Osteoblast-like cells with different embryologic origins behave differently in increasing zoledronic acid concentrations: a pilot study in pigs

Philipp Kauffmann, MD, DMD,[a] Wolfram Hahn, DMD, PhD,[b] Niklas Sievers, DMD,[a] Markus Troeltzsch, MD, DMD,[a] Karin Hohloch, MD, PhD,[c] Phillipp Brockmeyer, MD, DMD,[a] Robert Cordesmeyer, MD, DMD,[a] Henning Schliephake, MD, DMD, PhD,[a] and Rudolf Matthias Gruber, MD, DMD, PhD[a]

Objective. We compared the intraindividual effects of increasing zoledronic acid (ZA) concentrations on osteoblast-like cells with different embryologic origins.

Study Design. Cultured osteoblast-like cells from mandible and iliac crest bone samples of domestic pigs were exposed to increasing concentrations of ZA (0, 10^{-8}, 10^{-6}, and 10^{-4} M). Proliferation was assessed by cell counting. Receptor activator of nuclear factor kB ligand and osteoprotegerin (OPG) messenger RNA expression were assessed at 0, 1, 4, 7, and 10 days.

Results. The OPG expression level was higher in the iliac crest than in the mandible. Neither ZA concentration nor the cells' origin affected the expression of receptor activator of nuclear factor kB ligand. At 10^{-6} M, OPG expression from both locations reached the same level after 7 days of cultivation, as OPG expression increased to a greater extent in the mandible in comparison to the iliac crest.

Conclusion. Cultured mandibular osteoblast-like cells reacted more sensitively to high ZA concentrations than did osteoblast-like cells from the iliac crest. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123:20-28)

Bisphosphonates (BPs) are used worldwide to treat skeletal diseases such as osteoporosis and Paget disease or bone metastases in malignant disease. One of the serious side effects of BPs is BP-related osteonecrosis of the jaw (BRONJ).[1-3] The prevalence of BRONJ varies from 0.7% to 18.6% after/during intravenous application of BPs and from 0.01% to 4.3% after/during oral administration of BPs.

BPs have inhibiting effects on bone metabolism, predominantly on osteoclasts, in vitro and in vivo, whereas controversial results have been published concerning the influence of BPs on osteoblasts.[4,5] Additionally, the antiangiogenetic effect of BPs is considered to be one of the reasons for the development of avascular necrosis of the bone.[6]

Zoledronic acid (ZA), a third-generation BP, is a very potent inhibitor of bone resorption. ZA inhibits the enzyme farnesyl diphosphonate synthase and subsequent prenylation of small GTPases. This inhibition leads to decreased osteoclast function and apoptosis of osteoclastic cells and furthermore leads to localized macrophage immunosuppression.[7-11]

Osteoclasts are recruited by osteoblasts through the production of receptor activator of nuclear factor kB ligand (RANKL). RANKL binds to the osteoclastic membrane receptor RANK (receptor activator of nuclear factor kB) and activates cells that increase bone resorption. This effect is limited by osteoprotegerin (OPG), which is also produced by osteoblasts. OPG is a soluble decoy receptor (i.e., a receptor that binds to a ligand and prevents it from binding to its normal receptor), thus attenuating the effect of RANKL.[12,13]

BRONJ of bones almost always occurs in the jaw.[14] Only eight cases of external auditory canal osteonecrosis have been reported worldwide.[15] There are a number of reasons why BRONJ has been reported to occur mainly in jaw bones, including that the thin and vulnerable mucosa in the oral cavity may be responsible for the easy invasion of pathogens, leading to bone necrosis that is not remodeled/resorbed due to the decreased activity of osteoclasts.[16] Additionally, osteoclast recruitment occurs through communication with osteoblasts, and potential differences between bone cells from the jaw and other parts of the body have increasingly been the focus of

Statement of Clinical Relevance

This work provides the basis for further in vivo research in pigs, as they have a similar metabolism, comparable masticatory activity, and similar physiology with regard to therapeutic concentrations of bisphosphonates compared to human beings.

[a]Department of Oral and Maxillofacial Surgery, Georgia Augusta University, Göttingen, Germany.
[b]Department of Orthodontics, Georgia Augusta University, Göttingen, Germany.
[c]Department of Hematology and Oncology, Göttingen Comprehensive Cancer Center, Georgia Augusta University, Göttingen, Germany.

Received for publication May 1, 2016; returned for revision Jul 18, 2016; accepted for publication Aug 8, 2016.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2016.08.010

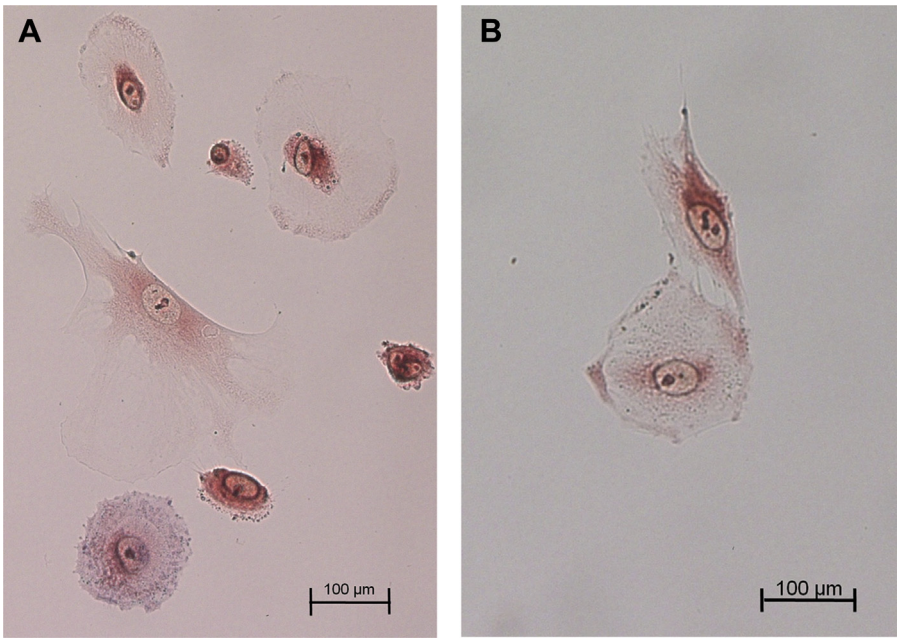

Fig. 1. (**A**) Immunohistochemical staining for alkaline phosphatase showing cultivated osteoblast-like cells from the iliac crest with red granules in a light-blue cytoplasm, indicating synthesis of alkaline phosphatase. (**B**) Immunohistochemical staining for alkaline phosphatase showing cultivated osteoblast-like cells from the mandible with red granules in a light-blue cytoplasm, indicating synthesis of alkaline phosphatase.

research on BRONJ.[17-19] Embryologic factors also are considered to be involved in the development of BRONJ.[18,20,21] In the skeleton, two different bone formation processes are described, endochondral ossification and intramembranous ossification. Osteoblasts of the jaw bone originate from the ectodermal cranial neural crest and are capable of intramembranous ossification, whereas osteoblasts of the uninvolved skeleton are derived from the mesodermal mesenchyme.[18,21,22] Parts of the proximal mandible also develop through endochondral ossification, and no BRONJ cases have been described in this region.[23]

Due to its similar metabolism, comparable masticatory activity, and similar physiology, we transferred therapeutic concentrations of ZA to cultured osteoblast-like cells from domestic pigs.[24-27] The aim of our study was to test the hypothesis that increasing ZA concentrations have different effects on the recruitment activity of osteoblast-like cells, depending on whether the cells come from the jaw or the iliac crest. Furthermore, we examined whether cultivation of porcine osteoblast-like cells from bone samples of the iliac crest and the mandible of adult domestic pigs was possible.

STUDY DESIGN

Tissue source

Fresh bone samples were taken from the mandible and iliac crest of five female domestic pigs 6-9 months of age using bone rongeur forceps (Aesculap, Tuttlingen, Germany). This sampling was performed to enable an intraindividual evaluation of the effect on osteoblast-like cells from the mandible and the iliac crest. In accordance with the Committee on the Ethics of Animal Experiments of the University of Göttingen, no ethics approval was required, as the bone samples were obtained from a local butcher. The domestic pigs, of an average age of 6-8 months and weighing approximately 100 kg, were sacrificed by the butcher, Sebert's, in Göttingen.

Cell culture

Six to eight fragments, average size 1 mm × 1 mm, from each site and animal were placed in Petri dishes (Sarstedt, Germany) and subsequently cultivated in Dulbecco's modified Eagle medium (DMEM) (Thermo Fisher, Waltham, MA). We ensured that no soft tissue, such as periost or bone marrow, was included. After attachment of the bone sample to the plastic surface of the dish, the osteoblast-like cells migrated out of the tissue. After 7 days, the bone fragments were removed from the primary culture.

The cells were cultured under controlled conditions (37°C, 5% CO_2, and 95% air) with DMEM containing 2% gentamicin and 1% amphotericin B, as well as 15% fetal calf serum to stimulate cell proliferation. Once the monolayer of the outgrowing cells reached confluence after 3-4 weeks, the cells were passaged twice and cultured in six well plates at a density of 100,000 cells per well (Greiner Bio-One, Germany).