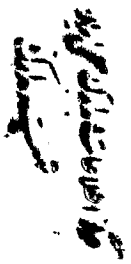
In the Name of God
the Compassionate the
Merciful



Shiraz University of Medical Sciences
School of Dental Medicine

THESIS FOR THE PARTIAL FULFILLMENT OF MASTER OF SCIENCE IN ENDODONTICS

Histological Evaluation of the Effect of Three Medicaments; Trichloracetic Acid, Formocresol and Mineral Trioxide Aggregate on Pulpotomized Teeth of Dogs

By:
Dr. Babak Karami

Advisors:
Dr. Akbar Khayat
Professor of Endodontics

Dr. Fariborz Moazami
Assistant Professor of Endodontics

April 2007

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to Dr. A. Khayat and Dr. F. Moazami for their invaluable advice and guidance during the course of this research. Without their generous and careful supervision over the past years, meticulous reading of grafts, continued belief in my ability, and friendship, this thesis would not have been completed. They are my inspiration for what academic life should be about.

Besides my advisors I would like to thank, Dr. S. Pardis for his help in the histological evaluation, Dr. E. Karami for his assistance with the statistical analysis of this study and finally the members of the Endodontics Department who had a great share in my education.

I would also like to thank the respectful committee of thesis.

Dedicated to:

My parents whose sacrifice made my education possible

My wife, Elham for her continuous understanding and support

My Brothers, for their kindness and friendship

and last but not least

My beloved children

Kiavash and Kamyar

whose attitude, despite their young and tender age was supportive and inspiring

ارزیابی پایان نامه

پایان نامه تخصصی شماره ۱۰۳۶ در رشته اندودانتیکس با عنوان :

Histological Evaluation of the Effect of Three Medicaments; Trichloracetic Acid, Formocresol and Mineral Trioxide Aggregate on Pulpotomized Teeth of Dogs

با نگارش دکتر بابک کرمی در تاریخ ۸۶/۱/۲۷ با نمره (۲۰) و درجه (ممتاز) مورد تایید قرار گرفت.

اساتید محترم راهنما:

۱.

۲.

هییت محترم داوران:

۱.

۲.

۳.

۴.

۵.

Contents

CHAPTER 3

CHAPTER 4

CHAPTER 1

INTRODUCTION

Introduction

Pulpotomy is a therapeutic procedure, which consists of the surgical amputation of coronally inflamed pulp. The wounded surface of the radicular pulp is treated with a medicament or dressing agent to promote healing or to cause fixation of the underlying tissue. The objective is to maintain vitality of the radicular pulp. Pulpotomy is a common procedure in the treatment of acutely inflamed primary teeth. It is also used in the management of young permanent teeth with open apices.[6]

The success of vital pulp therapy techniques in cariously exposed primary molar teeth is thought to be dependent upon the technique employed, the inflammatory status of the coronal and radicular pulp tissue, the type of pulp therapy agent used, the period of observation and the criteria used to diagnose success.

The features of a successful pulpotomy treatment can be described as:

1- Dentin bridging
2- Maintenance of pulp vitality
3- Lack of undue sensitivity or pain
4- Minimum pulpal inflammatory response
5- The ability of the pulp to maintain itself without progressive degeneration
6- Lack of internal resorption and/or intraradicular pathosis [92,123]

One of the outcomes felt to indicate a successful pulpotomy is the presence of a dentin bridge at the site of pulp amputation. This phenomenon was thought to indicate healing of the pulp tissue and to be promoted by the application of a material such as calcium hydroxide.[70] However, this supposition is now not without controversy as it is known that pulpal obliteration/dentin bridge formation results from deposition of reactionary dentin also termed irritation dentin.

Studies have indicated that an exposed pulp possesses inherent ability to produce dentin in response to operative procedures or trauma, irrespective of the agent applied

to the amputation site.[36] Infection control appears to be a key issue; however calcific barrier formation has also occurred after formocresol pulpotomies in primates.[209]

Historically various materials have been used in pulpotomy procedures. These include, but are not limited to: ivory, quill, gold - beaters skin, oiled skin, paper, plaster of Paris, Canada Balsam, asbestos, etc.[46] Other pulpotomy dressings have also been examined more recently such as formocresol, calcium hydroxide, freeze dried bone, gluteraldehyde, ferric sulfate, bone morphogenic protein, MTA, etc.

Formocresol has been a popular pulpotomy medicament in the primary dentition for the past sixty years. According to Avram and Pulver, in their 1989 survey, formocresol continues to be the most widely used pulpotomy medicament for vital primary teeth.[14] The search for a medicament to replace formocresol became imperative after several negative reports questioning both its local and systemic side effects.[75,155,177,207]

A newer material that has been advocated for vital pulp therapy is MTA. Many favorable features have been reported on the use of MTA. These are its excellent sealing ability, biocompatibility, ability to form dentin bridge, and cementum and periodontal ligament regeneration.[197,216,259] Hence, MTA has been recommended as a retrograde filling material in the repair of perforations, pulp capping and apexification. Although MTA is commonly used for a variety of endodontic indications, but the material is expensive and its clinical use is therefore restricted to more affluent patients.

TCA (trichloracetic acid) is the main pulpotomy dressing being investigated in this study. The use of TCA as a chemical cauterizing agent has been popular in medicine and dentistry since the 19th century. It is a cheap chemical substance and no carcinogenicity has been reported regarding this material.

It is the first time this chemical substance is being suggested as a pulpotomy dressing for pulpotomy of primary and young permanent teeth. The purpose of this study was to compare the dental pulp response in dog premolars when TCA, formocresol, MTA

and ZOE were used as pulpotomy agents. All four materials are investigated clinically, radiographically and histologically.

CHAPTER 2

HISTOPATHOLOLOGY OF THE PULP

Histopathology of the Pulp

Histology of the pulp

The dental pulp is a minute piece of connective tissue akin to any other in the body and consists of collagen fiber, cells, nerve fibers and blood vessels embedded in a gel-like ground substance. However, its unique characteristic is that it is encased in a rigid hard tissue called dentin.[248] The pulp is described as having two defined regions called central and peripheral.[28]

Peripheral pulp zone

The peripheral pulp zone is at the periphery of the pulp, adjacent to the calcified dentin. Next to the predentin lies, the palisade of columnar odontoblast cells and central to the odontoblasts is the subodontoblastic layer, termed the cell free zone of Weil.[13] Plexus of capillaries and small nerve fibers are present in this layer. Deep to the odontoblastic layer is the cell-rich zone, which blends with the stroma of the pulp. The cell-rich zone contains fibroblasts and undifferentiated cells, which sustain the population of odontablasts by proliferation and differentiation.[287]

Both of the mentioned zones are usually indistinct or absent in the embryonic pulp and usually appear when dentin formation is active. However, both of these zones are less constant and less prominent near the root apex.

Central pulp zone

The main body of the pulp is in the central zone and it contains the principal support system for the peripheral pulp, which includes the large vessels and nerves, which

supply the outer pulp layer. The principal cells in this layer are fibroblasts and the principal extracellular components are ground substance and collagen.

Cells of the pulp

The pulp contains a pool of cells which each is discussed separately as follows:

Fibroblasts

The basic cells of the pulp are the fibroblasts, similar to connective tissue fibroblasts found elsewhere in the body. They exhibit a wide variation in their degree of differentiation.[95] However the ability of these cells to form calcified tissues differentiates them from other regular connective tissue.

Fibroblasts in the pulp are spindle-shaped cells with ovoid nuclei. They synthesize and secrete the bulk of the extracellular components, that is, collagen and ground substance. The fibroblasts also eliminate excess collagen by resumption of collagen fibers which has been seen to occur intracellularly by the action of lysosomal enzymes.[264]

Odontoblasts

The odontoblast is a highly differentiated cell in the pulp. These cells arise from peripheral mesenchymal cells of the dental papilla during tooth development.[87] The odontoblast consists of two compartments, cell body and odonloblastic process. The cell body contains organelles that represent different stages of secretion of collagen, glycoprotein's and calcium salts.[82] The main function of the odontoblast is the production of dentin. At the coronal portion of the tooth the odontoblasts are tall and columnar in shape and in the root portion they are shorter and more or less cuboidal. At the apex they are flattened and look more like fibroblasts. Odontblasts at the

coronal region produce more regular dentin with regular dentinal tubules but in the apical portion, less tubular and more amorphous dentin is produced.[71]

The odontoblast has a process known as the odontoblastic process. The cell body manufactures the matrix material which is transported to and secreted from the odontoblastic process. When observed by light microscopes using a variety of special procedures and stains it has been said that the odontoblastic process extends to the dontinoenamel junction, a distance of 2-3 mm.[12] Although when the dentin was examined with a transmission electron microscope, the odontoblastic layer was determined to be limited to the inner third of the dentin and the outer two thirds was filled with extracellular fluid.[25,256] However in more recent investigations when examined with scanning electron microscope indicates that the processes may indeed extend to the dentionenamel junction.[121,156]

Defense cells

Histiocytes and macrophages

Undifferentiated mesenchymal cells around blood vessels can differentiate into histiocytes and macrophages under appropriate stimulation.[239] By the way macrophages are monocytes that have left the blood stream, entered the tissues and differentiated. These cells are highly phagocytic and can extravasate blood cells, dead cells foreign bodies (endodontic paste, zinc oxide, etc) or other debris.[276] However another subset of macrophages participates in immune reactions by processing antigens and presenting it to memory T-cells.[94] When activated by the appropriate inflammatory stimuli, macrophages are capable of producing a large variety of soluble factors, including IL-1, TNF, growth factors and other cytokines.[31]

Polymorphonuclear leukocytes

Neutrophils are not normally present in intact healthy pulp but with injury and cell death they rapidly migrate from nearby capillaries and venules.[45] The most common

form of leukocyte in pulp inflammation is the neutrophil. Although eosinophils and basophiles are occasionally detected, neutrophils are the major cell type in microabscesses formation and are very effective at destroying and phagocytizing bacteria or dead cells.

Lymphocytes and plasma cells

These inflammatory cell types generally appear following invasion into the area of injury by neutrophils. Although, Hahn et al. reported finding T-lymphocytes in normal pulps from human teeth.[94] T_8 (suppressor) lymphocytes were the prominent T-lymphocyte subsets present in these pulps. Lymphocytes have also been observed in the pulps of impacted teeth.[141] B-lymphocytes are scarcely found in the normal pulp.

Mast cells

Mast cells are seldom found in normal pulp tissue although they are routinely found in chronically inflamed pulps.[215] The granules of mast cells contain heparin, an anticoagulant, and histamine an important inflammatory mediator. The granules are released into surrounding tissue fluid during inflammation. When histamine is released near vascular smooth muscles, vasodilatation of these vessels are caused, therefore increased permeability allow fluids and leukocytes to escape from these vessels.

Dendritic cells

Dendritic cells are primarily found in lymphoid tissues but they are also widely distributed in connective tissues, including the pulp. These cells are termed as antigen presenting cells (APC). They are known to play a central role in the induction of T-Cell dependent immunity. These APC engulf protein antigens and then present an assembly of peptide fragments of the antigens and class II molecules. T-cells recognize this assembly and bind to it with the T-cell receptor therefore T-cell activation occurs.

Connective tissue fibers of the pulp

Collagen and elastin are the two types of structural proteins found in the pulp. Elastic fibers are confined to the walls of arterioles and unlike collagen are not a part of the extracellular matrix. Type I collagen is synthesized by odontoblasts and osteoblasts. This type of collagen is found in skin, tendon, bone, dentin and pulp. Type I, III, V and VII are synthesized by fibroblasts. Type III collagen is found in most unmineralized connective tissues. It is a fetal form found in the dental papilla and the mature pulp. Type VII collagen is a component of basement membrane and type V collagen is a constituent of interstitial tissues.

The presence of collagen fibers passing from dentin matrix between odontoblasts into the dental pulp has been reported in fully erupted teeth.[24] These bundles of collagen are termed by many as Korff's fibers. The highest concentration of large fiber bundles are usually found near the apex. Thus it is advised to engage the pulp with a barbed broach in the region of the apex during pulpectomy to remove these collagen fibers intact.[262]

Ground substance

The ground substance is a structure-less mass, gel-like in consistency which makes up the bulk of the pulp organ. It consists primarily of complexes of proteins and carbohydrates and water. These complexes are composed of combinations of glycosaminoglycans, that is, hyaluronic acid, chondroitin sulfate and other glycoproteins.[263]

Ground substance is a medium through which metabolites and waste products are transported to and from cells and vessels. The proteoglycan content of pulp tissue decreases approximately 50% with tooth eruption.[149] With tooth eruption, hyaluronic acid and dermatan sulfate increase and chondroitin sulfate decreases greatly.

The consistency of the pulp is largely determined by the proteoglycan components of the ground substance. The long glycosaminoglycan chains of the proteoglycan molecules form relatively rigid coils constituting a network that holds water, thus forming a characteristic gel. Water content of the young pulp is very high (~90%) thus the ground substance forms a cushion capable of protecting cells and vascular components of the tooth.

Blood supply of the pulp

Arterioles having diameters of 100 μm or less enter the tooth through the apical foramen or foramina supplying the dental pulp. Other small vessels may also supply the pulp by the way of lateral or accessory canals. At the apex and extending through the central pulp, one or more arterioles branch into smaller terminal arterioles or metarterioles that are directed peripherally toward the odontoblastic layer, beneath which they ramify to form a capillary plexus.[134] This network provides the odontoblasts with a rich source of metabolites.

In the coronal portion of the pulp, capillary blood follow is nearly twice that in the root portion,[127] and blood flow in the pulp horns region is greater than other areas of the pulp.[168] Capillaries in the subodontoblastic layer are frequently seen with fenestrations. These fenestrations promote rapid transport of fluid and metabolites from the capillaries to the adjacent odontoblasts.[206]

The vessels of the pulp have thinner muscular walls (tunica media) than vessels of comparable diameter in other parts of the body. Blood passing through the capillary plexus then passes into capillary venules and then into larger venules.[134] Venules in the pulp have very thin walls and the muscular coat of these venules is thin and discontinuous. The largest venules have a diameter that may reach a maximum of 200 μm which is considerably larger than arterioles of the pulp.

Arteriovenous anastomoses may be present in the coronal and radicular portions of the pulp. These vessels provide a direct communication between the arterioles and

venules thus bypassing the capillary bed. These small vessels have an approximate diameter of 10 μm. These anastomoses play an important role in the regulation of the pulp circulation. Theoretically they could provide a mechanism for shunting blood away from areas of injury or inflammation, where damage to the microcirculation may result in thrombosis and hemorrhage.[253]

Among oral tissues, young and normal pulp have the highest volume of blood flow but much lower than blood flow in major visceral organs. This reflects the fact that the respiratory rate of pulp cells is relatively low (3.2 ml O_2/min/100gr).[285] By the way pulp blood flow is greater in the subodontoblastic capillary plexus where the oxygen consumption is higher than the central pulp.[22]

Lymphatics of the pulp

The presence of pulpal lymphatics has been a matter of debate because it is difficult to distinguish between venules and lymphatics. However with the help of electron microscopy lymphatic capillaries have been detected.[23] Lymphatic capillaries arise in the peripheral pulp zone and join other lymph capillaries to form collecting vessels. These vessels finally leave the pulp throngh the apex with the other vasculature.

Nerves of the pulp

Nerve bundles containing myelinated and unmyelinated nerves enter each root through the apical foramen. The majority are unmyelinated nerves, most of which are part of the sympathetic division of autonomic nervous system and cause reduction in blood flow when stimulated.[49,118] The remaining nerves are myelinated sensory nerves of the trigeminal system.

Beneath the cell rich zone, myelinated nerve fibers branch extensively to form the plexus of Raschkow. From here many fibers lose their myelin sheath and pass through the cell free zone to terminate as receptors or as free nerve endings near