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Cytotoxicity of Root Canal Disinfecting Agents against Stem Cells of Apical Papilla – A Review

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Abstract:
The aim of this review was to systematically assess the literature on cytotoxicity of root canal irrigants and intracanal medicaments on stem cells of the apical papilla. A structured strategy was employed and 7 papers were identified. The papers were analysed for concentration of agent used, testing method and viability of stem cells of the apical papilla (SCAP). Irrigation protocols using a final revise of EDTA showed superior survival of SCAP. The results showed that the endodontic literature is inconsistent with regards to the effect of intracanal medicaments on SCAP. Further studies are recommended to determine the ideal disinfection regimen prior to regenerative endodontic strategies.
Keywords: Stem cells, regenerative endodontics, intracanal medicaments, intracanal irrigants.

INTRODUCTION

The present focus in endodontics demonstrates a shift towards regenerative approaches wherein biologically based procedures are used to regenerate tissues of the dentin-pulp complex. The procedure for regenerative endodontics (also termed revascularization) depends on satisfactory sterilization of the root canal system and creation of a microenvironment suitable for adhesion, survival and differentiation of undifferentiated mesenchymal cells (1). The mesenchymal cells that are of specific importance in this approach are the stem cells of the apical papilla (SCAP). SCAPs have a high rate of proliferation and odontogenic differentiation (2)

Probably the most important step in regenerative endodontic strategies is disinfection of the root canal system. Numerous clinical studies have been published focusing on the outcome of this procedure (3). However, a standardized or recommended protocol is yet to be definitively reported. Several in vitro studies have assessed the impact of intracanal disinfecting agents on the SCAP (4). The aim of this review was to critically appraise the literature on the cytotoxity of root canal irrigants and intracanal medicaments to SCAP.

Search strategy:

The following databases were searched until February 2016: MEDLINE (via PubMed), Science Direct, ISI Web of Science and Google Scholar by two independent reviewers. The following inclusion criteria were used: in vitro studies; use of SCAP; tested and compared the cytotoxicity of more than one irrigant or intracanal medicament; and quantitative results provided. Different combinations of key words were used in search queries (Table 1). With this method, 165 abstracts were obtained. In addition, the following journals were searched manually between January 2000 and February 2016: Journal of Clinical Periodontology; Journal of Dental Research; Journal of Periodontology; Journal of Periodontal Research; Periodontology 2000; Journal of Endodontics; International Endodontic Journal; Journal of Dentistry; Journal of American Dental Associations; Clinical Oral Investigations. No language restriction was applied. Finally, the references of all selected full-text articles and related reviews were scanned.

Results:

A total of 165 papers were obtained. After initial review and screening, 15 papers were excluded because they were out of scope of the present work. Only studies that evaluated the effect of irrigants or intracanal medicaments on SCAP were included in this work. In effect, a total of 7 papers were included in this review (5-11).

Discussion:

Pulpal necrosis of immature permanent teeth is a clinical challenge in endodontics. In these clinical cases, the tooth presents with a thin dentinal wall which predisposes the tooth to fracture (12-14). These teeth were treated with calcium hydroxide as the intracanal medicaments initially for apexification procedures until there was formation of a calcific barrier and it was followed by obturation of the root canal. Later, this procedure was modified to include the placement of an artificial barrier of mineral trioxide aggregate, which resulted in lesser visits and better outcomes (15). In the past few years, a new treatment protocol called revascularization or regenerative endodontics, is being advocated for such teeth. These reports claim natural root apical closure and formation of an intracanal tissue similar to pulp tissue although this is not confirmatory. Regenerative endodontics also showed positive responses for pulp vitality test after the treatment was done (1,3,16-21).

Regenerative endodontics starts with disinfecting the root canal with irrigants. Minimal or on instrumentation, placement of intracanal medicament. The duration of intracanal medicament placement depends on the signs and symptoms. In the next appointment, the dressing is removed and intracanal bleeding is induced. This bleeding creates a matrix for the growth of new dentin-pulp complex in the root canal space. Regenerative endodontics depend on three main factors: stem cells, scaffold, and growth factors (22).

Mesenchymal stem cells residing in the apical papilla of permanent teeth with immature roots are known as SCAP. These were discovered by Sonoyama *et al*. SCAP forms the odontoblast- like cells, which produces dentin *in vivo*, these act like cell source of primary odontoblasts for the formation of root dentin (2). SCAP supports apexogenesis, which can occur in infected immature permanent teeth with periradicular periodontitis or abscess. SCAP residing in the apical papilla survive such pulp necrosis because of their proximity to the periapical tissue vasculature. Hence even after endodontic disinfection, SCAP can generate primary odontoblasts, which complete root formation under the influence of the surviving epithelial root sheath of Hertwig. To realise this goal, maintaining the viability of SCAP is essential (2).

Root canal irrigants:

Ethylene diamine tetraacetic acid:

Trevino et al evaluated the effect of 17% of EDTA on SCAP using an immunomagnetic separation method and found 88.66% viability of cells, whereas NaOCl/EDTA group showed 74.35% viability of cells, while EDTA/CHX and NaOCl/EDTA/IPA/CHX group showed 0% viability of cells. *Martin et al* evaluated the effect of 17% of EDTA on SCAP using quantitative real time polymerase chain reaction method and found that 35% increase in SCAP cells.

The irrigation protocol evaluated in studies that contained EDTA as the final irrigant showed cell survival. EDTA with 17% concentration are often used in nonsurgical root canal treatment, this helps in removal of smear layer and opens the dentinal tubules and they help in maximising the bactericidal and bacteriostatic effect (23-25). Its chelating effect promotes the dentin derived growth factors. These growth factors presented with proliferation, survival, and differentiation of stem cells. Greater attachment of SCAP to the dentinal matrix after removal of smear layer can be because of greater survival of stem cells in the organotype root canal system after using EDTA (26,27). 17% EDTA promoted the growth of odontoblast like cells which resulted in new dentin formation.

Irrigation with 17% EDTA for one minute followed by a final rinse with NaOCl is the most common method to remove the smear layer. Longer exposure can cause excessive removal of both peritubular and intratubular dentin (28). EDTA has a little or no antibacterial effect (29). EDTA is also known for its solubility of dentin-derived growth factors which will thereby increase its bioavailability. In addition, it allows the inductive properties of dentin- derived morphogens and growth factors present in dentin (30).

Sodium hypochlorite:

Martin et al evaluated the effect of 6% of NaOCl on SCAP using quantitative real time polymerase chain reaction method and found greatly diminished SCAP survival (5,600± 5,500 cells). *Trevino et al* evaluated the effect of NaOCl/EDTA group showed 74.35% viability of cells whereas, NaOCl/EDTA/IPA/CHX group showed 0% viability of cells. *Essner et al* evaluated the effect of NaOCl with different concentrations (0.33%, 0.16%, 0.08%, and 0.04%) using the Cell Titer-Glo Luminescent Cell Viability Assay method and found that increase in cell viability with the lowering the concentration of NaOCl.

Sodium hypochlorite is the most commonly used root canal irrigants. It is an antiseptic and inexpensive lubricant that has been used in dilutions ranging from 0.5% to 5.25%. Free chlorine that is present in NaOCl dissolves the vital and necrotic tissues by breaking down proteins into amino acid. It is a non-specific proteolytic agent with an excellent tissue dissolving ability. The dentin conditioning with 6% NaOCl was not conductive for the survival of SCAP cells in organotype root canal system (5). With lower concentrations of NaOCl it retains the debridement properties while being conductive for survival of SCAP cells. A dilute concentration of NaOCl showed significant antimicrobial properties to promote the cell viability (31). But using the lower concentrations of NaOCl reduces its cytotoxicity effect in cases of inadvertent extrusion beyond the apical foramen. On other hand, decreasing the concentration of the solution reduces its toxicity, antibacterial effect and ability to dissolve tissues. Increasing its volume or warming it increases its effectiveness as root canal irrigant. The major disadvantage of this irrigant are its cytotoxicity when injected into periradicular tissues, foul smell and taste, ability to bleach clothes and ability to cause corrosion of metal objects (32). It alters the properties of dentin. NaOCl exerts deteriotive effects on mechanical properties and chemical compositions of dentine (33).

Intracanal medicaments:

Triple antibiotic paste (TAP):

Althumairy et al evaluated the effect of TAP in different concentrations (1 mg/mL, 1000 mg/mL) using Cell Titer-Glo Luminescent Cell Viability Assay method and found that there was no adverse effect on cell viability and no viability respectively to the concentrations. *Chuensombat et al* evaluated the effect of TAP in different concentrations (0.39 mg/mL, 25.00 mg/mL) using MTT assay method and found that 0.39 mg/mL 3Mix produced more than 90% cell viability,25.00 mg/mL 3Mix completely eliminated isolated bacteria. *Sabrah et al* evaluated the effect of TAP in different concentrations (0.125, 0.25, 0.5, 1, and 10 mg/ml) using LDH assay and cell viability assay methods and found that cell viability assays, all antibiotic dilutions except 0.125 mg/ml significantly reduced the viability of DPSC. For LDH assays, the two lowest concentrations of TAP (0.25 and 0.125 mg/ml) were non-toxic to DPSC. *Ruparel et al* evaluated the effect of TAP at different concentrations (1, 10, 100 mg/mL) using an automated method of detecting trypan blue dye and found that 58.0% ±12.4%, 8.0% _±1.8%, and 1.3% ± 0.5% SCAP survival, respectively.

TAP was successful in promoting the healing and repair of the periapical tissues. TAP can help promote functional development of dentin-pulp complex (34). TAP contains both bactericidal (metronidazole, ciprofloxacin) and bacteriostatic (minocycline) agents to allow for successful revascularization. A recent study reported that TAP are

able to significantly reduce new biofilm formation of E. faecalis and Porphyromonas gingivalis at concentrations of 0.03 mg/ml for TAP using a microtiter plate method (35). TAP was proved to be biocompatible, increases the level of interleukin-10, an anti-inflammatory cytokine. In addition, metronidazole and ciprofloxacin can generate fibroblast (36). The concern of this antibiotic paste is that it may cause bacterial resistance. Minocycline may cause tooth discolouration which is the major disadvantage. As a remedy for discolouration, they used dentin bonding agent and composite resin before placement of the triple antibiotic dressing to prevent discolouration,but the discolouration was only reduced (21). TAP can be effectively used for sterilization of canals and healing of periapical pathology. A concentration of 1 mg/ml of TAP was also effective in eradicating more than 99.9999 %. The cytotoxicity of TAP was greater than the single drugs used in the combination. (37).

Double Antibiotic Paste (DAP):

Ruparel et al evaluated the effect of DAP at different concentrations (1, 10, 100 mg/mL) using an automated method of detecting trypan blue dye and found that 53.0% ±12.4%, 7.0% ±1.8%, and 0.9%±0.5% SCAP survival, respectively. Althumairy et al evaluated the effect of DAP in different concentrations (1 mg/mL, 1000 mg/mL) using Cell Titer-Glo Luminescent Cell Viability Assay method and found that there was no adverse effect on cell viability and no viability respectively to the concentrations. Sabrah et al evaluated the effect of DAP in different concentrations (0.125, 0.25, 0.5, 1, and 10 mg/ml) using LDH assay and cell viability assay methods and found that cell viability assays, all antibiotic dilutions except 0.125 mg/ml significantly reduced the viability of DPSC. For LDH assays, the three lowest tested concentrations of DAP (0.5, 0.25, 0.125 mg/ml) were non-toxic to DPSC.

Double antibiotic paste plays a significant role in revascularization (or) regenerative endodontics. DAP is a combination of ciprofloxacin, and metronidazole, initially minocycline was added with these two components but the main disadvantage is that it causes discolouration of tooth. So in order to overcome this disadvantage, minocycline was removed. DAP at a 10 mg/mL concentration were able to eradicate all the established biofilm though did not eradicate completely (8). DAP are not toxic to dental pulp fibroblasts and SCAP cells. Dentin conditioning with DAP at a paste-like consistency triggers the complete destruction of SCAP placed in a scaffold within the root canal space. This is an indirect effect because the dentin disks were extensively washed before seeding SCAP cells (38). DAP at concentrations even lower than the 1 mg/mL value retain their antibacterial effectiveness against endodontic pathogens (39). Thus, it appears that the detrimental effects of DAP on SCAP survival are largely prevented when they are used at a concentration of 1 mg/mL or lower (38).

Chlorhexidine:

Trevino et al evaluated the effect of 2% of CHX on SCAP using an immunomagnetic separation method and found EDTA/CHX and NaOCl/EDTA/IPA/CHX group showed 0% viability of cells. CHX is bacteriostatic at a concentration of 0.2% and bactericidal at a concentration of 2%. CHX is considered as an effective agent against microbial biofilms.

The challenge that prevents the use of CHX as a routine irrigant in endodontics is its lack of tissue solubility during chemomechanicsl preparation (39). The major advantage of CHX over NaOCl is its lower cytotoxicity and lack of foul smell and taste. After irrigation of NaOCl, CHX is added and is known to form a cytotoxic precipitate. Even though CHX shows both bactericidal and bacteriostatic properties, it lacks the tissue dissolution capacity of other irrigants. The tested protocols in the study done by *Trevino*, show survival of SCAP, yielding no viable cells (5). It has broad spectrum antibacterial action, sustained action and low toxicity. Because of the above properties of this, it has been recommended as a potential root canal irrigant. Dentin medicated with CHX acquires antibacterial substantivity. The adsorption of positively charged ions released by CHX prevents bacterial colonization on the dentin surface, and the duration of this effect exceeds the period of medicament application (40).

Calcium hydroxide:

Ruparel et al evaluated the effect of 1 mg/mL of calcium hydroxide using an automated method of detecting trypan blue dye and found that 68.3% ± 15% SCAP survival. *Althumairy et al* evaluated the effect of calcium hydroxide using Cell Titer-Glo Luminescent Cell Viability Assay method and found that there was greater survival of SCAP.

The antibacterial effect of calcium hydroxide is due to its alkaline pH. It dissolves the bacteria and necrotic tissue and by products (41). Because of its toxicity ,it should be placed within the canal with a help of a file or needle. Extrusion of the material into the periapical tissues can cause tissue necrosis and pain for the patient. (42) Calcium hydroxide promoted survival and proliferation of cells. Dentin conditioning with calcium hydroxide had no detrimental effect on SCAP survival. Instead, greater survival and proliferation were detected when SCAP were placed in contact with the conditioned dentin(11).

Calcium hydroxide has been shown to promote survival and proliferation of SCAP when directly added to the culture medium. All concentrations of this material promoted cell survival. This finding was unique among the experimental drugs evaluated in this study and is consistent with several studies that have shown the potential of Ca(OH)2 to induce repair by the formation of a hard-tissue barrier in vital pulp therapy or the formation of reactionary dentin when used as a cavity liner in indirect pulp-capping procedures (43-46). It is possible that it remains in the dentin after irrigation, having a direct effect on the dentinal wall. In addition, Ca(OH)2 has been shown to solubilize and release transforming growth factor- beta 1 and other growth factors embedded in dentin. Ca(OH)2 can be removed from the canal by using irrigants such as saline, NaOCl,EDTA or MTAD (42).

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