate significantly reduced femoral bone loss. Hilding and Aspenberg (268) showed that local application of a bisphosphonate during total joint surgery reduces migration of metal prostheses as measured by radiostereometry. The authors applied 1 mg

ibandronate (1 mL) to the tibial bone surface 1 min before cementation. The role of bisphosphonate as an adjunct to conventional periodontal therapy in management of periodontal disease has recently been in focus. In a prospective investigation on possible effects of alendronate on alveolar bone, 335 patients with moderate or severe periodontal disease were randomized to either placebo or 70 mg alendronate once a week (269). After 2 years of treatment with bisphosphonate, there was no detectable effect on alveolar bone loss, except in those patients with low mandibular bone mineral density at baseline. In contrast, local treatment appears to be efficacious. In a recent series of three randomized controlled trials, local treatment of parodontitis with a gel containing a very high concentration of alendronate was successful in regenerating a large part of the lost bone, whereas placebo had little effect (270-272).

Osteonecrosis of the jaw (ONJ)

Definition

There is strong evidence for a link between the use of systemic bisphosphonates (especially those given intravenously) and osteonecrosis of the jaw in cancer patients (273-275). This condition of the jaw is defined as non-healing, exposed bone for more than 8 weeks in patients receiving a bisphosphonate and without any history of local radiation therapy (276, 277). Clinically, the disease presents as exposed alveolar bone (Figure 3) that occurs spontaneously or becomes evident following a surgical procedure such as tooth removal, periodontal surgery, apicoectomy or dental implant placement (273-275). These lesions most often become frequently symptomatic when surrounding tissues are inflamed or when there is clinical evidence of infection. Signs and symptoms that may occur before the development of clinically detectable osteonecrosis include pain, tooth mobility, mucosal swelling, erythema, and ulceration. The incidence of ONJ is estimated to be 1-12% in cancer patients receiving high-dose intravenous bisphosphonates (273-275). The frequency of ONJ in bone malignancy cases, mainly treated intravenous bisphosphonates, was found to be 1 in 100 (278). If tooth extractions were carried out, the calculated frequency of ONJ was 1 in 10 (278). In a retrospective study, Wang et al (279) found that the incidence of ONJ associated with intravenous bisphosphonates was at least 3.8 per 100 patients with multiple myeloma, 2.5 per 100 patients with breast cancer, and 2.9 per 100 patients with prostate cancer. In osteoporosis patients, bisphosphonate-associated osteonecrosis of the jaw is rare and the incidence may not be greater than the natural background incidence of the condition. Epidemiological studies have indicated an estimated incidence of

less than 1 case per 100 000 person-years of exposure to oral amino bisphosphonates (273-275).

Figure 3. Exposed alveolar bone in the mandible of a patient with intravenous bisphosphonate therapy.

Pathogenesis

The etiopathogenesis of ONJ remains uncertain. When the condition known as bisphosphonate-related osteonecrosis of the jaw (BRONJ) was first described, its similarities with radiation-induced osteonecrosis led to the assumption that the condition started with sterile necrosis of the jaw bone, which acquired a clinical appearance of classical chronic osteomyelitis after exposure to the oral cavity (273, 274). Hence the name osteonecrosis, a term otherwise reserved for sterile bone death, usually because of impaired blood supply. At that time, it was speculated that bisphosphonates could cause osteonecrosis through effects on blood vessels in bone, possibly by inhibition of vascular endothelial growth factor (280-282). It was soon

suggested that BRONJ does not begin as a form of classical osteonecrosis, but is in fact osteomyelitits from the start (283, 284). Bacterial contamination with *Actinomyces* appears to play an important role in maintaining osteomyelitic wounds (285-286). Because jaw bone containing bisphosphonates will be resorbed slowly, it is conceivable that bacterially contaminated bone cannot be removed fast enough to prevent the development of chronic osteomyelitis. This view is supported by the observation that similar lesions appear after treatment with an anti-RANKL antibody which reduces osteoclast recruitment (287). Thus, it appears that reduced resorptive activity is a key factor behind the reduced ability of these lesions to heal.

Corticosteroids and chemotherapeutic drugs have been suggested as factors that can predispose to ONJ or increase the risk of developing ONJ (288). The duration of bisphosphonate therapy also appears to be related to the likelihood of developing necrosis with longer treatment regimens associated with a greater risk (289). The mean time to ONJ after zoledronate treatment was calculated to be 1.8 years and the minimum was 10 months; after pamidronate, the mean time was 2.8 years and the minimum was 1.5 years; and after oral BP therapy, the mean time was 4.6 years and the minimum was 3 years (290). Similar findings have been reported by others (291, 292).

Treatment

The optimal treatment strategy for ONJ is still to be established. No effective treatment has been developed yet and interrupting drug therapy does not seem to be sufficient. In general patients with suspected ONJ should be evaluated and managed by a team including a dental specialist, an oral and maxillofacial surgeon, and an oncologist. The presentation and

symptomatology of ONJ can vary in patients despite similar disease processes, bisphosphonate dosage regimens and treatment duration. A clinical staging system (Figure 4) has been developed in order to more accurately categorize patients with ONJ (277, 293).

Staging

Stage 1	Exposed, necrotic bone that is asymptomatic and has no evidence of infection
Stage 2	Exposed, necrotic bone associated with pain and infection
Stage 3	Exposed, necrotic bone in patients with pain, infection, and pathological fracture, extraoral fistula, or osteolysis extending to the inferior border

Figure 4. Clinical staging of BRONJ according to the American Association of Oral and Maxillofacial Surgeons (AAOMS).

In early stages, surgical debridement and coverage has been successful (294). Segmental osteotomies are recommended only for severe cases (295-297). However, this controversial treatment has a high morbidity and affects the quality of life of patients (298). The difficulty in treating ONJ has highlighted the importance of prevention. Before starting on bisphosphonate therapy, patients should be screened for dental comorbidities and invasive dental procedures should be performed.

Prevention

There are currently no evidence-based guidelines on the management of bisphosphonate- induced ONJ; therefore, emphasis is placed on preventive measures.

Before the start of bisphosphonate therapy, the patient should be referred for a thorough dental evaluation to identify and treat any potential sources of infection. The dentist should emphasize oral hygiene instructions and routine dental prophylaxis to ensure optimal dental health. If dental health is suboptimal and tooth extraction is required, bisphosphonate therapy should be delayed by 4–6 weeks to allow appropriate bone healing (293).

If a patient is already on bisphosphonate therapy with no evidence of osteonecrosis, it is paramount to emphasize good oral hygiene and preventive dental care. However, oral surgical procedures should be avoided, as bone healing may be compromised. If dental extraction is necessary, immediate mucoperiosteal coverage of the alveoli might be performed to minimize bacterial contamination and the risk of ONJ.

If ONJ has already developed, dental management will depend on the severity of the lesion. Treatment objectives for these patients will be directed at eliminating pain, and controlling soft and hard tissue infections. At early stages, however, surgical debridement and coverage has been successful (especially for patients on oral bisphosphonates). Segmental osteotomies are recommended only for severe cases. However, this controversial treatment has a high morbidity and affects the quality of life in patients. Antibiotics can be useful in preventing the development of ONJ, at least in an animal model, but they have not been tested in clinical trials.

Although there is no reason to stop bisphosphonate treatment in patients who are about to receive routine dental care, there is a debate about whether treatment should be withheld temporarily (drug holiday) when more invasive dental care, such as a surgical procedure, is needed. Given the long half-life of bisphosphonates in bone (measured in years) whether or not temporary cessation of treatment with these agents would reduce associated risks is not known. These questions require further study.

Thoughts behind the start of the project

Bisphosphonates have been tested extensively for treating osteoporosis and they are in clinical use. These drugs can also be used to reduce peri-implant resorption allowing orthopedic implants to achieve a stronger primary fixation. In a series of experimental studies, Aspenberg and co-workers found better fixation of metal implants with local delivery of bisphosphonates. Once released from the surface of a coated implant, bisphosphonates reduce osteoclast activity, thereby changing the balance of bone turnover in favor of bone formation, leading to a net gain in local bone density. Clinically, this idea has been tested in orthopedics but not in dentistry. For dental implants, improved fixation would enable surgeons to push the limits regarding the quality of bone (in a surgical sense) in which implants can be inserted. One could possibly also widen the indications for immediate loading. The possibility to use resonance-frequency analysis to estimate postoperative stability opened the door for studies of implant fixation in a way that would be impossible in orthopedics, and we decided to explore this in a pilot study followed by a randomized trial. While these studies were proceeding, there were many reports about the development of ONJ in patients who are on bisphosphonate therapy. However, our team believes that local treatment of bone tissue with bisphosphonate is beneficial, while systemic treatment may be associated with complications, such as ONJ. We therefore developed a rat model from the literature to study the pathogenesis of ONJ.

Hypotheses

A bisphosphonate coating improves the fixation of dental implants

An ONJ-like lesion can be reproducibly produced in a rat model

Bone exposure is required for development of an ONJ-like lesion in rats

An immune deficiency (modelled by corticosteroid treatment) is required for development of an ONJ-like lesion in rats

A bisphosphonate coating improves implant fixation also under conditions where there is a high risk of developing ONJ-like lesions in rats

Mucoperiostal coverage prevents the development of ONJ-like lesions after molar extraction in rats

Material and methods

Study designs (I and II), clinical studies

Study I

Is it feasible to use bisphosphonate-coated implants in the human jaw?

In total, 35 implants (Brånemark MK III Ti Unite, 3.75 mm in diameter) were inserted in 5 patients with completely edentulous jaws by a two stage protocol. The coated implants were 10 mm long. The other implants varied between 11.5 and 13 mm. Implant stability was measured by using resonance-frequency analysis at the implant placement and 6 months later, at abutment connection. Radiographic intraoral films were obtained in a standardized manner using a long-cone technique preoperatively, after 8 weeks, and after 6 months at abutment connection. Finally, coated implants were removed en bloc for histological examination (Figure 5).

Study II

Does bisphosphonate coating improve the fixation of dental implants?

A randomized clinical trial with internal controls was performed in 16 patients. In total, 61 implants (Brånemark MK III Ti Unite, 3.75 mm in diameter) were inserted in 16 patients with both completely and partially edentulous jaws by a two-stage protocol. The coated and control implants were both 11.5 mm long and visually indistinguishable. The other implants varied between 11.5 and 13 mm. The resonance frequency analysis and dental radiography were performed as in study I. However, in study II, radiological examination was also performed one year after functional loading (Figure 5).

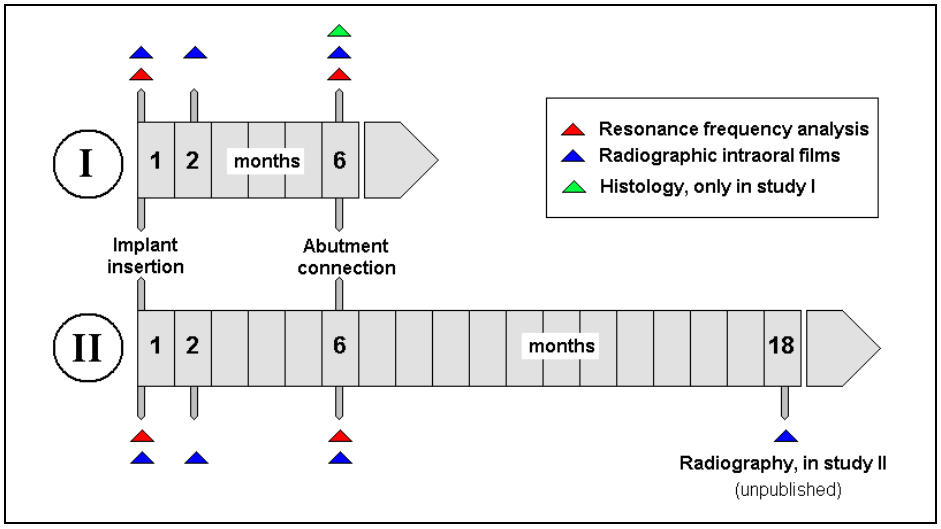

Figure 5. Summary of clinical studies I and II.

Resonance-frequency measurement (studies I and II)

The present RFA technique (Osstell Mentor, Integration Diagnostics, Sävedalen, Sweden) in these studies is based on magnetic pulses (3,500 to 8,500 kHz) instead of electrical excitement. The resonance frequency instrument converts kHz units to implant stability quotient (ISQ) value running from 1 (lowest) to 100 (highest).

Following implant insertion, a SmartPeg was connected to the implant and the registration was then performed by holding a probe near to the peg. SmartPeg is a small aluminium rod with a magnet attached to its top. To excite the SmartPeg, magnetic pulses with four different frequencies are sent by the coil in the measurement probe. As a consequence the SmartPeg starts to vibrate mostly in two directions perpendicular to each other. These two directions correspond to the lowest and the highest frequencies possible.

Vibration of the SmartPeg generates an alternating magnetic field that is detected by a receiving coil in the probe. To suppress electromagnetic "noise" in the environment, each pulse frequency is sent out 4 times and an average signal is created. So for each measurement, a total of 16 pulses are sent. The average signal of each set of four pulses is converted into a frequency spectrum by using Fast Fourier Transform. These four spectra are analyzed by the instrument in order to find the two highest peaks, which are in turn used to calculate the two ISQ values. If the difference between the two peaks is less than 3 ISQs or if only one peak is detected, only one ISQ value is presented. Measurements were repeated three times for each implant with the probe oriented perpendicular to the long axis of the implant. The mean of these measurements was recorded.

Study design (III-V), experimental studies

Study III

Can an ONJ-like lesion be reproducibly produced in a rat model?

After a number of preliminary experiments, forty rats were randomly allocated to four groups of 10. All animals underwent unilateral molar extraction and received different drug treatments (Figure 6). The animals were euthanized 2 weeks after tooth extraction, using carbon dioxide. The presence of osteonecrosis was determined by clinical and histological observations of groups I–III. To test the hypothesis that ONJ can develop without preceding sterile bone death, osteocyte viability in the contralateral mandibular was examined using lactate dehydrogenase histochemistry (LDH) in group IV.

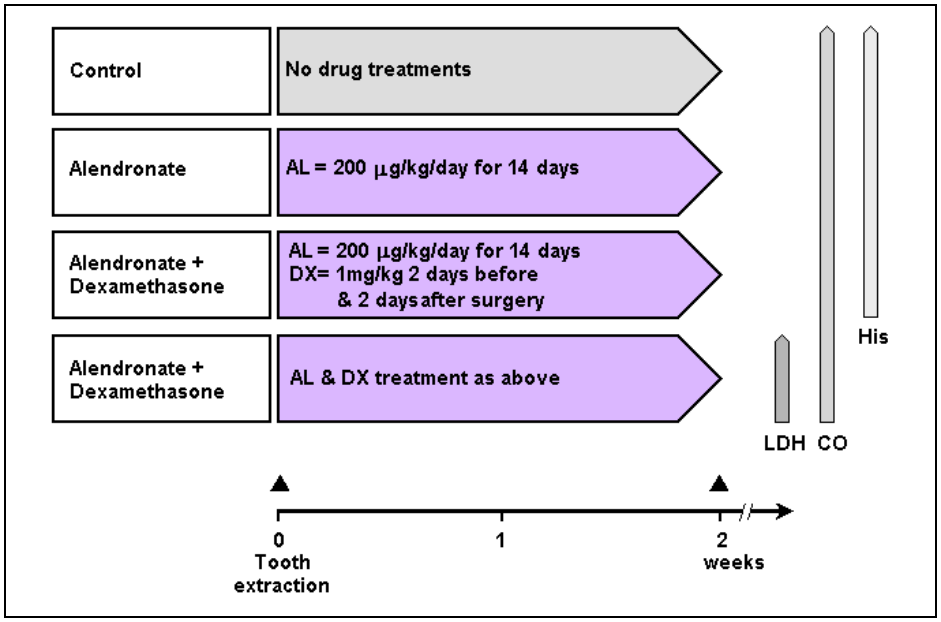

Figure 6. Summary of experimental study III (AL: Alendronate; DX: Dexamethasone; LDH: Lactate dehydrogenase; CO: Clinical observation; His: Histology).

Study IV

Can a bisphosphonate coating improve implant fixation also under conditions with a high risk of ONJ-like lesions in rats?

In this study we used 4 groups of 10 rats. All rats underwent extraction of the left maxillary first molar, a titanium screw was implanted into the socket of the mesiopalatal root of the molar and the animals received different drug treatments (Figure 7). We measured removal torque as the primary variable. As a secondary endpoint, we noted the presence of any ONJ-like lesions. Finally, we estimated the amount of remaining bone at the entire extraction site, as well as in the immediate vicinity of the implant, by micro-CT.

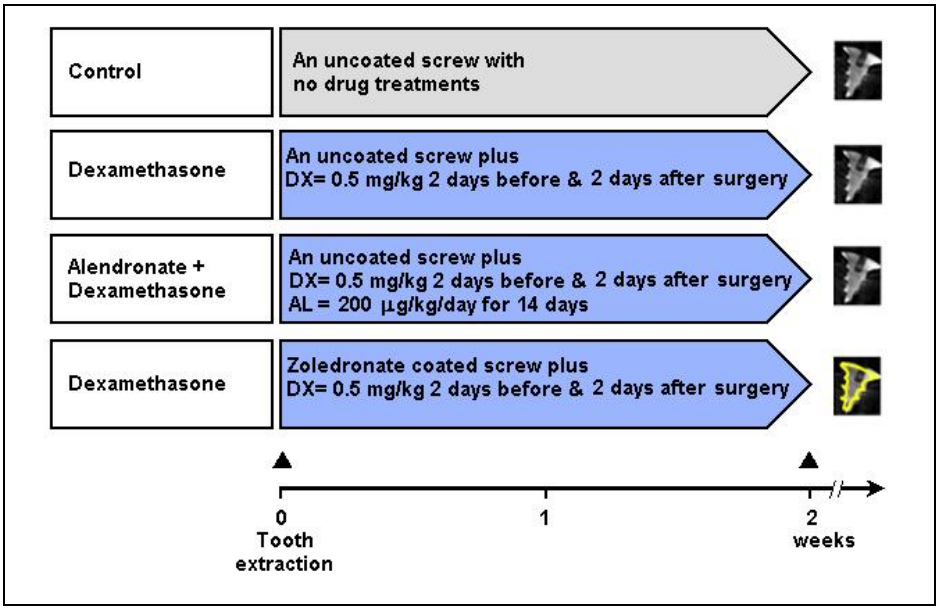

Figure 7. Summary of experimental study IV (AL: Alendronate; DX: Dexamethasone).

Study V

Can early mucoperiostal coverage prevent ONJ-like lesions?

In this study we used 3 groups of 10 rats, which underwent extraction of the left maxillary first molar and received different treatments (Figure 8). The animals were anesthetized and inspected after 1, 2 and 3 weeks. To reduce suffering in the animal, those with ONJ-like lesions were euthanized using carbon dioxide after 2 weeks. The others were kept alive until 3 weeks, to ensure that no wounds developed. The presence of ONJ-like lesions was evaluated by clinical observation, and this was confirmed by histology.

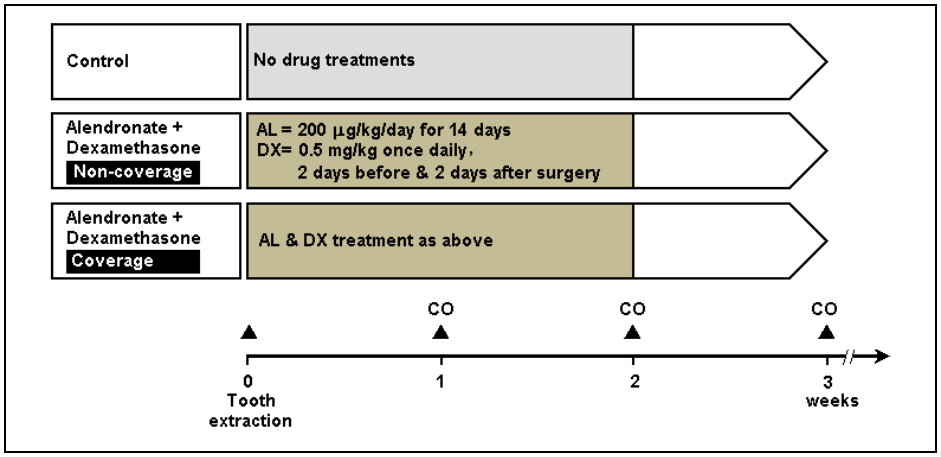

Figure 8. Summary of experimental study V. Animals with ONJ-like lesions were euthanized using carbon dioxide after 2 weeks. The others were kept alive until 3 weeks, to make sure that no wounds developed (AL: Alendronate; DX: Dexamethasone; CO: Clinical observation).

Coating technique

The coating procedure in studies I and II was performed as described by Tengvall et al (260). Briefly, a cross-linked layer of fibrinogen was covalently bound to the metal, and then small amounts of pamidronate and ibandronate were bound and adsorbed to the fibrinogen matrix. The thickness of the fibrinogen and bisphosphonate layers was estimated to be 23 nm as measured by ellipsometry (299). The amount of bisphosphonate, approximately 60% pamidronate and 40% ibandronate, on similarly treated surfaces has been measured to be less than 1µg per cm^2. The coating used in clinical studies is a "first-generation " bisphosphonate coating (260). While these studies were proceeding, improved bisphosphonate coatings were developed using zoledronate. The screws in study IV were coated with fibrinogen matrix into which zoledronate was embedded. By using of

isotope-labeled bisphosphonate in a parallel series, the amount of bisphosphonate per screw was found to be approximately 400 ng.

In study IV, the stability of the bisphosphonate layer was also been tested by screwing and unscrewing an implant in extraction sockets of rats. Implant-insertion trauma did not have any influence on this layer, as measured by ellipsometry and scintillator.

Results

Short summary of results of clinical studies (I and II)

Is it feasible to use bisphosphonate-coated implants in the human jaw?
Does bisphosphonate-coating improve the fixation of dental implants?
There was no loss to follow-up. No complications were seen from insertion to abutment connection and 1 year after functional loading in any of the all together 21 patients.

Marginal bone height (I and II)

In study I, 105 intraoral radiographs were taken and no significant differences were found for this variable.

In study II, 244 intraoral films were taken. At 6 months, the marginal bone loss was less with bisphosphonate coating than in the controls ($p = 0.012$) (Figure 9). The difference was already apparent at 2 months ($p = 0.017$). The independent observer also found a significant treatment effect at 6 months ($p = 0.003$). The measurements for the bisphosphonate implants at 6 months were in complete agreement between the two observers for 10 of the patients, and the difference did not exceed 0.25 mm for the remaining 6 patients. Study II extends only to abutment connection at 6 months. However, we have now followed these patients with a new radiography at 18 months. The difference between coated and control implants remained significant ($p = 0.04$).

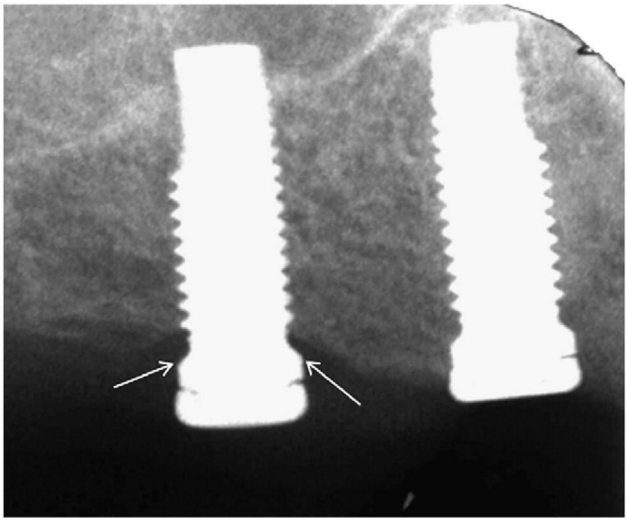

Figure 9. Dental radiograph (patient 16) showing a bisphosphonate coated implant (right) and a control (left). Arrows show reference points for measurement of marginal bone level.

Resonance-frequency analysis (I and II)

In study I, Comparing the seven implants in each patient, the bisphosphonate-coated implants always had the largest increase in ISQ value, although in one patient there was a tie (Figure 10). Although bisphosphonate-coated implants were inserted in bone of poor quality (at posterior site), they had the largest increase in ISQ values.

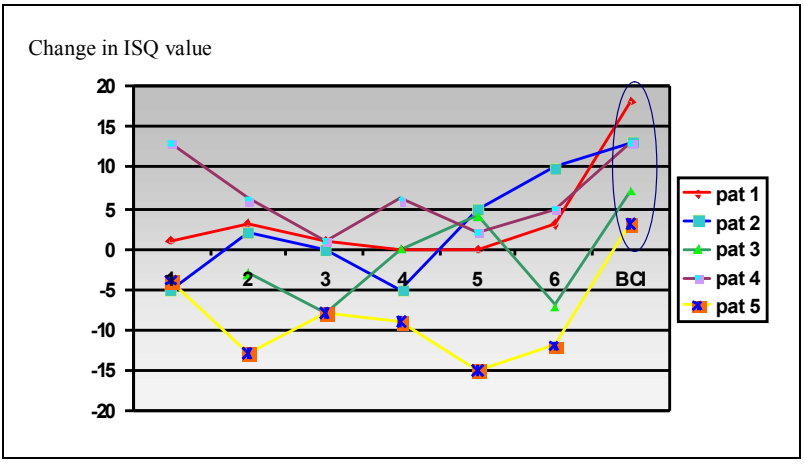

Figure 10. Change in ISQ values between insertion and abutment connection. All implants in each patient are connected by a line. Implant values are in the same order as inserted, starting opposite to the bisphosphonate implant, which is encircled.

In study II, the bisphosphonate-coated implants showed a larger increase in ISQ value from baseline to 6 months than did the controls (a difference in increase of 6.9 units between experimental and control implants; 95% CI: 4.1–9.8; p =0.0001) (Figure 11). All the coated implants except two showed a higher increase in ISQ value at 6 months than their paired controls. The absolute ISQ value at 6 months was higher for the coated implant in all cases but one.

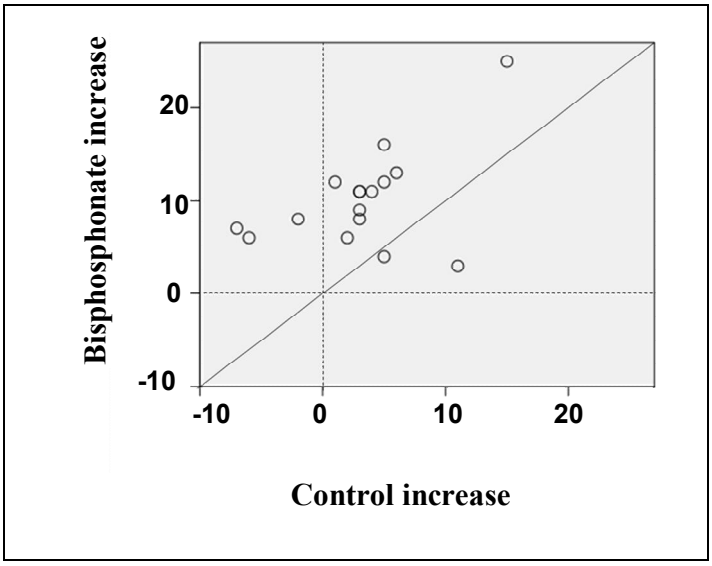

Figure 11. Increase in ISQ from baseline to 6 months. Each point describes the control implant (horizontal axis) and the bisphosphonate implant (vertical axis) in the same patient. Points above the diagonal line indicate a higher increase in ISQ in the bisphosphonate implant than in the control.

Histology (I).

The dental titanium implant coated with bisphosphonate was inserted in the molar region, at a minimum distance of 5–6 mm distal to the last regular implant. Histological ground sections of bisphosphonate-coated implants showed that the screws were fully osseointegrated (Figure 12). Mature, lamellar bone trabeculae had formed in intimate bone contact with the implant. New bone had formed around the implant 1–2 mm into the maxillar sinus. At the abutment end, bone resorption was seen adjacent to the implant. These areas extended about 1 mm down and less than 1 mm out from the implant. There were no signs of active resorption and no signs of necrosis.

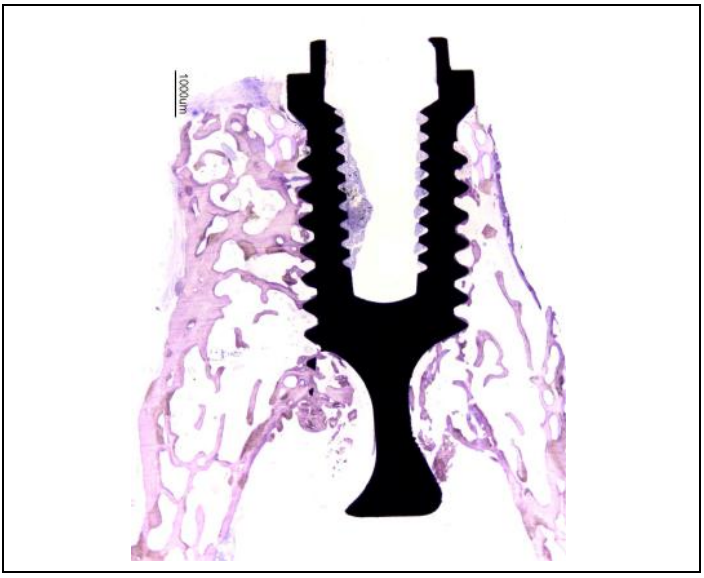

Figure 12. Histological cross-sectional image of a bisphosphonate-coated implant after 6 months.

Short summary of results of experimental studies (III)

Can an ONJ-like lesion be reproducibly produced in a rat model?

All 10 animals in the systemic group developed large ONJ-like lesions (Figure 13). The alendronate and control groups showed an intact overlying mucosa in all rats (Figure 13). This was confirmed by histology. All animals with ONJ-like lesions showed discontinuity of the overlying epithelium, bony sequestra and inflammatory cells. Lactate dehydrogenase assay showed living osteocytes in the maxillae without tooth extraction. On the side of tooth extraction, most osteocytes also appeared normal, except in regions close to the necrotic wound. Because of technical problems, mainly with cutting of the specimens, only six of 10 samples could be examined. The negative controls showed no living (i.e. stained) osteocytes.

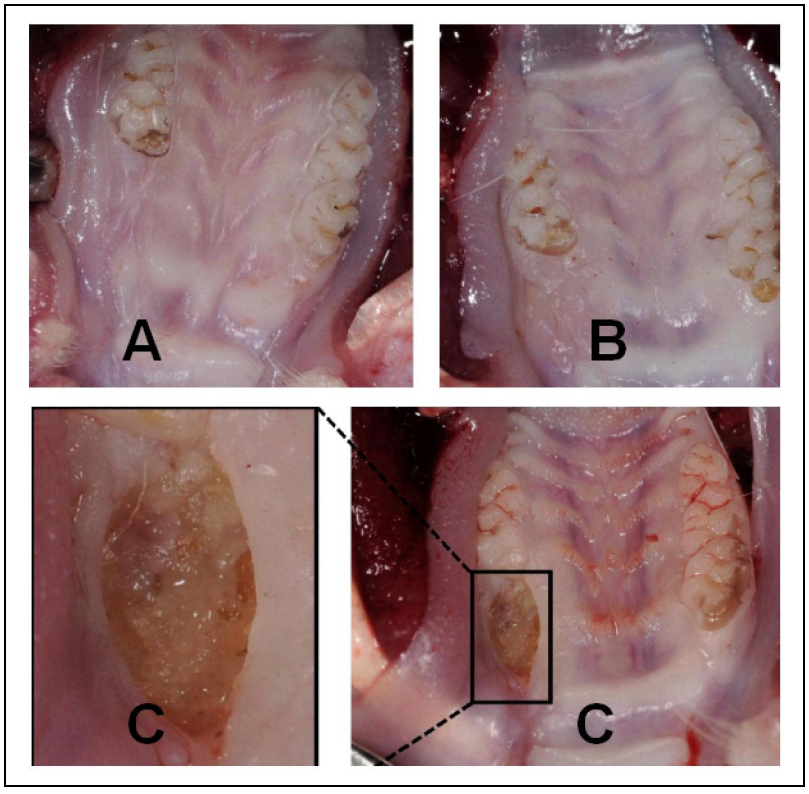

Figure 13. Normal healing after extraction of first molar in the Control and Alendronate groups (A and B). Bone exposure in rat treated with dexamethasone and alendronate (C**).**

Short summary of results of experimental studies (IV)

Can a bisphosphonate coating improve implant fixation also under conditions where there is a high risk of ONJ-like lesions in rats?

All 10 animals with systemic alendronate treatment developed large ONJ-like changes, while all of them with local treatment were completely healed (Figure 14). Implant removal torque was higher for the bisphosphonate-coated implants than in the other groups ($p < 0.03$ for each comparison).

Micro-CT of the maxilla showed more bone loss in the systemic alendronate group than in groups receiving local treatment (p = 0.001). The bone density in the immediate vicinity of the implant was higher for the Local group than for the Controls, and lower for the Systemic group compared with controls (p = 0.001 for both comparisons).

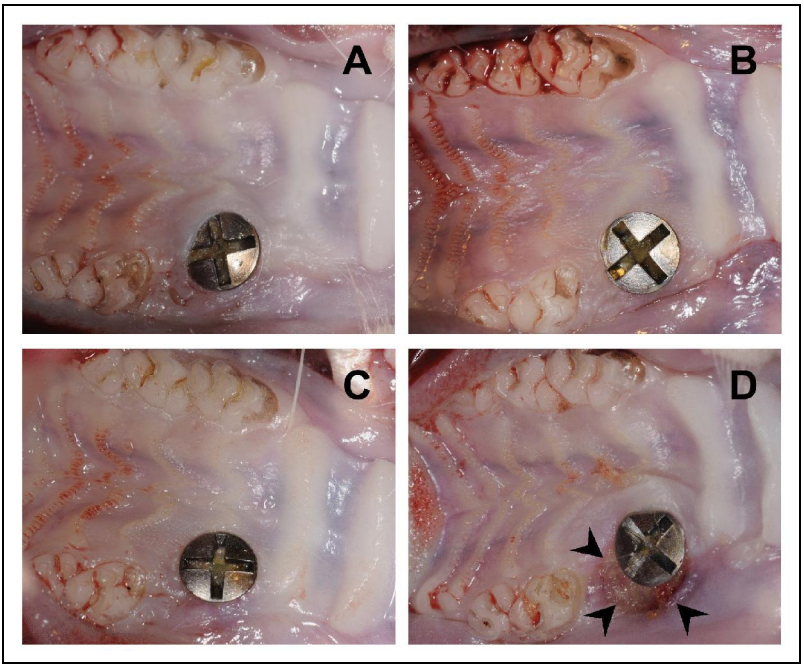

Figure 14. Normal healing after extraction of first molar and insertion of oral implant into the Control group (A), the Local group (B), and the Dexamethasone group (C). Bone exposure in rat treated with dexamethasone and alendronate (Systemic) (D).

Short summary of results of experimental studies (V)

Can early mucoperiostal coverage prevent ONJ-like lesions?

All animals in the non-coverage group developed large ONJ-like changes (Figure 15). The coverage and control groups showed an intact overlying

mucosa in all rats (Figure 15). Findings were confirmed with histology. Due to technical problems, mainly with cutting the specimens, only 9 of the control and coverage samples could be examined. These two samples were then excluded from histological examination. The blinded evaluation showed epithelial discontinuity in all non-coverage cases and in none of the others. Large sequestrae or areas with empty osteocyte lacunae were seen in all non-coverage cases. In the other groups, minor sequestrae were seen occasionally. Inflammatory cells were also more common in the non-coverage group.

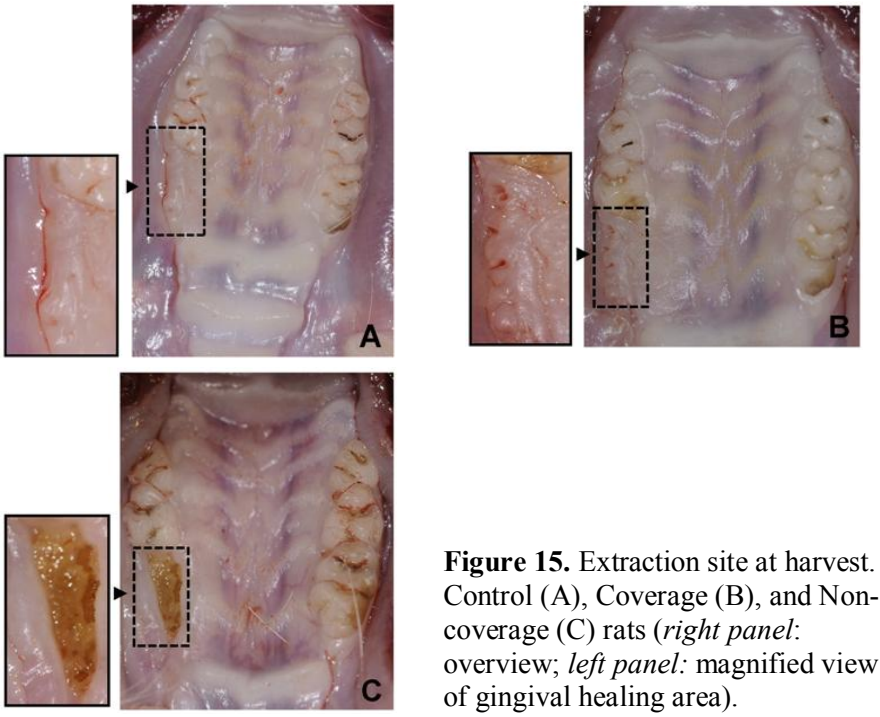

Figure 15. Extraction site at harvest. Control (A), Coverage (B), and Non-coverage (C) rats (*right panel*: overview; *left panel:* magnified view of gingival healing area).

Discussion

Implants and local delivery of bisphosphonate

Throughout history, humans have sought ways to replace lost teeth. However, it is only within the past 100 years that members of the dental and medical professions have made substantial progress in the permanent replacement of missing teeth by intraosseous anchorage of artificial metal fixtures. Fifty years later, the term osseointegration was coined by Brånemark. Direct contact between living bone tissue and titanium implants can lead to biological adhesion. Osseointegration is observed in several areas, not only with dental implants, but also with maxillofacial implants, replacement of damaged joints, and placement of artificial limbs. The success of these operations is dependent of the fixation of the implants, which, in turn, depends on the strength of the bone that holds them. If bone quality is poor, surgical procedures can be modified to provide sufficient mechanical fixation by adding more screws or larger devices, or by protecting the implant from mechanical loading for a considerable time after surgery, for osseointegration. Thus, if the quality of the bone holding an implant could be improved locally, surgical procedures would become simpler and rehabilitation would become faster.

To improve bone-to-implant contact, most manufacturers of dental implants have focused on implant surfaces (101). Common methods of treating titanium dental implant surfaces are blasting, acid-etching, and chemical modification (124, 300).

Bisphosphonates are anti-resorptive drugs that act specifically on osteoclasts, thereby maintaining bone density and strength (202). Once released from the

surface of a coated implant, bisphosphonates reduce osteoclast activity, thereby changing the balance of bone turnover in favor of bone formation, leading to a net gain in local bone density.

During the last decades, the stability of implants with local bisphosphonate treatment has been tested in clinical studies (268) and animal studies (255-259), but not in human jaws. One problem is to find a method to measure the effect of bisphosphonate treatment on implant fixation in terms of stability. To our knowledge, this is available only for dental implants, by measurement of mechanical resonance frequency, which provides unique opportunities for implant research. One reason for this is that dental implants are accessible for examination without surgical exposure, which is in the case with orthopedic implants.

Resonance-frequency analysis is a reliable and non-invasive method to measure the quality of fixation in humans. The method, its validation, and clinical use have been comprehensively reviewed (179, 182-184). The changes in implant stability expressed by differences in ISQ value over time reflect the biologic events associated with the bone-implant interface. In two clinical studies, a total of 96 implants were inserted in 21 patients. In a randomized trial with a paired design, one implant of each pair was coated with a thin fibrinogen layer containing two bisphosphonates (pamidronate and Ibandronate). The bisphosphonate-coated implants showed a larger increase in ISQ value from baseline to 6 months than did the controls. It is unclear at what time the bisphosphonate exerted its effect during the healing process. It is a weakness of these studies that early stages of the healing period of the implants were not studied by resonance-frequency analysis. The reason is that the studies were conducted as two-stage procedures.

However, the positive effect on radiographic structure already at 2 months might suggest that early changes in stability could be seen in the initial phase of the healing process.

Periapical radiographs have been used routinely to evaluate longitudinal bone loss around implants. The obvious advantages are that they are non-invasive, non-destructive techniques that can be used at multiple sites and several times in clinical trials. The disadvantage is that radiographs provide a two-dimensional representation of a three-dimensional volume. Despite this, differences around implants over time can be detected in radiographs and are included in the success criteria in most clinical trials.

The level of marginal bone around the implant is widely considered to be one of the most important reference criteria for monitoring of peri-implant health and for evaluation of the long-term success of dental implants. Several factors may contribute to marginal bone loss. It may be the result of bacterial contamination, promoting the occurrence of peri-implantitis and progressive bone resorption (301). Incorrect fixture positioning may also cause marginal bone loss. The most coronal portion of peri-implant bone may tend to resorb if the fixture is placed with a residual buccal wall that is too thin. Following implant placement, a small amount of marginal bone resorption occurs and these small changes are considered to be part of a physiological process.

Study II extends only to abutment connection at 6 months. We have now followed these patients with a new radiography at 18 months. The difference between coated and control implants remained significant ($p = 0.04$).

The largest available follow-up material after routine implant insertion (n = 431) showed a mean marginal resorption from insertion to 18 months of 0.7 mm with an SD of 1.0 (302). Judging by the SD, the distribution is quite skewed. Our figures for uncoated implants are similar to these, with a mean of 0.63 and SD of 0.58 (although we prefer to report median and range). Based on their findings in submerged implants, Albrektsson et al. (139, 303) proposed criteria for implant success, including the absence of implant mobility and absence of pain. Thus, 1 mm of bone loss was considered acceptable during the first year of function, and 0.2 mm annually thereafter. In our studies, the marginal bone loss was less with bisphosphonate- coated implants than with the controls at 2, 6 and 18 months. The difference between coated and control implants did not change significantly between 6 and 18 months (Figure 16). A clinical difference of 1 mm can be regarded as negligible. However, in highly demanding cases such as implants in the anterior maxilla, preservation of the marginal bone level is critical for an "esthetic" result.

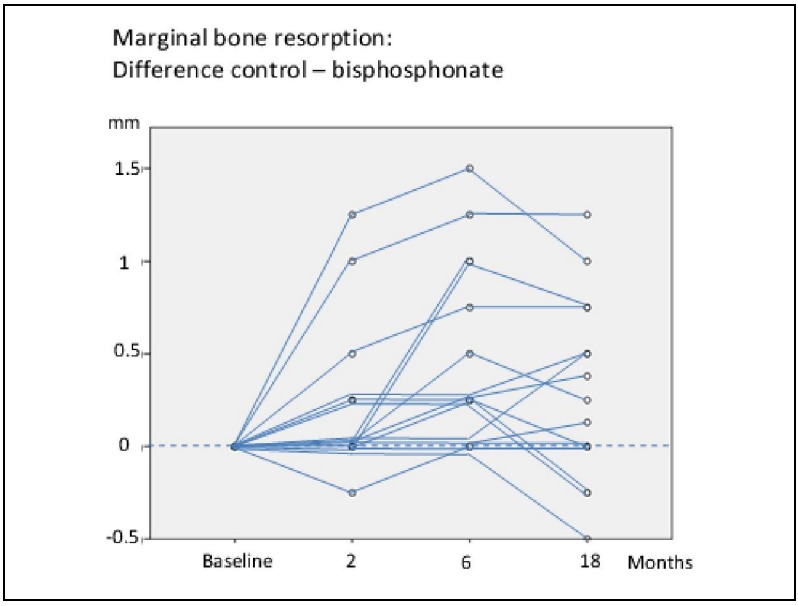

Figure 16. Difference in marginal bone loss between control implants and bisphosphonate-coated implants at baseline and 2, 6, and 18 months postoperatively. Note that study II reports only 2 and 6 months.

Resonance-frequency analysis

Resonance-frequency analysis (RFA) is a non-invasive, objective method for evaluation of implant stability and has been validated through several *in vitro* and *in vivo* studies (178-180). This technique does not correlate with the insertion torque, as the torque is mostly due to surface friction (304, 305). However the method correlates well with cortical bone thickness (306) and the cutting resistance at the time of implant placement (183). The use of resonance- frequency analysis may provide the possibility of identifying implants with higher risk of failure and the ability to individualize implant treatment. For example, early overload of an implant may cause the resonance frequency to decrease, which serves as a warning, so that

loading can be interrupted and the implant allowed to regain fixation (307). Furthermore, in a longitudinal study by Glauser et al. (192), ISQ values of 72 stable implants was compared with those of nine implants that had lost stability over 1 year according to an immediate/early-loading protocol. The implants that failed during the course of the study showed significantly lower stability already after 1 month. Similar findings have been published by Atieh et al. (308).

Rat model of ONJ

The Pathophysiology of ONJ is not well understood. An animal model of ONJ has therefore been developed to mimic bisphosphonate-related osteonecrosis in cancer patients. Following tooth extractions, a combination of zoledronate and dexamethasone caused ONJ-like lesions in the majority of rats (309). The importance of surgical trauma in the development of ONJ has been emphasized by these authors (309). It is important to note that the animals underwent a rigorous dental procedure (extraction of all three molars in any given jaw bone) that generally exceeds the sort of dental trauma usually experienced by humans who develop BRONJ. In our animal studies, only one molar was extracted, but we added partial excision of gingiva adjacent to the extraction site, to increase the area of bone exposure. This might explain our higher success rate, with lesions in all cases.

As with any animal study involving pharmaceutical agents, it is important to consider the doses administered with respect to those used clinically. Clinically, cancer patients are given zoledronate as an intravenous infusion every 4 weeks at a dose of 4 mg for an individual weighing 60 kg (~66 µg/kg) (310). In an attempt to mimic dosing for multiple myeloma, animals

in our studies were dosed daily with alendronate using a subcutaneous injection of 200 μg/kg for 14 days.

Previous experience in our group has shown that strong inhibition of osteoclastic activity in rats can be achieved with this dose (311). When calculated as dose per body weight per day, the rat dose is 100 times higher than the human dose. However, the serum concentration after each injection may be quite similar, as the metabolic rate of rats is estimated to be 3 times higher than that of humans (312).

The relevance of our model for human ONJ is not certain. One important difference between rats and humans may be size. Vascular outgrowth, epithelial proliferation and other processes of healing are likely to occur at roughly the same speed (in mm per day) in animals of different sizes. Thus, our model may be relevant mainly for millimeter-sized lesions rather than for complete extraction defects in humans. On the other hand, similar rat models are regarded as relevant, at least for the principal aspects of ONJ (309, 313).

A weakness in our studies was that the bisphosphonate treatment in our model started at tooth extraction, whereas in clinical ONJ the treatment has lasted for a long time before this. However, if ONJ starts with a contaminated lesion rather than primary sterile osteonecrosis, the pathophysiological processes will take place under the influence of bisphosphonates in both cases. Moreover, this timing was chosen because it further demonstrates that no bone pathology pre-dating tooth extraction is necessary for the development of BRONJ.

Implants and coating technique

The coating technique for dental implants in this study was presented by Tengvall et al. (260). Briefly, a crosslinked layer of fibrinogen was covalently bound to the metal, and then small amounts of pamidronate and ibandronate were bound and adsorbed to the fibrinogen matrix. In an animal model, the thickness of this bisphosphonate layer (a few nm) was measured by ellipsometry (255). Ellipsometry is an optical method that is often used to measure the thickness of thin films adsorbed to flat surfaces. However, because the surface area of the screw is much greater than a corresponding flat surface, it may be difficult to translate the amount of drug on a flat surface to that of the screw.

The recommended human dose of alendronate for treatment of osteoporosis is 10 mg/day. The gastrointestinal uptake of oral bisphosphonates is about 0.7% for alendronate (225) and 0.3% for pamidronate (226). For individuals weighing 75 kg, this means that of the prescribed human daily dose of bisphosphonates, approximately 0.5-1 µg/kg/day reaches the bloodstream. For comparison, the total amount of bisphosphonate on coated human dental implants is in the order of 1 µg ibandronate (less than 1µg /cm^2). Clinically, osteoporosis patients are given ibandronate as an oral dose of 50mg per day for individuals weighing 60 kg (~ 833 µg/kg) (314). Thus, the amount of bisphosphonate on the surface of the dental implant corresponds to the total body dose of one day of osteoporosis treatment.

After insertion of bisphosphonate-coated implants, the bisphosphonate is released from the surface and rapidly accumulates in the surrounding bone tissues. In an animal model using a fibrinogen immobilization matrix and

^{14}C-alendronate, 60% of the immobilized bisphosphonate was released after 8 h, but the release continued slowly for up to 8 days (255).
Once released from the surface of a coated implant, bisphosphonates reduce osteoclast activity, thereby changing the balance of bone turnover in favor of bone formation, leading to a net gain in local bone density (314). Fast formation of a shell of new woven bone surrounding the implant is seen, which becomes slowly remodelled into lamellar bone (315). Furthermore, it has been shown that the amount of bone increases adjacent to the implant, with a maximum density 250 μm from the implant surface (316).

One question regarding local bisphosphonate therapy of any type is the nature of the systemic side effects that the drug may have on skeletal remodeling. Following elevation of the mucoperiosteal flap Yaffe et al. (317) applied 22 μg of ^{14}C-labeled alendronate directly to alveolar bone with a soaked gelatin sponge in a rat model. The local absorbtion of alendronate and its disposition in the contralateral side of the mandible as well as in the tibia bone were analyzed. The mean total amount of drug measured in the entire left tibia after 60 min was 3.2% of the dose applied (0.7 μg). McKenzie et al. (318) inserted hydroxyapatite-coated implants with 100 μg ^{14}C-labeled zoledronate in dogs' femoral bone. Bone samples adjacent to and distant from the implant were harvested and the concentration of radiolabeled bisphosphonate in each sample was quantified using liquid scintillation spectrophotometry. The mean zoledronate concentration in the cortical bone adjacent to the implant (peri-implant bone) was 733 ng/g bone at 6 weeks and 377 ng/g bone at 52 weeks. At 6 weeks small amounts of zoledronate ($\leq$ 7.2 ng/g) were detected throughout the skeleton, indicating some escape into the circulation after local elution. This indicated that most of the bisphosphonate remained at the implant site. By using the isotope-

labeled bisphosphonate in a parallel series in our animal model (study IV), the bisphosphonate amount per screw was found to be approximately 400 ng. This is a very low dose compared with the dose currently used in clinical practice.

These studies support our notion that local delivery of bisphosphonates and their affinity for bone may become an important treatment modality to prevent resorption of bone during dental and orthopedic procedures. It seems that the risk of systemic side effects of local delivery of bisphosphonate would be small.

Lactate dehydrogenase analysis

Bisphosphonates have been associated with osteonecrosis of the jaw, and it has been suggested that this condition starts with sterile bone death. We hypothesized that BRONJ can develop without being preceded by sterile bone death. The viability of osteocytes was therefore the subject of this examination. Lactate dehydrogenase (LDH) is a cytoplasmic enzyme found in most living cells. The lactate dehydrogenase assay is a popular method to detect cell viability in bone sections. The major advantage of the LDH assay is the stability of the LDH enzyme for up to 36 h after cell death, eliminating any false negative viability results due to processing of the tissue. With this method, viable osteocytes react to form non-reversible tetrazolium-formazan granules, while non-viable osteocytes are distinguished with methyl green stain.

The use of staining of LDH activity as a measurement of osteocyte viability goes back to 1982 when Wong et al. (319) introduced this histological

method for use on bone sections derived from human femoral heads. They demonstrated that viable osteocytes stained positive for LDH activity, while it was already known that necrotic tissue does not show any staining of LDH activity staining. Sambrook et al. (320) showed that the number of viable osteocytes was significantly reduced in femoral head bone of glucocorticoid-induced osteoporosis patients compared with controls. They use frozen bone samples for this verification. We used a lactate dehydrogenase assay according to a modified protocol by Phillips et al. (321). By increasing the concentrations of coenzyme and tetrazole, these authors obtained excellent reactivity. Fresh, unfixed specimens were used to preserve LDH activity. Since the enzyme persists only for 36–48 h at 37 °C after sacrifice (319), the specimens should be processed within 24 h to minimize artifact (321). However, freezing results in disruption of the structural integrity of the cell (322).

Reported specimen thicknesses for sawn fresh bone vary from 200 µm (320, 323) to 300 µm (320, 324). Phillips et al. (319) used 400-µm-thick fresh bone and demonstrated that section thickness may not affect osteocyte reactivity. However, it may affect the length of time required for decalcification. In the present study, we used 800-µm-thick fresh bone. However, sawing a thinner section would probably cause damage due to the low density of the maxillary bone.

Pathophysiology of ONJ

The pathophysiology of ONJ is not well defined, and the condition is probably multifactorial in nature. While it this is most likely associated with bisphosphonate use, a causal effect has yet to be demonstrated. There are

several features that make the oral cavity a unique environment. The alveolar bone in both the mandible and the maxilla is covered by a thin layer of periosteum and epithelium, which are subjected to a wide variety of stresses such as mastication forces, dental procedures, and periodontal disease. This combination of constant stress predisposes the thin mucosa to trauma, leading to exposure of bone.

When bisphosphonate-related osteonecrosis of the jaw (BRONJ) was first described, its similarities with radiation-induced osteonecrosis led to the assumption that the condition started with sterile necrosis of the jaw bone. At that time, it was speculated that bisphosphonates could cause osteonecrosis through effects on blood vessels in bone, possibly by inhibition of vascular endothelial growth factor (VEGF) (280-282). It has been demonstrated that patients with advanced solid cancers and associated bone metastases have significantly reduced levels of serum VEGF when treated with zoledronate or pamidronate (280, 325, 326). However, a recent clinical trial compared the incidence of ONJ in breast cancer patients receiving bevacizumab (avastin, anti-VEGF-1 monoclonal antibody) or placebo (327). The analysis failed to show any increased incidence of ONJ in the risk patients. The anti-angiogenic role of bisphosphonate is still unclear. The notable incidence of ONJ in the maxilla appears to refute the idea of the anti-angiogenic effect of bisphosphonate being the sole cause of ONJ.

It was later suggested that BRONJ does not begin as a form of classical osteonecrosis, but is in fact osteomyelitits already from the beginning (283, 284). Microbial contamination with *Actinomyces* appears to play an important role in maintaining the osteomyelitic wounds (285, 286). Hansen et al (285) examined histopathological specimens from patients with

actinomycosis of the jaw. Out of a total number of 45 patients with actinomycosis, 43 (93%) suffered from ONJ (58%) or infected osteoradionecrosis (35%). Furthermore, the oral cavity and teeth are colonized by a complex microbial flora, many of which are pathogenic organisms. The intimate relationship between teeth and jaws allows a portal of entry for microbes and other inflammatory products to the underlying bone, a situation that is not found in any other part of the body. Hence, oral surgical procedures exposing bone to the oral cavity increase the risk of ONJ. In a rat model, we showed that ONJ-like lesions can be prevented when extraction sockets are covered with a mucoperiosteal flap (328). This shows the importance of bacterial role in the development of this condition.

Because jaw bone containing bisphosphonates will resorbed slowly, it is conceivable that bacterially contaminated bone cannot be removed fast enough to prevent the development of chronic osteomyelitis. This view is supported by the observation that similar lesions appear after treatment with an anti-RANKL antibody that reduces osteoclast recruitment (287).

Thus, it appears that resorptive activity is a key factor in bone healing.

Based on these considerations, this condition might be called bisphosphonate-induced osteomyelitis of the jaw.

Conclusions

Studies I and II

A thin, bisphosphonate-eluting fibrinogen coating can improve the fixation of metal implants in human bone. This might lead to new possibilities for orthopedic surgery in osteoporotic bone and for dental implants.

Study III

ONJ-like lesions were reproducibly induced in a rat model at sites of tooth extraction, whereas there were no signs of osteocyte death in uninjured sites. Osteonecrosis of the jaw appears to arise first after the bone has been exposed.

Study IV

In a rat model, local bisphosphonate treatment with zoledronate improved implant fixation in a setting where systemic treatment caused ONJ. Improved fixation of bisphosphonate-coated dental implants in humans might be achieved with a smaller risk of such complications in comparison with systemic treatment.

Study V

Mucoperiosteal coverage of newly exposed bone prevented ONJ-like lesions in rats. Immediate coverage of extraction sites might be recommended for patients at risk of ONJ.

This treatment has now been implemented at our department.

What next?

We started this project by showing that local treatment of implants with bisphosphonate may have a future place in orthopedic surgery and dental surgery, since bisphosphonate coatings improved the fixation of dental implants in the human jaw. However, the clinical benefits of this technique are still not understood. One could speculate that if bisphosphonates have an effect in the early phase of healing, then rehabilitation after implantation would become faster. This hypothesis still has to be tested.

Hypothetical positive long term effects would include reduced rates of mechanical loosening and peri-implantitis. In order to study such effects, it will be required a very large number of patients that is not currently within reach. Another important issue is the risk that patients might be exposed to with the use of locally delivered bisphosphonates. A potential risk would be the peri-implantitis, which may jeopardize the entire perception of local bisphosphonate treatment. However, to exclude that such risks exist (with sufficient confidence intervals), again a very large number of patients would be required. At present, we can only say that if any such local adverse effects would appear, the problem would be easily solved by removing the bisphosphonate-containing bone in the immediate vicinity of the implant.

The posterior maxilla has been described as the most difficult and problematic part of the mouth for the implant practitioner, and it requires the most ingenuity for achievement of successful results. Anatomical considerations include reduced bone quantity, especially in patients who have experienced alveolar resorption in the wake of tooth loss. In these cases, it may be necessary to perform a bone augmentation procedure before placement of the implant. However, one drawback in using autogenous bone

is the unpredictable resorption of bone, which might be reduced by the use of bisphosphonates, perhaps locally. More short- and long-term data are needed for us to fully evaluate the benefits of bisphosphonate use in oral surgery.

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