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Role of oxidative and nitrosative stress in autogenous bone grafts to the mandible using Guided Bone Regeneration and a Deproteinized Bovine Bone Material

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ABSTRACT

The aim of this study was to evaluate the role of oxidative and nitrosative stress in autogenous bone grafts to the mandible based on immunohistochemical analysis.
Material and methods: Using a well-established sheep model autogenous bone grafts were harvested form the iliac bone. A combination of a Collagen Membrane (CM) and Deproteinized Bovine Bone Material (DBBM) was used to cover the bone graft (Experiment 2). This modification was compared with simple onlay bone grafts (Experiment 1). Immunohistochemically, the expression of specific stable degradation products of oxidative and nitrosative stress was compared between the two experimental groups.
Results: Specific markers for oxidative and nitrosative stress showed statistically significant differences in expression in the different experimental groups. The influence of oxidative and nitrosative stress on osteoblasts (OB), osteoclasts (OC), and osteocytes (OCy) was analysed. Experiment 2 showed increased expression of markers in OB and decreased expression in OC.
Conclusions: Taking the result of this study and reports from the literature into consideration grafts in Experiment 2 showed less resorption and atrophy, higher activity of OB and inhibition of OC, and less expression of Reactive Oxygen and Nitrogen Species (RONS) as markers of oxidative stress within the graft. These data illustrate the improved remodelling processes in grafts using CM and DBBM.

1. Introduction

The loss or deficiency of bone because of trauma, tumour, infections or syndromes is a common situation in oral and maxillofacial surgery. Adequate bone volume is an indispensable prerequisite for the structural and functional rehabilitation of patients. These indications have been expanded using bone tissue transplants prior to the placement of endosseous implants. The transplantation of autogenous bone is still considered to be the gold standard in reconstructive surgery due to its osteogenic properties and its immunological inertness (Bauer and Muschler, 2000; Khan et al., 2005).

The successful incorporation of autogenous bone grafts depends on mechanical stability within the defect, the size of the defect, and the quality of the host side as well as the surrounding soft tissues. Bone grafts undergo unpredictable resorption and structural collapse during the process of remodelling. Authors describe resorption rates of up to 70 % in non-fixed autogenous bone grafts (Maiorana et al., 2005).

Modifications of the host side such as Guided Tissue Regeneration (GTR) is commonly used to cope with the problem of the graft's resorption (Donos et al., 2005; Jardini et al., 2005; Sculean et al., 2000). The concept of GTR uses different membranes, which act as barriers and prevent undesired cells to migrate into the defect (Gassling et al., 2013; Karring et al., 1993). As a

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continuative development of GTR, Guided Bone Regeneration (GBR) employs the concept of GTR (Dahlin et al., 1991; Jovanovic et al., 1992; Kostopoulos and Karring, 1994; Lundgren et al., 1992; Simion et al., 1994). In oral surgery the use of GBR in a clinical setting does not always result in a predictable bone fill to the defect (Jovanovic et al., 1992; Kostopoulos and Karring, 1994; Lundgren et al., 1995; Simion et al., 1994).

The combination of autogenous bone grafts with bone substitution materials such as Deproteinized Bovine Bone Material (DBBM) shows less resorption and a retained dimension of the transplant (Araujo et al., 2002; Maiorana et al., 2005). DBBM is biocompatible and has osteoconductive properties, making it ideal to be used in augmentation procedures (Chaves et al., 2012; Kolk et al., 2012; Schlegel et al., 2003).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as the superoxide anion (O_2^-), hydroxyl radical ($OH^\bullet$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^\bullet$), and peroxynitrite anion ($ONOO^-$) are highly reactive, and therefore potentially harmful (Davies, 1995; Droge, 2002; Valko et al., 2007). Endogenous and exogenous factors lead to the activation of signal cascades and transcription factors resulting in senescence and apoptosis (Valko et al., 2007). Due to short half life period and extreme responsiveness, a direct analysis of reactive oxygen and nitrogen species (RONS) is not possible (Droge, 2002). Stable degradation products are used for immunohistochemical analysis.

1.1. 3-Nitrotyrosine

Nitrotyrosine is a product of tyrosine nitration mediated by $ONOO^-$ and O_2^-. Newly formed stable nitrotyrosinated proteins can be detected immunohistochemically as 3-nitrotyrosine. As the nitration of tyrosine is mediated by RNS, 3-nitrotyrosine is considered to be a biomarker of nitrosative stress and oxidative protein damage. Along with others, nitrotyrosine has been found to be elevated in atherosclerosis, lung disease, inflammatory diseases, and post transplantation (Mohiuddin et al., 2006; Shigenaga et al., 1997; Skinner et al., 1997).

1.2. 8-Isoprostane

8-isoprostane is a stable isomer of prostaglandin-like compounds formed *in vivo* by lipid oxidation catalysed by free radicals (Liu et al., 1998; Morrow et al., 1990). 8-isoprostane can also be found as a side-product of prostaglandin H_2-synthase (PGH_2-synthase) by cyclooxygenase (COX). It is considered to be a very stable and specific marker for oxidative stress (Lehnig, 1999; Longmire et al., 1994; Morrow et al., 1990). Moreover, 8-isoprostane shows direct biological activity and acts as a highly potent vasoconstrictor and as a platelet thromboxane/endoperoxide receptor antagonist (Kromer and Tippins, 1998; Morrow et al., 1992).

1.3. P-ERK

Phosphorylated extracellular signal-regulated kinase (p-ERK) belongs to the mitogen-activated protein kinases (MAPK). MAPK cascade is a key intracellular pathway regulating cellular functions, such as proliferation, migration, angiogenesis, and cell survival and can be activated either receptor-mediated or by RONS H_2O_2 and $ONOO^-$. High levels of p-ERK protein expression have been found in various types of cancers, such as melanomas, gliomas, bladder, endometrial, and esophageal cancer (El-Habr et al., 2010; Puhringer-Oppermann et al., 2007; Tasioudi et al., 2012).

As shown elsewhere, p-ERK and p-ERK induced oxidative stress is responsible for the inhibition of osteoblastic differentiation (Bai et al., 2004).

1.4. P-AKT

Phosphorylated AKT also known, as Protein Kinase B (PKB) is such as ERK a serine/thyreonine-specific protein kinase. P-AKT is involved in the PI3K-pathway (Phosphoinositid-3-Kinase) and can be activated by heat shock, hypoxia, UV- and γ-radiation, and oxidative stress (H_2O_2) (Chen et al., 2001; Crossthwaite et al., 2002; Konishi et al., 1996; Shaw et al., 1998). AKT regulates fundamentally important cellular functions such as proliferation, cell survival, glucose balance and protein synthesis. Moreover, endothelial nitric oxide synthase (eNOS) as an important molecule mediating angiogenesis is activated by AKT (Zeng et al., 2000).

PI3K/AKT-pathway and MAPK/ERK-pathway both build a complicated network, which regulates cell proliferation and apoptosis significantly.

Our own research suggests the superiority of autogenous bone grafts in combination with GBR and a DBBM in terms of reduced resorption and better revascularization within the bone graft (Adeyemo et al., 2008a, b; Koerdt et al., 2013). The aim of the present study is to illustrate the role of oxidative and nitrosative stress in mandibular augmentation analysing PI3K/AKT-pathway and MAPK/ERK-pathway as well as 3-nitrotyrosine and 8-isoprostane as direct biomarkers of oxidative stress.

2. Materials and methods

Twelve adult female sheep were used for this study (mean weight and Standard Deviation (SD), 73.6 ± 8.6 kg, range from 63 to 90 kg). Local authorities approved all experimental procedures. The animals were randomized into four groups of three animals each, based on the time there were euthanized: 4, 8, 12, or 16 weeks after surgery.

2.1. Harvesting and transplantation of iliac bone graft

All experimental procedures were performed by one of the investigators and followed a standardized protocol.

Under general anaesthesia (2% propofol i.v.), a bi-cortical bone graft (2.0 × 2.0 × 1.5 cm) was harvested from the iliac bone of each sheep (Fig. 1). The harvested corticocancellous graft was then divided into two equal parts (1.0 × 2.0 × 1.5 cm), following by splitting into two mono-cortical grafts each (1.0 × 2.0 × 0.75 cm). For all experiments the lateral surface of the mandible was carefully exposed through an extraoral approach without perforation of the oral cavity (Fig. 1). Each sheep received the three experimental grafts on the lateral surface of the mandible (Fig. 1). Surgical wounds were closed in layers with interrupted resorbable sutures (Vicryl® 2.0, Ethicon, Norderstedt, Germany). All procedures were carried out in a strict aseptic technique. All animals were treated with prophylactic antibiotics (penicillin-dihydrostreptomycin, ani-Medica, Germany), started during the surgery process and continued for at least 3 days after surgery. The animals also received analgesic treatment (Carprofen, Pfizer, Germany) for 3 days postoperatively to avoid pain associated with the surgical procedure.

2.2. Experiment 1

The cancellous surface of a mono-cortical bone graft was placed tightly against the mandibular cortical bone and fixed with two titanium screws (1.0 × 12 mm). The screws were inserted into the graft until the under surface of the screw heads came into contact with the outer surface of the graft (Fig. 2).