Int. J. Oral Maxillofac. Surg. 2006; 35: 1127–1132
doi:10.1016/j.ijom.2006.07.002, available online at http://www.sciencedirect.com

International Journal of
Oral & Maxillofacial Surgery

Research Paper
Osteobiology

Osteopontin and bone metabolism in healing cranial defects in rabbits

M. Gordjestani[1], L. Dermaut[1], L. De Ridder[2], P. De Waele[3], W. De Leersnijder[4], F. Bosman[4]

[1]Department of Orthodontics, University of Ghent, Gent, Belgium; [2]Department of Histology, University of Ghent, Gent, Belgium; [3]Xellentis, Biotechnology Company, Belgium; [4]Innogenetics, Biotechnology Company, Belgium

M. Gordjestani, L. Dermaut, L. De Ridder, P. De Waele, W. De Leersnijder, F. Bosman: Osteopontin and bone metabolism in healing cranial defects in rabbits. Int. J. Oral Maxillofac. Surg. 2006; 35: 1127–1132.

Abstract. Non-collagen proteins such as bone sialoprotein and osteopontin (OPN) form 10% of the extracellular bone matrix. In this study, the influence of OPN on bone repair was investigated. Human OPN (Innogenetics®) was produced by a recombinant technique and bonded onto the surface of hydroxyapatite (Interpore 200®). Thirty rabbits were divided into six equal groups. A circular defect (10 mm) was prepared in each parietal bone. In four rabbits of each group the left and right defects were filled with either OPN-coated hydroxyapatite (OPN-HA) or non-coated hydroxyapatite (HA). One sham animal of each group received no implants. The animals were killed after 1, 2, 6, 12, 18 and 30 weeks. The histological sections were scanned and analysed digitally. There were no statistically significant differences in total bone formation between defects filled with OPN-HA and HA. Bone formation at the borders of the healing area was significantly higher in defects filled with OPN-HA than in those filled with HA. Less bone formation was noted in the OPN-HA and HA groups at the centre of the healing area than at the borders of the healing area and the dural area. Although some animals in the sham group showed a high level of bone formation in the dural area, this was not significantly different to that in the dural area of the other groups. There was no sign of infection or tissue rejection of the graft.

Key words: osteopontin; bone; bone metabolism; rabbits; hydroxyapatite.

Accepted for publication 3 July 2006
Available online 2 October 2006

The extracellular bone matrix contains collagen and non-collagen proteins. The non-collageneous proteins such as bone sialoprotein and osteopontin (OPN) account for 10% of the matrix[10,13,14]. OPN, a glycoprotein with a glycine–arginine–glycine–aspartate–serine (GRGDS) cell-binding sequence, has an attachment capacity that promotes the attraction and distribution of bone formation cells[2,6,12,17,22]. The concentration of OPN reaches its maximum as a function of stage of mineralized tissue formation[16]. In the initial stage of spongiosa formation of the tibial metaphysis, OPN was detected at the calcified cartilage line just below the zone of vascular invasion at the growth-plate level. Plasma proteins, such as alpha-2HS-GP, interact with OPN at sites of mineralized tissue[15]. Various growth factors regulate the production of OPN in the bone matrix[11,12]. At the same time, transforming growth factors stimulate the production of bone matrix and OPN[23]. Extracellular phosphate activates OPN production in osteoblasts. This regulation may be a control mechanism to attract and differentiate osteoblast-like cells.

Bone resorption factors such as 1,25 dihydroxyvitamine D3 and retinoid acid

0901-5027/121127 + 06 $30.00/0

activate the production of OPN[9,20,21]. OPN binds strongly to the calcium phosphate crystals in mineralized tissue, which inhibits crystal proliferation in bone. OPN plays a role in the recruitment of osteoclasts as in its absence the mobility of osteoclasts decreases[4]. These studies show the value of this protein during the various stages of osteogenesis and bone remodelling.

The presence of a thrombin cleavage site induces certain physiological processes of OPN. B lymphocytes show a more effective attachment to thrombin-cleaved OPN than to full-length OPN[8]. SENGER et al.[18] showed that endothelial cell migration during angiogenesis is affected by the interaction of thrombin-cleaved OPN with $\alpha_v\beta_3$ integrin receptors. The structural changes of OPN as a result of thrombin cleavage alter cell signal transduction. The OPN fragments are strongly chemotactic for the endothelial cells and may help promote new blood vessel formation. According to these studies, OPN plays an important role in bone formation based on its influence on the bone matrix and endothelial tissue. OPN is not a bone-specific protein, and its presence has been shown in various tissues such as milk, kidney, liver, ...[19]. This study aims to investigate the influence of OPN attached to HA on bone metabolism during the healing of calvarium defects in adult rabbits.

Materials and methods

Human OPN (Innogenetics NV, Belgium) was produced by a recombinant technique. OPN has a high affinity for HA[7] and was coated onto HA granules (Interpore 200®). Interpore 200® consists essentially of pure HA with a tiny amount of tricalcium phosphate[25]. Interpore 200® granules, with a mean size of 425–1000 μm, have similar biomechanical characteristics to spongious bone. Interpore 200® is stable in body fluids, can be moulded into a functional shape and size, and withstands sterilization procedures. The interconnected microchannels encourage rapid ingrowth of connective tissue and subsequent deposition of bone. HA is resorbed by giant cells and not by osteoclasts. Its resorption pattern is slow and complete resorption may take up to 10 years[5]; this can inhibit the natural ingrowth of bone. The coating procedure was carried out by immersing sterile HA granules in the OPN solution at a concentration of 0.614 μg OPN/ml for a total of 21 h at different temperatures between 10 and 21 °C. After removing the coating solution, the granules were left to dry on a flow bench. The drying procedure was completed when the weight of the granules stabilized. The dried granules were aliquoted under sterile conditions into 100 ± 0.5 mg portions and stored at 70 °C until use. By comparing the OPN concentration in the coating solution before and after the coating process, the amount of coated OPN was calculated: 1.047 μg OPN per mg HA granules. As 4 ml of the coating solution could not be recovered from the beads, an additional 0.872 mg of OPN had dried onto them. This increased the final density of OPN on the beads to 1.23 μg/mg. SDS–PAGE analysis of the post-coating supernatant confirmed the drop in OPN concentration and showed that the OPN had not suffered proteolytic degradation during the coating process (Fig. 1).

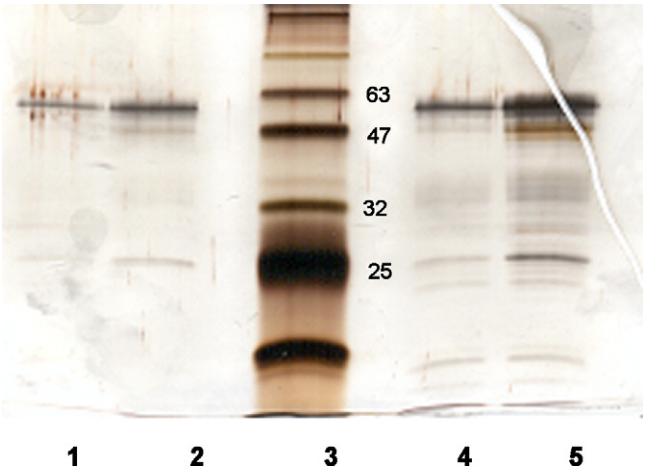

Fig. 1. SDS–PAGE analysis of OPN solution before and after coating onto Interpore-200® granules (silver staining). Lanes 2 and 5: 0.76 and 2.29 μg, respectively of the OPN solution before coating. Lanes 1 and 4: equivalent amounts of the OPN solution after coating (to be compared with lanes 2 and 5, respectively). Lane 3: prestained Mr marker (kDa) (Biolabs).

Circular tablets (diameter 9.9 mm) formed of HA (100 ± 0.5 mg) and coated with OPN were used (OPN-HA); for the control HA group the tablets were not coated with OPN. Fibrin glue (Tissue Coll®) was used to shape the HA granules into tablet form in an attempt to prevent displacement (Fig. 2). Thirty 8-month-old rabbits were divided into six groups of five animals. All the animals were kept according to laboratory animal guidelines. The animals were anaesthetized with Domitor®. Both parietal bones were exposed through a full-thickness median incision. Using a trephine bur designed for this experiment a circular defect of 10 mm was made in each of the parietal bones. The defects were standardized in shape to make comparative measurements reliable. During preparation the dura mater was kept intact, but all bone debris was removed and the surgical site was thoroughly rinsed with saline.

In four rabbits of each group the left and right defects were filled with either OPN-HA or HA, such that each rabbit carried both types of filling. One animal of each group was used as a sham, with the parietal defects not filled with any implant material. The animals of the different groups were killed after 1, 2, 6, 12, 18 and 30 weeks. After killing the animals, the defects with bony surroundings were dissected and transversally cut through the centre. These defects were decalcified and embedded in paraffin. Histological sections of 15 μ were made and stained using haematoxylin and eosin. Haematoxylin and eosin is a common staining method to study decalcified bone matrix formation (osteoid).

The central histological section in each defect was magnified microscopically (×100). The magnified images were scanned digitally. The images were saved in TIF format with an average size of 26 Mb (Fig. 3).

Measurements

Three different measurements were carried out to calculate the amount of new bone formation. The digital pictures were analysed by means of Adobe Photoshop® software (version 7).

For the first measurement, the total healing area was traced manually onto the digital images. The number of pixels within this area with the same colour properties as the intact calcified bone tissue outside the defect zone was calculated as a reference (Fig. 4). By doing so, a bone formation ratio was defined, dividing the amount of calci-