Cairo University
Faculty of Veterinary Medicine
Department of Surgery, Anesthesiology and Radiology

Simvastatin as a Growth Promoting Material on the Healing of Critical Sized Bone Defect in Dogs

(An Experimental Study)

Thesis presented by

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Key words: Critical sized bone defect, Simvastatin, healing, dogs, osteopontin, Alizarine, Masson Trichrome.

Abstract

Critical size defect represents a major challenge in orthopedic surgery. The aim of the work was decided to study the effect of locally applied simvastatin as a growth promoting material on the healing of experimentally induced critical size bone defect in dogs.

A prospective experimental study was done on 18 skeletally mature male mongrel dogs. A 21 mm mid-shaft femoral defect was induced in all dogs. Dogs were randomly allocated into one of the two groups; group 1:- simvastatin was locally applied into the defect site, group 2:- defects were kept without simvastatin (sham-operated group). All defect sites were stabilized using bone plate and screws. Dogs were evaluated clinically and radiographically every two weeks till 12 weeks. The dogs were euthanized at 4,8 and 12 weeks for gross pathologic and histopathologic examination (Hematoxyline & Eosin, Masson Trichrome, Alizarine red and immune histochemical evaluation (osteopontin).

Simvastatin resulted in acceleration of bone healing on the induced critical size defect, the defects healed in a consistent pattern of consolidation, incorporation and remodeling . the femoral cortex opposite the bone plate demonstrated most mature remodeling, evident both radiographically as well as hitopathologically.

Local application of simvastatin may be advised for acceleration of bone healing in critical bone defects otherwise the empty critical defect resulted in an atrophic nonunion.

"DEDICATION"

To my parents.

To my siblings

Amani M. Nagi

Amani M. Nagi

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*First and foremost, I thank **ALLAH** for giving strength to complete my young dream and for giving me happiness to finish the work.*

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List of abbreviations

Abbreviation	Denote
ALP	Alkaline phosphatase
BMD	Bone mineral density
BMPs	Bone morphogenetic proteins
Cbfa 1	Core- binding factor 1
Cm	Centimeter
CPR	C- reactive protein
Cyr 61	Cysteine- rich 61
DCP	Dynamic compression plate
DJD	Degenerative joint disease
DNA	Dinucleic Acid
ECs	Endothelial cells
EDTA	Ethylene diamine tetra-acetic acid
eNOS	Endothelial nitrous oxide synthesase
FFD	Focal film distance
FGFs	Fibroblast growth factors
G	Gauge
H&E	Hematoxyline & Eosin
HMG-CoA	Hydroxymethylglutaryl- CoA
I.M.	Intramuscular
I.V.	Intravenous
IACUC	Institutional Animal Care and Use Committee
IGF	Insulin- like growth factors
IL	Interleukin
iNOS	Inducible nitrous oxide synthesase
Kg	Kilogram
kVp	Kilo voltage
LDL	Low-density lipoproteins
LPS	Lipo-poly saccharide

mAs	Milliampair second
Max	Maximum
MCP-1	Monocyte chemotactic protein-1
Mg	Milligram
Min	Minimum
Mm	Millimeter
MMP-9	Matrix metalloproteinase-9
mRNA	messenger Ribonucleic acid
MSCs	Mesenchymal stem cells
MTC	Masson Trichrome
NO	Nitric oxide
NRS	Numerical Rating Scale
OPG	Osteoprotegerin
PDGF	Platelet-derived growth factor
RANK	Receptor activator of nuclear factor kappa β
RANKL	Receptor activator of nuclear factor kappa β ligand
S	Second
SD	Standard deviation
SIM	Simvastatin
TCP	Tricalcium phosphates
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
VEGFs	Vascular endothelial growth factors

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Introduction

A large segmental long bone defect constitutes a challenging problem in orthopedic surgery. Although bone tissue usually heals spontaneously, but in complicated conditions such as pathological fractures or those situations leading to large bone defects, the healing process fails **(Johnson, Austin and Breur, 1994).**

Numerous surgical techniques have been advocated, to enhance the healing and fill the bone defects. Among considerations are restoration of skeletal continuity with biologically and biomechanically sound bone; the early return to unrestricted limb function, minimal patient's compromise, and/or compliance throughout the course of the treatment without the need for specialized surgical skills and/or equipment. Therefore, the optimal reconstruction modality for large segmental defects in long bones has yet to be established **(Oryan, Alidadi and Moshiri, 2013).**

Different types of glycosaminoglycans, growth factors, stem cells, natural grafts (auto-, allo- or xenograft) and biologic- and synthetic- based tissue engineered scaffolds are some of the examples **(Oryan, *et al.*, 2013)**.

Mundy, Garrett, Harris , *et al.*, 1999 who mentioned firstly tested the effects of more than 30.000 compounds on bone formation and found that the addition of statins, including simvastatin almost 50% increase new bone formation in rats through enhancing the expression of bone Morphogenetic Protein-2 (BMP-2) gene mRNA.

Statins are potent compound widely used for inhibiting hepatic cholesterol biosynthesis by blocking 3-hydroxy-3-methylglutaryle coenzyme A (HMG-CoA), a key enzyme in the cellular mevalonate pathway **(Goldstein and Brown, 1990).**

Subsequent promising clinical and experimental studies were done on the effect of simvastatin on bone formation with significant radiographic, mechanical, biochemical and histological differences in fracture healing. **(Nyan, Sato, Oda, *et al.*, 2007; Skoglund and Aspenberg, 2007; Wang, Xu, Yang and Lv, 2007; Ayukawa, Yasukawa, Moriyama, *et al.*, 2009; Liu, Wu and Sun, 2009).**