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Densitometry of calus mineralization in a critical size defect of a rabbit radius

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ABSTRACT

This study aims to investigate the use of a photodensitometry to analyze plain radiographic images and correlate them with the rate of new bone formation in a critical size defect of a rabbit radius filled with an autologous omental graft. The computer program MCID Evaluation 7.0. was used for photodensitometric processing of X-ray images taken at 2, 4, 6 and 8 weeks after surgery. The study was conducted on 20 adult New Zealand white rabbits under general anaesthesia, critical-sized osseous defect was created in the right radius and in treated group filled with autogenous omental graft. Optical densitometry of radiographs revealed statistically significant differences between the experimental and control sites. The study showed that autogenous omental grafts promoted healing of the critical-sized defect of the rabbit radius.

Key words: bone, densitometry, omentum, radiogram

Introduction

Bone is a composite material consisting of mineral collagen, water, noncollagenous proteins, lipids, vascular elements, and cells. The absolute amounts of these constituents vary with animal age, sex, tissue site, health, and dietary status. The mineral found in bone is an analogue of the naturally occurring geologic mineral hydroxyapatite $Ca_{10}(PO_4)6(OH)2$ (GLIMCHER, 1998). Various biological and mechanical factors play important roles in the mineralization process of the fracture callus (BUCKWALTER et al., 1995a; BUCKWALTER et al., 1995b). There are many variables that influence callus formation and its initial radiographic appearance. These include: the patient's age, the

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type and site of the fracture, comorbid conditions, management of the fracture, and blood supply (McKINLEY and CHAMBLISS, 2000). Adequate concentrations of calcium and phosphate ions, the presence of a calcifiable matrix and control of regulators is required for mineralization.

On the other hand, angiogenesis is considered essential for proper fracture healing and callus mineralization. Some studies in animal models have showed that the inhibition of angiogenesis can completely prevent fracture healing (HAUSMAN et al., 2001).

It is well known that greater omentum has a great ability in revascularization of tissues. Its use has been reported in complex head and neck reconstruction (LOKSEN et al., 2002), in reconstruction of cervical soft tissue and the oesophagus (MIXTER et al., 1990), in chest wall reconstruction (MANSOUR et al., 2002), in reconstruction of the osteoradionecrosis of the mandible (MORAN and PANJE, 1987), in healing of segmental bone defect in rabbit tibia (KOS et al., 2006), in healing of critical size defect in rabbit radius (KOS et al., 2008). Densitometry gives important information about the healing of bone. Densitometry has proved to correlate with the biomechanical status of the healing callus, at least in the early phase of fracture healing. Densitometry of the healing callus has been performed traditionally by inspecting radiographs; the optical density of a radiograph is theoretically an indirect measure of bone mineral content (BMD), although it involves several limitations (MARKEL and CHAO, 1993). In spite of these limitations, good precision and accuracy have been reported for calibrated radiographic absorptiometry (YANG et al., 1994). Dual-energy X-ray absorptiometry (DXA) is the method of choice for defining BMC more accurately. However, it is also a projectional method, which may have some of the limitations of radiographic photodensitometry. Computed tomography has been applied to the volumetric evaluation of callus size (DEN BOER et al., 1998) and mineral density (MARKEL and CHAO, 1993; REICHEL et al., 1998). Peripheral quantitative computed tomography (pQCT), introduced in 1976, is a method for the assessment of volumetric bone mineral density (BMD), bone mineral content (BMC) and bone cross-sectional geometry (AUGAT et al., 1998). Even though it was developed for human studies, pQCT has also proven an effective and highly precise tool for evaluating the densitometric and geometric properties of bone in experimental animal studies (FERRETTI et al., 2000).

The aim of this study was investigate influence of a free omental graft on callus mineralization by densitometric analysis of radiograms.

Materials and methods

The study was conducted on 20 adult New Zealand white rabbits under general anaesthesia. The critical-sized osseous defect was created in the right radius and filled with an autogenous omental graft. The animals were divided into 2 groups, with 10 animals in each.

The first radiograph of the excavated area was made two weeks postoperatively, in order to monitor the dynamics of creating the callus every two weeks, ending with the eighth week from the operation. All examples were recorded with standard film for screening mammogram from the "Fotokemika" factory. The changes within the area of the macrostructure of the radius bone on both groups of animals were analysed with:

- visual processing of X-ray images
- photodensitometry of X-ray images

The photodensitometry method. The standardization of X-ray images was carried out by adding to the recorded area a material of known density or similar density as the sample being recorded, but of greater density than any other sample. In this way, the material becomes a reference point on X-ray images and the density of other areas can be set in relation to that reference point. In order to be most precise in calculation of the density of the sample, it is important to use many levels of materials of known density as reference points. This material is made of several different levels but of an exact known density and is called a calibration wedge. It is common to use an aluminium calibration wedge for the standardization of X-ray images (ELLWOOD, 1997).

Calibration wedge. A calibration wedge is composed of one or more levels of known density. It is usually set on the lower part of X-ray images or sensors so that it does not cover the bone structures. Therefore, the measured values of the density are in relation with the density of calibration wedge. After recording and developing the X-ray images, we see the differences in brightness between the calibration wedge and X-ray images. It happens because the greater density material absorbs more X-rays and omissions of X-rays to the images are less. When we have determined that the calibration wedge is the brightest on X-ray images it is possible to relate it to other points on X-ray images. Therefore, primarily the grey-levels for other points have to be determined. It is common to use a scale of grey-levels from 0 to 255 where 0 represents the brightest point (the point of greater density of sample or calibration wedge) and 255 represents the darkest point (the point of the least density of sample). Since the scale is defined as such, other densities of other points can be related to the density of calibration wedge. Taking into consideration that the scale of grey-levels is not linear i.e. if one material is twice the density of another one, it does not mean that the grey-level will be half the size. This is a consequence of characteristics of X-ray images where the relationship between the amount of absorbed rays and darkness is not linear. In order to correct it, it is necessary to bring the grey-levels into relationship with the real amount of absorbed X-ray images. This is achieved by transformation of grey-levels into relative optical density.

Photodensitometric processing of X-ray images. The X-ray images obtained were exposed to photodensitometric processing. The computer program MCID Evaluation 7.0. was used for the photodensitometric processing of X-ray images. The bone was densitrometric proximal and distal and one field was in the centre of the defect made. The relative optical (ROD) density was measured at these mentioned points. All obtained results were analysed statistically. The medium values and standard deviations of the optical density of the measuring fields were calculated within the same group, and between the groups. The results are displayed graphically.

Results

Visual evaluation of the radiographs: Radiographic examination identified disturbed healing with pour callus formation in rabbits in the control group after 6 and 8 weeks of the postoperative period. (Figs. 1 and 2). At the first 6 weeks, resorption cavities at the osteotomy gap were evident. Although in the control group not only a lower rate of callus formation than in the treated group was observed, no signs of mineralisation could be detected. In the experimental group, more intensive callus formation was observed by serial radiographs at 6 and 8 weeks of the postoperative period (Figs. 4 and 5). During this time, a mild periostal reaction was also noticed. While these radiographic alterations had a tendency to increase in rabbits of the experimental group at 8 weeks, the size of the callus and its opacity increased. A large amount of callus with bone opacity was seen on radiographs at 8 weeks. Therefore, density occupied the entire area of the defect.

Fig. 1. Radiograph of bone without omentum made six weeks after operation

Fig. 2. Radiograph of bone without omentum made eight weeks after operation

Fig. 3. Radiograph of bone with omentum made six weeks after operation

Fig. 4. Radiograph of bone with omentum made eight weeks after operation

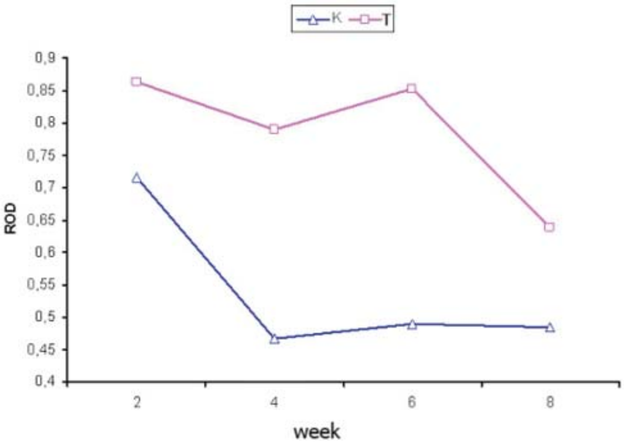

Fig. 5. The statistic notability of the difference between the controlled and treated population grows from the second to the sixth week of observation. During the eighth week no statistically significant difference was found between the control and treated population.

Discussion

Determining the density of bone tissue is important for monitoring the healing and diagnosing and planning changes of bone mass during sickness or treatment (YANG et al., 2002).

The bones absorb 30-40 times more radiation than soft tissue, which is why the density of blackness on the film is considerably smaller than the penetration of the X-ray through soft tissue

The level of mineralization of a callus is determined by densitometry of X-ray images.

The standardization of X-ray images is conducted by adding some material of known density to the recorded area, i.e. approximately the same density as the recorded sample, but always of more density than any other part of the sample. On that way, the material becomes the reference point on X-ray images and we can determine the density of other fields in relation to that reference point. (ELLWOOD et al., 1997; SHROUT et al., 1993).

Radiography is a basic method for evaluating fracture healing, both in clinical use and in animal studies: radiographs are able to visualise callus formation after mineralisation (REICHEL et al. 1998). Radiographs are usually taken immediately after surgery to examine the location of the fracture and the quality of fixation. After the sacrifice of animals, high-resolution X-ray images are usually taken, which can be used for a variety of measurements, such as bone density or bone dimensions. For long bone fractures, the healing parameters, such as periosteal reaction (callus formation), quality of union and bone remodelling, can be quantified on radiographs by different scoring systems (LANE and SANDHU, 1987).

Follow-up radiographs are important to assess proper alignment and adequate healing of a fracture. The formation of a callus is one characteristic used to monitor these features, but the timing and size of its appearance are variable. Therefore, when to expect to see a callus on radiographs requires a general understanding of the fracture healing process and the many variables that can affect callus formation. The callus, or immature bone, results from a precise chronological process (FROST, 1989; ROCKWOOD et al., 1991).

Bone fracture healing is known to be affected by various factors which are still under extensive investigation. Mineralization of the callus is one of the major and crucial steps in this process.

There are two not necessarily mutually exclusive theories concerning how mineral deposition starts at discrete sites in bone. During endochondral ossification, cartilage calcification is widely believed to start within extracellular membrane-bound bodies, known as matrix vesicles (ECMVs) (TAKAHASHI et al., 1998; SIMONET, 1997).

ECMVs released from the chondrocyte, are seen as the site where mineral first appears in the extraterritorial matrix, adjacent to the hypertrophic cells of the calcifying cartilage. ECMVs provide protected areas for the accumulation of mineral ions away from the space-filling proteoglycan aggregates that keep the cartilage matrix hydrated, and, by virtue of their negative charge and bulk, are effective inhibitors of mineral nucleation and growth (TSUDA et al., 1997). ECMVs contain a nucleational core consisting of acidic phospholipids, calcium, inorganic phosphate, and a Ca-transport protein, annexin. This nucleational core is believed to be responsible for membrane associated mineral formation within the ECMVs. The ECMVs also contain enzymes that can modulate inhibitors of mineral formation found in the extracellular matrix. (DEAN et al., 1996; ANDERSON et al., 2004).

Physiological bone mineralization in mammals involves the ordered deposition of apatite on a type I collagen matrix. The bone apatite crystals are always deposited such that their longest dimension lies parallel to the axis of the collagen fibril. The cascade of events includes the formation of that matrix and the oriented deposition of these crystals. (DICKSON et al., 1975).

The simplest way to determine the density of bone mass is with the help of X-ray images. The X-ray images of the healing of bone fractures, i.e. filling the bone flaw, are not constant in all cases; there exist several differences in speed and range of some healing phases which are dependent on several factors. Therefore, we tried in our research to reduce the differences in some factors to the smallest extent, for instance by using animals of equal age and weight. Operating procedures for all animals were observed on the right radius bone in the same area and the same bone defects were made.

Photodensitometry was performed proximally and distally from the defect made and one field in the centre of the defect made. Relative optical density (ROD) was measured at these points.

On the basis of visual observation of X-ray images over a period of eight weeks, the creation can be traced of the callus in the controlled group and the group treated with a self-transplant of greater omentum. It was noticed with visual observation that the process of healing of the critical bone defect was more intensive and of wider range in the treated group than in the control. Also the phenomenon was noticeable of larger callus volume in the treated group at the place where the self-transplant was set during the operating procedure.

Photodensitometric processing clearly confirmed the visual interpretation on the X-ray images. Based on the value of relative optical density, it is possible to observe the period in which the creation or mineralization of the callus is most intensive. Therefore, by observing the total population it was established that affiliation to the treated or controlled group contributed statistically more to the difference in comparison to the time of exposure. The statistical relevance of relative values of optical density between the controlled and treated group rose from the second to the sixth week of observation, while in the period of the eighth week no statistical difference between the groups was found. From these facts it is clear that in the treated group the mineralization of the callus was more intensive in the period from four to six weeks, and during the last two weeks the level of mineralization was low in both groups. Under normal conditions, one should expect bony callus formation to show radiographically anywhere between 7 days to 4 weeks. Most authorities suggest taking follow-up radiographs in the first 1 to 2 weeks to assess for alignment and radiographic evidence of healing. After 4 weeks, if no bony callus is evident, suspicion should be raised about delayed union or non-union (McKINLEY, 2000)

References:

ANDERSON, H. C., J. B. SIPE, L. HESSLE, R. DHANYAMRAJU, E. ATTI, N. P. CAMACHO, J. L. MILAN (2004): Impared calcification around matrix vesicles of growth plate and bone in a alkaline phosphatase deficient mice. Am. J. Pathol. 164, 841-847.

AUGAT, P., C. GORDON, T. F. LANG, H. IIDA, H. K. GENANT (1998): Accuracy of cortical and trabecular bone measurements with peripheral quantitative computed tomography (pQCT). Phys. Med. Biol. 43, 2873-2888.

BUCKWALTER, J. A., M. J. GLIMCHER, R. R . COOPER (1995a): Bone biology (part I): structure, blood supply, cells, matrix and mineralization. J. Bone Joint Surg. Am. 77, 1256-1275.

BUCKWALTER, J. A., M. J. GLIMCHER, R. R. COOPER (1995b): Bone biology (part II): formation, form, modeling, remodeling, and regulation of cell function. J. Bone Joint Surg. Am. 77, 1276-1289.

DEAN, D. D., B. D. BOYAN, O. E. MUNIZ, D. S. HOWELL, Z. SCHWARTZ (1996): Vitamin D metabolites regulate matrix vesicle metalloproteinase content in a cell maturation-dependent manner. Calcif. Tissue Int. 59, 109-116.

DEN BOER, F. C., J. A. M. BRAMER, P. PATKA, F. C. BAKER, R. H. BARENTSEN, A. J. FEILZER, E. S. M. de LANGE, H. J. T. M. HAARMAN (1998): Quantification of fracture healing with three-dimensional computed tomography. Arch. Orthop. Trauma Surg. 117, 345-350.

DICKSON, I. R., E. A. MILLAR, A. VEIS (1975): Evidence for abnormality of bone-matrix proteins in osteogenesis imperfecta. Lancet 2, 586-587.

ELLWOOD, R. P., R. M. DAVIES, H. V. WORTHINGTON (1997): Evaluation of dental subtraction radiography system. J. Periodontal. Res. 32, 241-248.

FERRETTI, J. L., R. F. CAPOZZAL, G. R. COINTRY, R. CAPIGLIONI, E. J. A. ROLDAN, J. R. ZANCHETTA (2000): Densitometric and tomographic analyses of musculoskeletal interactions in humans. J. Musculoskel. Neuron. Interact. 1, 31-34.

FROST, H. M. (1989): The biology of fracture healing: an overview for clinicians: part I. Clin Orthop. 248, 283-293.

GLIMCHER, M. J. (1998). The nature of the mineral phase in bone: biological and clinical implications. In: Metabolic Bone Disease and Clinically Related Disorders (Avioli L. V., S. M. Krane, Eds.), Academic Press, San Diego, pp. 23-50.

HAUSMAN, M. R., M. B. SCHAFFLER, R. J. MAJESKA (2001): Prevention of fracture healing in rats by an inhibitor of angiogenesis. Bone 29, 560-564.

KOS, J., V. NADINIĆ, D. HULJEV, I. NADINIĆ, J. TURČIĆ, D. KOŠUTA, T. ANIĆ, T. BABIĆ, D. VNUK, M. KRESZINGER, O. SMOLEC (2006): Healing of bone defect by application of free transplant of greater omentum. Vet. arhiv 76, 367-379.

KOS, J., O. SMOLEC, D. KRPAN, T. BABIĆ, D. VNUK, M. KRESZINGER, B. PIRKIĆ, K. HOCK (2008): Healing of critical size defect on diaphiseal bone in rabbit by using free omental graft. Bone 43 (S1), p.78.

LANE, J. M., H. S. SAHDHU (1987): Current approaches to experimental bone grafting. Orthop. Clin. North Am. 18, 213-225.

LOKSEN, A., G. W. CARISON, J. H. CULBERSTON, C. SCOTT HULTMAN, A. V. KUMAR, G. E. JONES, BOSTWICK III, M. J. JURKIEWICZ (2002): Omental free flap reconstruction in complex head and neck deformities. Head Neck 24, 326-331.

MANSOUR, K. A., V. H. THOURANI, A. LOKSEN, J. G. REEVES, J. I. MILLER Jr., G. W. CARLSON, G. E. JONES (2002): Chest wall resection and reconstruction: a 25-year experience. Ann. Thorac. Surg. 37, 1720-1726.

MARKEL, M. D., E. CHAO (1993): Noninvasive techniques for quantitative description of callus mineral content and mechanical properties. CORR Journal 293, 9-15.

McKINLEY, D. W., M. L. CHAMBLISS (2000): Follow-up radiographs to detect callus formation after fractures. Arch. Fam. Med. 9, 373-374.

MIXTER, R. C., V. K. RAO, J. KATSAROS, J. NOON, E. TAN (1990): Simultaneous reconstruction of cervical soft tissue and esophagus with a gastro-omental free flap. Plast. Reconstr. Surg. 86, 905-908.

MORAN, W. J., W. R. PANJE (1987): The free greater omental flap for treatment of mandibular osteoradionecrosis. Arch. Otolaryngol. Head Neck Surg. 113, 425-427.

REICHEL, H., S. LEBEK, C. ALTER, W. HEIN (1998): Biomechanical and densitometric bone properties after callus distraction in sheep. CORR Journal 357, 237-246.

ROCKWOOD, C. A., D. P. GREEN, R. W. BUCHOLZ (1991): Healing of musculoskeletal tissues. In: Rockwood and Green's Fractures in Adults. 3rd ed. Philadelphia, Pa: JB Lippincott.

SIMONET, W. S. (1997): Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89, 309-319.

SHROUT, M. K., B. J. POWELL, C. F. HILDEBOLT, M. W. VANNIER, N. M. AHMED (1993): Digital radiographic image-based bone level measurements:effect of film density. J. Clin. Periodontol. 20, 595-600.

TAKAHASHI, N., H. YAMAHA, S. YOSHIKI, G. D. ROODMAN, G. R. MUNDY, S. J. JONES, A. BOYDE, T. SUDA (1988): Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 122, 1373-1382.

TSUDA, E., M. GOTO, S. MOCHIZUKI, K. YANO, F. KOBAYASHI, T. MORINAGA, K. HIGASHIO (1997): Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem. Biophys. Res. Commun. 234, 137-142.

YANG, S. O., S. HAGIWARA, K. ENGELKE, M. S. DHILLON, G. GUGLIELMI, E. J. BENDAVID, O. SOEJIMA, D. L. NELSON, H. K. GENANT (1994): Radiographic absorptiometry for bone mineral measurement of the phalanges: precision and accuracy study. Radiology 192, 857-859.

YANG, J., R. CHIOU, A. RUPRECHT, J. VICARIO, L. A. MacPHAIL, T. E. RAMS (2002): A new device for measuring density of jay bones. Dentomaxillofacial Radiology 31, 313-316.

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SAŽETAK

Istražena je upotreba fotodenzitometrije za analizu rengenograma u usporedbi sa stopom novostvorene kosti u kritičnom defektu kunićje palčane kosti ispunjene autolognim presatkom velikoga omentuma. Računalni program MCID 7.0. rabljen je za obradu rengenograma koji su snimljeni 2, 4, 6 i 8 tjedana nakon operacije. Istraživanje je provedeno na 20 odraslih novozelandskih kunića u općoj anesteziji. Denzitometrija rengenograma pokazala je statistički značajne razlike između pokusne i kontrolne skupine. Istraživanje je pokazalo da autologni presadak omentuma pospješuje cijeljenje kritičnoga defekta kunićje palčane kosti.

Ključne riječi: kost, denzitometrija, omentum, rendgenogram