

Modulatory Effects of Zoledronic Acid on Methylprednisolone-Induced Osteoporosis of Alveolar Bone in Albino Rats

(Histomorphometric and Immunohistochemical study)

Thesis

Submitted for partial fulfilment of the requirements of
Doctorate Degree in Oral Biology

By

Dina Mohamed Abdel-Khalek

M.Sc (Ain Shams University-2010)

B.D.S (Ain Shams University-2002)

*Faculty of Dentistry
Ain Shams University
2012*

Under Supervision of

Prof. Dr. Medhat A. El-Zainy

Professor of Oral Biology

Former Vice Dean

Faculty of Dentistry – Ain Shams University

Co-Supervisor

Dr. Reham Magdy Amin

Associate Professor of Oral Biology

Faculty of Dentistry – Ain Shams University

*Faculty of Dentistry
Ain Shams University
2012*

()

Acknowledgement

*Endless grateful thanks to Allah
for helping me to finish this work,*

*First of all, I'd like to express my deep gratitude and appreciation to **Prof. Dr. Medhat Ahmed El-Zainy**, Prof. of Oral Biology and former vice dean, Faculty of Dentistry, Ain Shams University for his extremely precious help, continuous support and valuable guidance throughout this work and since I was a first year student and until today.*

*I am also equally thankful to **Ass. Prof. Dr. Reham Magdy Ameen**, Ass. Prof. of Oral Biology, Faculty of Dentistry, Ain Shams University for her effort, time and indeed valuable notices during all the different stages of this work,*

*And never forget my endless thankfulness to **Prof. Dr. Souzi Farid Shinashin**, head of Oral Biology Department, Faculty of Dentistry, Ain Shams University, for her immeasurable backing & encouragement.*

Sincere thanks are also extended to all my senior staff and colleagues in the Oral Biology Department for their valuable remarks and faithful cooperation.

Dedication

To my Family

Contents

<i>Subjects</i>	<i>Page No.</i>
List of Abbreviations	i
List of Figures.....	ii
List of Tables	vii
Introduction.....	1
Review of Literature	3
Aim of the Work.....	33
Materials and Methods.....	34
Results	40
Discussion.....	112
Conclusions	121
Summary.....	123
References	130
Arabic summary.....	—

List of Abbreviations

AGA	: Arginine-Glycine-Aspartate
BMD	: Bone mineral density
CC	: Cutting cone
DEXA	: Dual energy Xray absorptiometry
FC	: Filling cone
GC	: Glucocorticoids
H&E	: Hematoxylin and Eosin
IAP	: inhibitor of apoptosis protein
IGF-I	: Insulin-like growth factor I
Mpsl	: Methylprednisolone
MT	: Masson Trichrome
NIH	: National Institute of Health
PBS	: Phospate buffer saline
PDL	: Periodontal ligament
TBS	: Tris buffered saline
ZOL	: Zoledronic acid

List of Figures

<i>Figure</i>	<i>Title</i>	<i>Page No.</i>
Fig (A):	Molecular formula of Bisphosphonates and Pyrophosphates	5
Fig (B):	Molecular formula of Zoledronic acid	7
Fig (C):	Molecular formula of Methylprednisolone.	11
Fig (D):	The role of caspase-3 in apoptosis	31
Fig. (1):	A photomicrograph of the control group showing alveolar bone proper (H&E Org. mag. X100)	42
Fig. (2):	A photomicrograph of the control group showing smooth alveolar bone proper, Zucker kandle & Hershfeld canal. (H&E X200)	42
Fig. (3):	A photomicrograph of the control group showing cortical plate and spongiosa (H&E Org. mag. X100)	43
Fig. (4):	A photomicrograph of the control group showing the spongy bone (H&E Org. mag. X400)	43
Fig. (5):	A photomicrograph of the control group showing the compact cortical bone (H&E Org. mag. X400)	45
Fig. (6):	A photomicrograph of the control group showing the spongy bone with resting lines (H&E Org. mag. X200)	45
Fig. (7):	A photomicrograph of the control group showing osteoblasts lining the bone surface (H&E Org. mag. X1000)	45
Fig. (8):	A photomicrograph of the control group showing osteocytes in lacunae (H&E Org. mag. X1000)	45
Fig. (9):	A photomicrograph of the control group showing mononucleated and multinucleated osteoclasts (H&E Org. mag.X1000)	46
Fig. (10):	A photomicrograph of the control group showing reversal lines (H&E Org. mag. X200)	46
Fig. (11):	A photomicrograph of a marrow cavity in the control group (H&E Org. mag.X400)	47

Fig. (12):	A photomicrograph of of group IIA showing an uneven socket wall (H&E Org. mag.X100)	49
Fig. (13):	A higher magnification of the previous fig. detached osteoblasts (H&E Org. mag. X200)	50
Fig. (14):	A photomicrograph of group IIA showing flattened osteoblasts (H&E X1000)	50
Fig. (15):	A photomicrograph of group IIA showing periodontal ligament space (H&E Org. mag.X200)	51
Fig. (16):	A photomicrograph of group IIA showing periodontal ligament with dilated and congested blood vessels (H&E Org. mag. X400)	51
Fig. (17):	A photomicrograph of interradicular region of group IIA (H&E Org. mag.X100)	52
Fig. (18):	A photomicrograph of bone trabeculae of group IIA showing thin and disorganized trabeculae (H&E Org. mag. X200)	52
Fig. (19):	A photomicrograph of group IIA showing most osteocytic nuclei (H&E Org. mag. X400)	53
Fig. (20):	A photomicrograph of group IIA showing hyperchromatic osteocytic nuclei and multiple reversal lines (H&E Org. mag.X400)	53
Fig. (21):	A photomicrograph of group IIA showing osteoclasts (H&E Org. mag. x1000)	54
Fig. (22):	A photomicrograph of group IIA showing a marrow cavity (H&E Org. mag.X100)	54
Fig. (23):	A photomicrograph of group IIA showing numerous reversal lines (H&E Org. mag. X200)	55
Fig. (24):	A photomicrograph of group IIA showing resting lines and wide vascular canals (H&E Org. mag. X100)	55
Fig. (25):	A photomicrograph of subgroup IIA showing a smooth and regular socket wall (H&E Org. mag.X100)	58
Fig. (26)	A photomicrograph of subgroup IIA showing osteoblasts lining the socket wall (H&E Org. mag.X X200)	58
Fig. (27):	A photomicrograph of cortical bone of subgroup IIA (H&E Org. mag.X400)	59

Fig. (28):	A photomicrograph of subgroup IIA showing flattened osteoblasts. (H&E Org. mag.X1000)	59
Fig. (29):	A photomicrograph of subgroup IIA showing osteocytes (H&E Org. mag.X1000)	60
Fig. (30):	A photomicrograph of subgroup IIA showing few osteocytes in bone matrix (H&E Org. mag.X1000)	60
Fig. (31):	A photomicrograph of subgroup IIA showing few giant, multinucleated osteoclasts (H&E Org. mag. X200)	61
Fig. (32):	A higher magnification of the previous figure showing multinucleated osteoclasts (H&E Org. mag. X1000)	61
Fig. (33):	A photomicrograph of subgroup IIA showing areas of bone formation (H&E Org. mag. X200)	62
Fig. (34):	A photomicrograph of subgroup IIA showing marrow cavity with widened blood vessels (H&E Org. mag. X200)	62
Fig. (35):	A higher magnification of a marrow cavity with numerous megakaryocytes (arrows) (H&E Org. mag. X400)	63
Fig. (36):	A photomicrograph of subgroup IIA with reversal lines (H&E Org. mag.X100)	63
Fig. (37):	A photomicrograph of subgroup IIA showing widened vascular canals (H&E Org. mag. X200)	64
Fig. (38):	A high magnification showing discrete bone islands (H&E Org. mag. X200)	64
Fig. (39):	A photomicrograph of group IIB showing irregular socket wall (H&E Org. mag.X100)	66
Fig. (40):	A photomicrograph of group IIB showing thin bony trabecula (H&E Org. mag.X100)	66
Fig. (41):	A higher magnification of the previous figure showing flattened and spaced osteoblasts (H&E Org. mag.X1000)	67
Fig. (42):	A photomicrograph of group IIB showing flattened osteoblasts (H&E Org. mag.X200)	67
Fig. (43):	A photomicrograph of group IIB showing numerous osteocytic lacunae (H&E Org. mag.X1000)	68

Fig. (44): A photomicrograph of group IIB showing giant osteoclasts (H&E Org. mag.X1000)	68
Fig. (45): A photomicrograph of bone trabeculae of group IIB (H&E Org. mag.X200).....	69
Fig. (46): A photomicrograph of group IIB showing vascular canals (H&E Org. mag. X100).	69
Fig. (47): A photomicrograph of group IIB showing a marrow cavity (H&E Org. mag. X100).....	70
Fig. (48): A photomicrograph of group IIB showing a fatty marrow cavity (H&E Org. mag. X200).....	70
Fig. (49): A photomicrograph of subgroup IIB showing a smooth socket wall (H&E Org. mag. X100).	72
Fig. (50): A photomicrograph of subgroup IIB showing an even periodontal ligament space (H&E Org. mag. X200)	72
Fig. (51): A photomicrograph of subgroup IIB showing thin bone trabeculae (H&E Org. mag. X100)	73
Fig. (52): A photomicrograph of subgroup IIB showing bone trabeculae (H&E Org. mag. X1000).....	73
Fig. (53): A photomicrograph of subgroup IIB showing cutting cone (CC) & filling cone FC (H&E Org. mag. X400)	74
Fig. (54): A photomicrograph of subgroup IIB showing osteocytic lacunae (H&E Org. mag.X400)	74
Fig. (55): A photomicrograph of osteocytes of subgroup IIB (H&E Org. mag. X400)	75
Fig. (56): A photomicrograph of subgroup IIB showing vascular canals (H&E Org. mag. X200).	75
Fig. (57): A photomicrograph of subgroup IIB showing discrete bone islands (H&E Org. mag. X200).	76
Fig. (58): A photomicrograph of bone trabeculae of subgroup IIB (H&E Org. mag. X200)	76
Fig. (59): A photomicrograph of control group with areas of new collagen formation (MT X200).	78
Fig. (60): A photomicrograph of group IIA showing intermingling blue and red areas (MT X200).	79

Fig. (61):	A photomicrograph of subgroup IIA showing a histological picture almost similar to control (MT X200).....	80
Fig. (62):	A photomicrograph of group IIB showing absence of blue colour stained areas (MT X200).	81
Fig. (63):	A photomicrograph of subgroup IIB showing intermingling blue and red areas (MT X200).....	82
Fig. (64):	A photomicrograph of osteoblasts from the control group showing almost no reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	83
Fig. (65):	A photomicrograph of osteocytes from the control group showing almost no reaction to anti-active caspase3 (Antiactive- caspase3 X400) counterstain: Hematoxylin	84
Fig. (66):	A photomicrograph of osteoclasts from the control group (Antiactive- caspase3 X400) counterstain: Hematoxylin	84
Fig. (67):	A photomicrograph of osteoblasts & osteocytes of group IIA (Antiactive- caspase3 X400) counterstain: Hematoxylin.	85
Fig. (68):	A photomicrograph of osteoclasts of group IIA (Antiactive- caspase3 X400) counterstain: Hematoxylin	86
Fig. (69):	A photomicrograph of osteoblasts & osteocytes of subgroup IIA (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	87
Fig. (70):	A photomicrograph of osteocytes of subgroup IIA (Antiactive- caspase3 X400) counterstain: Hematoxylin	88
Fig. (71):	A photomicrograph of osteoclasts of subgroup IIA (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	88
Fig. (72):	A photomicrograph of osteoblasts of group IIB showing (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	89
Fig. (73):	A photomicrograph of osteocytes of group IIB (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	90
Fig. (74):	A photomicrograph of osteoclasts of group IIB (Antiactive- caspase3 X400) counterstain: Hematoxylin.....	90
Fig. (75):	A photomicrograph of osteoblasts & osteocytes of subgroup IIB (Antiactive- caspase3 X400) counterstain: Hematoxylin.	91

List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (1):	Correlation between control and group IIA regarding mandibular cortical thickness	92
Table (2):	Correlation between control and subgroup IIA regarding mandibular cortical thickness	93
Table (3):	Correlation between control and group IIB regarding mandibular cortical thickness	94
Table (4):	Correlation between control and subgroup IIB regarding mandibular cortical thickness	95
Table (5):	Correlation between control and group IIA regarding the surface area percentage of spongy bone trabeculae	96
Table (6):	Correlation between control and subgroup IIA regarding the surface area percentage of spongy bone trabeculae	97
Table (7):	Correlation between control and group IIB regarding the surface area percentage of spongy bone trabeculae	98
Table (8):	Correlation between control and subgroup IIB regarding the surface area percentage of spongy bone trabeculae	99
Table (9):	Correlation between control and group IIA regarding the number of osteocytes per unit area in cortical bone	100
Table (10):	Correlation between control and subgroup IIA regarding the number of osteocytes per unit area in cortical bone.....	101
Table (11):	Correlation between control and group IIB regarding the number of osteocytes per unit area in cortical bone	102

Table (12):	Correlation between control and subgroup IIB regarding the number of osteocytes per unit area in cortical bone.....	103
Table (13):	Correlation between control and group IIA regarding the number of megakaryocytes per unit area in bone marrow.....	104
Table (14):	Correlation between control and subgroup IIA regarding the number of megakaryocytes per unit area in bone marrow	105
Table (15):	Correlation between control and group IIB regarding the number of megakaryocytes per unit area in bone marrow.....	106
Table (16):	Correlation between control and subgroup IIB regarding the number of megakaryocytes per unit area in bone marrow	107
Table (17):	Correlation between control and group IIA regarding BMD per unit area in cortical bone.....	108
Table (18):	Correlation between control and subgroup IIA regarding BMD per unit area in cortical bone.....	109
Table (19):	Correlation between control and group IIB regarding BMD per unit area in cortical bone.....	110
Table (20):	Correlation between control and subgroup IIB regarding BMD per unit area in cortical bone.....	111

Bisphosphonates are drugs that have early beneficial effects as to prolonging the life span of osteoblasts and preventing glucocorticoid-induced osteoblast apoptosis. They are potent analogues of inorganic pyrophosphates that are completely resistant to enzymatic hydrolysis and have a strong affinity for calcium phosphate (*Weinstein et al., 2002*).

Glucocorticoids are important drugs in treatment of variety of diseases, but long-term period use can lead to various adverse effects, including osteoporosis. Glucocorticoid-induced osteoporosis is mainly caused by inhibition of osteoblastic bone formation by inhibiting osteoblastogenesis and promoting osteoblast apoptosis, which results not only in decreased bone mineral density, but reduction of bone strength by trabecular thinning in bone microstructures. The evidence suggests that daily oral glucocorticoid doses higher than 5 mg prednisolone or equivalent increase the risk of fracture within 3–6 months after the start of therapy. High-dose inhaled glucocorticoids may also increase fracture risk. Treatment with vitamin D, calcitonin, sex hormone replacement, and bisphosphonates has been shown to be effective (*Mastaglia et al., 2006*).

Bisphosphonates have proven to be the most valuable drugs for glucocorticoid-induced osteoporosis. There are several lines of evidence indicating that they are effective in preventing and treating low bone mineral density and in reducing fracture risk (*Yasuhiro et al., 2004*).