بسم الله الرحمن الرحيم

(وَمَا تَوْفِيقِي إِلا بِاللهُ عَلَيْهِ تَوَكَلْتُ اللهُ عَلَيْهِ تَوَكَلْتُ اللهُ عَلَيْهِ تَوَكَلْتُ (حُبَيالُهُ عَلَيْهِ عِلْهِ عَلَيْهِ عِلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْهِ عَلَيْه

صدق الله العظيم سورة مود آيه (٨٨)

Effectiveness of various disinfectants, and their combinations used as endodontic intracanal medications

(An in vitro study)

Thesis submitted to the Faculty of Oral and Dental Medicine, Cairo
University, in the partial fulfillment of the requirements for the Master's

Degree in Endodontics

By

Soha Adel Abdou Sayed

B.D.S. Cairo University

Department of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

2011

Supervisors

Prof. Dr. Medhat A. Kataia

Professor of Endodontics,

Faculty of Oral and Dental Medicine

Cairo University

Ass. Prof. Dr. Maha M. Sallam

Assistant Professor of Oral and Maxillofacial Surgery,
Research Institute of Ophthalmology

Dr. Heind Abou El Nasr

Lecturer of Endodontics,

Faculty of Oral and Dental Medicine

Cairo University

Dedication

My Father, My Mother,

My brother karem,

My sister Mahy

For their endless love and support

I wish them all the best forever

Acknowledgement

Before all and above all thanks to Allah

I would like to express my deepest appreciation and gratitude to *Prof. Dr. Medhat A. Kataia*, Professor of Endodontics, Faculty of Oral and Dental Medicine Cairo University, for his supervision, encouragement, cooperation and help in conducting this study.

I would also like to thank *Ass. Prof. Dr. Maha M. Sallam*, Assistant Professor of Oral and Maxillofacial Surgery, Research Institute of Ophthalmology, for her great help in conducting this study, supervision cooperation, support and mother-dealing sympathy.

I wish to express my sincere thanks to *Dr. Heind Abou El Nasr*, Lecturer of Endodontics, Faculty of Oral and Dental Medicine Cairo University, for her generous help, continuous encouragement, constructive effort and valuable technical advice.

I would also like to thank *Dr. Sahar Esmael A. Negm*, Lecturer of Medical Microbiology and Immunology, Research Institute of Ophthalmology, for her support and help in microbiological work.

Great thanks and gratitude to Research Institute of Ophthalmology especially the head of dental department *Dr. Lobna Agamia* for great help and support.

List of contents

Title Page	e
List of contents	i
List of tablesi	i
List of figuresii	i
Introduction1	1
Review of Literature)
• Intra canal medications2	2
-Calcium hydroxide2)
-Doxycycline12	2
• Intra canal irrigants17	7
-Sodium hypochlorite17	7
-Chlorhexidine25	5
• Effect of intra canal medications on the sealing ability after root en resection	
Aim of the study4	1
Materials and Methods4	2
Results6	2
Discussion	5
Summary and conclusions8	35
References8	9
Arabic summary	

List of tables

Table	e No. Page
1.	The mean, standard deviation (SD) values and results of comparison
1	between microbial counts (log ₁₀ values of CFU) of <i>E.faecalis</i> in all
;	groups
2.	The mean, standard deviation (SD) values and results of comparison
1	between microbial counts (log ₁₀ values of CFU) of Candida albicans
j	in all groups66
3. '	The mean, standard deviation (SD) values and results of comparison
1	between microbial counts (log ₁₀ values of CFU) of E.faecalis and
	C.albicans with each group of intra canal medicament71
4. '	The mean, standard deviation (SD) values and results of comparison
1	between dye extraction in the five experimental groups

List of figures

Figure No.	Page
1: Ca(OH) ₂ powder	43
2: Doxycycline	43
3: 2% CHX	43
4: Teeth in Eppendorf tubes	48
5: Eppendorf in sterilizing bag	48
6: Laminar air flow	48
7: 2 MacFarland	48
8: Chart showing the classification of the samples	51
9: Eppendorf carrier	52
10: Pipettes, sterile blue and yellow tips	52
11: Infection of the tooth	53
12: H-files in sterile Weatherman tubes containing 1ml of broth	
13: Sterile glass rods	57
14: A microplate Spectrophotometer	61
15: Plate of spectrophotometer	61

16: Sample taken from infected tooth with E.Faecalis without any
medicament treatment with serial dilution 1/100000 (+ve control)64
17: E.faecalis on blood agar after treatment with Ca(OH)2 with serial
Dilution 1/10000 (group 1)
18: E.faecalis on blood agar after treatment with doxycycline paste without
dilution (group 2)65
19: E.faecalis on blood agar after treatment with doxycycline and Ca(OH) ₂
paste without dilution (group 3)65
20: Sample taken from infected tooth with <i>C.albicans</i> without any
medicament treatment with serial dilution 1/100000 (+ve control)68
21: C.albicans on blood agar after treatment with Ca(OH) ₂ paste with serial
dilution 1/100000 (group 1)
22: C.albicans on blood agar after treatment with doxycycline paste with
serial dilution 1/100000 (group 2) 69
23: C.albicans on blood agar after treatment with doxycycline/Ca(OH)2
paste with serial dilution 1/100000 (group 3)69
24: C.albicans on blood agar after treatment with Ca(OH) ₂ /doxycycline
/NaOCl with serial dilution 1/100000 (group 4) 70
25: C.albicans on blood agar after treatment with Ca(OH)2 /doxycycline
/CHX With serial dilution 1/100000 (group 5) 70

ABSTRACT

Abstract

The purpose of this study was to evaluate the antimicrobial efficacy of the following disinfectants and their combinations; Ca(OH)₂, doxycycline, NaOCl and CHX, and their effect on the sealing ability after root end resection. 120 extracted, human, single rooted teeth were decoronated and mechanically prepared. After sterilization of the roots, half of them were contaminated with Enterococcus faecalis and the other half with Candida albicans for 3 days. The roots were classified into five experimental groups according to the type of medicament used and two control groups. The experimental groups were; group 1: Ca(OH)₂ paste, group 2: doxycycline paste, group 3: Ca(OH)₂ doxycycline paste, group 4: Ca(OH)₂, doxycycline and NaOCl paste, group 5: Ca(OH)₂, doxycycline and CHX paste. The medications were left inside the root canals for 3 days. After removal of the medicaments, samples were taken, serially diluted, inoculated on agar plates and incubated for 48 hours at 37 ° C. The colony forming units were counted. In the 2nd part of the study, six roots were selected randomly from each experimental group. They were obturated and root ends were resected. The leakage was measured using dye extraction method. The results were statistically analyzed using ANOVA test, *Tukey's post-hoc* test and Student's t-test. Results showed that Ca(OH)₂ paste and doxycycline paste were not able to kill *E.faecalis* and *C.albicans* completely after 3 days of exposure. However, doxycycline paste showed significantly higher reduction in *E.faecalis* count than Ca(OH)₂ paste while Ca(OH)₂ paste showed non significantly higher reduction in C.albicans count than doxycycline paste. The Ca(OH)₂/ doxycycline

paste killed *E.faecalis* completely but with *C.albicans* they lower their account. The addition of doxycycline and irrigants to Ca(OH)₂ increased the antimicrobial effect, even if this was of no statistical significance. Ca(OH)₂ paste and doxycycline paste recorded the highest leakage value with no statistical significance difference between them while Ca(OH)₂, doxycycline paste showed the lowest leakage values. A combination of two or more disinfecting agents seems to be a valuable option in the battle against maximum amount of microorganisms and the addition of irrigating solutions to the mixture of intracanal medication was found to be a beneficial to improve the antimicrobial effect.

Key words: Intracanal medication, Calcium hydroxide, doxycycline, *E.faecalis*, *C.albicans*, sealing ability.

INTRODUCTION

Introduction

The established role of microorganisms in the pathogenesis of pulp and periradicular diseases has rendered the eradication of bacteria from the root canal system a primary objective of root canal therapy. It has been demonstrated that mechanical instrumentation cannot sufficiently disinfect root canals. Therefore, irrigation solutions and intracanal medication are required to eradicate microorganisms. The ideal irrigant must kill bacteria, dissolve necrotic tissue, lubricate the canal, remove the smear layer, and not irritate healthy tissues. The ideal medicaments must complete the disinfection of the root canal.

However, some cases are resistant to routine therapy and the infection may persist. *Enterococcus faecalis* and *Candida albicans* have been frequently isolated from persistent root canal infection and resist various intracanal irrigants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX), as well as commonly used intracanal medicament such as calcium hydroxide Ca(OH)₂. This may be the reason for a search of another intracanal medicament.

BioPure MTAD (a mixture of tetracycline, citric acid, and detergent) has been shown to be effective against *E.faecalis*. Doxycycline is the primary ingredient contributing to the antibacterial activity of MTAD. In an attempt to gain benefit of the high alkalinity of Ca(OH)₂ and the strong antimicrobial activity of doxycycline, this work aims to shed light on the efficacy of different combinations of these agents with endodontic irrigants against resistant microorganisms.

REVIEW OF LITERATURE