The Role of Simvastatin in Improving Bone Healing in Surgically Created Bony Defects: An Experimental Study

Thesis submitted to the Faculty of Dentistry, Ain Shams University for partial fulfillment of the requirements of master degree in Oral and *Maxillofacial Surgery*

By

Ahmed Mohamed Bahaa El-Din Abou El-kassem Ahmed

B.D.Sc. (2009)

Ain Shams University

Supervisors

Prof.Dr. Mohammad Diaa Zein El-Abedien Ismaiel

Professor of Oral and Maxillofacial Surgery
Faculty of Dentistry

Ain Shams University

Assoc.Prof.Dr. Heba Abdel Moniem Abdel Rahman Gad

Associate Professor of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmacy

Ain Shams University

Faculty of Dentistry
Ain Shams University

(2018)

“This thesis was a part of an experimental project conducted to assess the effect of the implantation of simvastatin on bone healing and bone morphogenic protein expression in surgically created defects in long bones of rabbits. This project included the thesis of my dear colleague, Dina Abd El Aziz Metwally.”

Dedication

This work is dedicated to

My Dear parents

The light that leads my way

My true friends for their encouragement and cooperation

Acknowledgment

Special thanks are due to Mahmoud Yehia, Mahmoud Abdelaziz, Omnia Adel, Yasser El Hadidy, Hossam El-Deen Hany and Aseel Asar for their support and help with this work,

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Finally, I must express my very profound gratitude to all my professors, mentors at the department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Ain Shams University and to my colleagues and the younger generation of surgeons at the department, for their support and encouragement throughout my years of study and through the process of researching and writing this thesis.

Thank you

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List of Abbreviations

Item	Abbreviation
(rhFGF-2)	recombinant human fibroblast growth factor-2
(HMG COA)	3-hydroxy-2-methyl-glutaryl coenzyme A
BMP	bone morphogenetic proteins (BMPs),
(LMHFV)	Low-magnitude high-frequency vibration
BMD	bone mineral density
FGF-1	fibroblast growth factor-1
RANK	Receptor Activator of Nuclear Factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
ACS	atelocollagen sponge
rhBMP-2	recombinant human BMP-2
SLN	solid lipid nanoparticles
EDTA	Ethylene diamine tetra-acetic acid
CBCT	Cone beam computed tomography

Osseous reconstruction of bony defects caused by cyst, neoplasm, trauma, infection and congenital defects is considered a challenge for maxillofacial surgeons. The aim of reconstruction is to restore continuity, shape and strength of the jaws achieving better life for the patients.

The healing process initiates an orderly but complex sequence of events that re-establish the integrity of the damaged tissues. If the result of the healing process is the formation of tissue that is structurally and functionally the same as the original tissue; then regeneration has taken place successfully. However, if healing resulted in formation of fibrous connective tissue or scar; then repair has occurred. Whereas a fibrous scar may be normal for soft tissue healing, it is suboptimal in the case of bone healing.

Bone healing is a complex dynamic process that can restore the shape, function and the mechanical integrity of the injured tissues to the pre-injured condition. Primary bone healing occurs in cases of extreme stability and negligible gap size, involving a direct attempt by the bone to form itself directly, while secondary bone healing occurs when there is no enough stabilization and gap size is moderate. Secondary bone healing has a series of sequential stages that can overlap to a certain extent, including inflammation, callus differentiation, ossification and remodeling. [(2)]

There is an interest in treatments that could enhance the rate of bone healing, providing a shorter rehabilitation time and a more rapid return to an active life style. Bone grafting and several osteoinductive factors, including bone morphogeneic protein, transforming growth factor β 1,2, activin, recombinant human fibroblast growth factor-2 (rhFGF-2) are locally applied to enhance bone healing. Also 1,2 dihydroxy vitamin D3, growth hormone, parathyroid hormone and some other compounds are considered as systemic anabolic agents that could enhance bone healing. [3]- [6]

A growing number of clinical studies have demonstrated the efficacy of statins as a promoting agent for bone healing. Statins are specific competitive inhibitors of 3-hydroxy-2-methyl-glutaryl coenzyme A (HMG COA) reductase and include lovastatin, simvastatin, pravastatin, atorvastatin, fluvastatin and cervistatin. All of these agents are widely used to lower cholesterol level providing an important approach toward the treatment of hyperlipidemia and arteriosclerosis. There are many studies that revealed that statins have an anabolic bone effect when it is used systemically or locally. [20] [22] [25]

Local statins application presented a major therapeutic advantage by delivering a higher drug concentration locally and preventing systemic side effects as myalgia, rhabdomyolysis,

liver failure and kidney failure. As a result, researchers are now investigating the possibility of incorporating statins into scaffolds for local delivery in treatment of bone defects. [40] [43]

This study was conducted to evaluate histologically and radiographically the bone healing after implantation of simvastatin in surgically created defects in long bones of rabbits.

Bone healing is a complex dynamic process that involves the migration, differentiation and proliferation of the inflammatory cells, angioblasts, fibroblasts, chondroblasts and osteoblasts. There are two types of bone healing: primary and secondary. In contrast to secondary intentional healing of the soft tissue, secondary bone healing can regenerate the injured bone to its pre-injury state. Secondary bone healing has a series of sequential stages that can overlap to a certain extent, including inflammation, callus differentiation, ossification and remodeling. (1)

In the first stage (the inflammatory stage), blood eminates from the ruptured vessels and a blood clot is formed throughout the defect acting as a scaffold for the migrating inflammatory cells. This is followed by an inflammatory process with the presence of macrophages, neutrophils and monocytes and formation of granulation tissue for the migration of undifferentiated mesenchymal cells. In the next stage, the mesenchymal cells may differentiate into chondrocytes, osteoblasts or fibroblasts, depending on the biological and mechanical conditions. These differentiated cells begin to synthesize the extracellular matrix of their corresponding tissue and the formation of the cartilaginous soft callus is initiated. (2)

Once the callus is formed, endochondral ossification begins; where, the cartilage is replaced by woven immature bone. The

ossification continues until all the cartilaginous soft callus has been replaced by immature woven bone. This typically occurs within three to four weeks. Once the bony defect has been ossified, bone remodeling begins gradually in order to restore the shape, form and internal structure to the pre-injured state; resulting in conversion of the woven bone to mature lamellar bone. [2]

Several local and systemic factors can affect the physiologic process of bone healing. Size of the defect, blood supply, bone stock, soft tissue scarring, infection, prior radiation therapy are examples of local factors that can affect bone healing. [3] [4]

On the other hand, patient's age, the nutritional status, smoking, vitamin D and calcium deficency, osteoporosis, diabetes and some medications as corticosteroids are considered as systemic factors that can affect bone healing too. [3] [4] [5] [6]

Despite the biologically optimized nature of the repair process, patients still require substantial time before bone restores its original form, structure and function. Consequently, there is an interest in treatments that could enhance the rate of repair, providing a shorter rehabilitation time and a more rapid return to an active lifestyle and work and overcome the previously mentioned systemic and local factors that affect this optimal repair.

Numerous techniques have been advised to improve bone healing. Autogenous bone grafting is considered to be the gold standard due to its osteogenic, osteoinductive and osteoconductive properties. However, due to many drawbacks such as donor-site morbidity, vital structure injury along with the limited amount; a variety of bone graft substitutes with osteoinductive or osteoconductive properties and other biologically based strategies, including electrical, ultrasound, and shockwave stimulation, have been developed over the years. [4] [5]

Many osteoinductive factors that are applied either locally or systemically, including bone morphogenic proteins (BMPs), platelet-derived growth factors, and parathyroid hormone have been used to improve bone healing. [3]

Physical means for bone healing enhancement:

Low-magnitude high-frequency vibration (LMHFV) is one of the physical means that has been proven to be osteogenic in osteoporotic intact bone. LMHFV is a form of biophysical stimulation which has been introduced as a non-pharmacological intervention for the treatment of osteoporosis, with proven positive effects on bone mineral density (BMD) and blood circulation in limbs. Shi et al. [4] proved that LMHFV was able to promote fracture healing in osteoporotic bone in rats by enhancing callus formation, remodeling, and mineralization. Radiographic and histomorphometric assessment revealed that LMHFV