

Citric Acid in Endodontics: A Review

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Abstract

Naturally occurring in citrus fruits, citric acid is categorized as a weak organic acid, which may exist both as a monohydrate or in an anhydrous form. In biochemistry, this organic acid acts as an intermediate in an essential metabolic process in all aerobic organisms called the Krebs cycle. The literature on citric acid in the context of endodontics up to June 2020 was reviewed using PubMed and MEDLINE. This review aimed to address the antibacterial efficacy of citric acid on endodontic microbiota, its effectiveness on the smear layer, the effects of its toxicity on fracture resistance of dentin, as well as the effectiveness of this acid on the removal of intra-canal medicaments. This report also addressed citric acid-containing endodontic irrigants.

Keywords: Antibacterial Activity, Calcium Hydroxide, Citric Acid, Smear Layer, Toxicity.

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Introduction

Citric acid, a weak organic tribasic acid occurring naturally in citrus fruit, was first isolated from lemon juice in the form of crystals by Carl Wilhelm Scheele (1). In biochemistry, it serves as an intermediate in an essential metabolic process called the Krebs cycle, which takes place in all aerobic organisms. Citric acid is mass-produced annually and is commonly used as an acidifier, a flavoring agent, as well as a chelator (1, 2).

A diverse set of fruits and vegetables, citrus fruit, in particular, contain greater than trace amounts of this acid. Founded on the fruit industry in Italy, industrial-scale processing of citric acid was initiated in 1890, where adding hydrated lime to the juice would initiate the precipitation of calcium citrate. This calcium salt was then collected and turned back into acid using a dilute sulfuric acid solution (1, 2).

In 1893, Wehmer (3) was credited for the discovery of the production of citric acid from sugar with the help of *Penicillium* mold. However, the industrial importance of the microbial production of citric acid was not revealed until the disruption of Italian citrus exports during World War I. Later in 1917, Currie (4) discovered the citric acid production efficiency of some *Aspergillus Niger* mold strains. Two years later, the Pfizer Company began the industrial-level production of citric acid using this process, which remains the prime industrial technique for the production of citric acid to this day. In this production technique, *Aspergillus Niger* cultures fed on sucrose or glucose-enriched medium are utilized to produce citric acid. After the filtration of the molds from the resultant solution, calcium hydroxide is used to precipitate calcium citrate salt. Similar to its extraction from citrus fruit, citric acid is regenerated by the addition of sulfuric acid to the salt solution. Citric acid can also be synthesized under high pressure from other calcium salt solutions, including calcium aconitate, isocitrate, and allosictrite (5). As well as addressing citric acid-containing endodontic irrigants, this review aimed to address the antibacterial efficacy of citric acid on endodontic microbiota, its effectiveness on the smear layer, the

effects of its toxicity on fracture resistance of dentin, and the effectiveness of this acid on the removal of intracanal medicaments.

Search Strategy

The literature on citric acid in the context of endodontics up to June 2020 was reviewed using PubMed and MEDLINE.

Chemical Characteristics

Citric acid may exist as a monohydrate or in an anhydrous form. Hot water causes the anhydrous citric acid to crystallize, whereas the monohydrate is crystallized with cold water. The conversion of the monohydrate into the anhydrous takes place at 78 °C. Additionally, Citric acid is highly soluble in absolute ethanol (76 parts of citric acid per 100 parts of ethanol) at 15 °C. When above 175 °C, citric acid decomposes while releasing carbon dioxide (6).

Silva et al. (7) showed that citric acid is commonly categorized as a tribasic acid, with pKa values of 5.21, 4.28, and 2.92 at 25 °C. Using spectroscopy, Maniatis et al. (8) showed that the hydroxyl group has a pKa value of 14.4. According to the speciation diagram, solutions of citric acid are buffers with pH values in the range of pH=2 and 8. Generally, citrate and mono-hydrogen citrate are the two present ions in biological systems, where pH is around 7. Metallic cations assist the citrate ion in forming complexes with quite large stability constants due to the chelate effect, as a result of which, the ion is even capable of forming complexes with positively charged alkali metal ions. However, 7 to 8-membered chelate rings will form when all three carboxylate groups are used to form a chelate complex. These rings are thermodynamically less stable than similar rings with fewer members. Therefore, to form a more stable ring with 5-members $(\text{NH}_4)_5\text{Fe}(\text{C}_6\text{H}_4\text{O}_7)_2 \cdot 2\text{H}_2\text{O}$, the hydroxyl group can undergo a deprotonation process (9).

Antimicrobial Activity

Based on the findings of Yamaguchi et al. (10), solutions of citric acid were shown to have antibacterial properties, when were tested on root canal bacteria. For *Enterococcus faecalis*, Arias-Moliz et al. (11) calculated the minimum bactericidal concentration and reported it to be 20%. Additionally, based on their report, even one hour after use, ethylenediaminetetraacetic acid (EDTA) showed no bactericidal activity.

Smear Layer Removal

In order to remove the smear layer, citric acid can be utilized as an irrigant for the canal system (12). Different concentrations, from 1% to 50%, have been used (13). The superior effectiveness of 10% citric acid over ultrasound in removing the smear layer from apical root-end cavities was reported by Gutmann et al. (14). In the study of Yamaguchi et al. (10), the chelating properties of citric acid were compared to those of EDTA, concluding that the powdered mixture of dentine–resin was more soluble in 0.5M, 1M, and 2M citric acid solutions than in a 0.5M EDTA. According to Liolios et al. (15), the commercial EDTA preparations were better smear layer removers when compared to 50% citric acid solution. The findings of both Di Lenarda et al. (16) and Scelza et al. (17) showed a minor to no difference in smear layer removal properties of 15% EDTA and citric acid. In the study of Machado-Silveiro et al. (18), dentin was immersed in 1% and 10% citric acid solutions as well as 10% sodium citrate, and 17% EDTA for the duration of 5-15 minutes in order to measure the demineralization capabilities of the named solution. Based on their results, 10% citric acid was a better demineralizer than 1% citric acid, which was, in turn, better than EDTA. Takeda et al. (19) showed that irrigation with 6% citric acid, 17% EDTA, and 6% phosphoric acid was not effective in removing the smear layer from the canal. According to Reis et al. (20) upon 60 seconds of application, citric acid solutions were capable of removing the smear layer.

Toxicity

Using the MTT assay, Prado et al. (21) revealed higher cell viability in 10% citric acid relative to other studied irrigation solutions. The ability of genetic damage or cell death on fibroblasts was evaluated by Marins et al. (22). According to their observations, although not genotoxic, citric acid, sodium hypochlorite (NaOCl), and EDTA were discovered to be cytotoxic when administrated in specific doses. Kang et al. (23) assessed the biocompatibility of the mineral trioxide aggregate (MTA) combined with certain hydrating accelerators, such as calcium chloride, citric acid, and calcium lactate gluconate solution. Findings demonstrated the best results for the mixture of MTA with 0.1% citric acid.

Effect on Fracture Resistance

Arslan et al. (24) measured the impact of varying concentrations of citric acid irrigation on root fracture at various time exposures. Their results showed the highest fracture resistance upon 10 minutes of irrigation with 50% citric acid, while the lowest resistance was observed when the root fracture was irrigated with 10% citric acid for 1 minute.

Effect on Calcium Hydroxide Removal

Arslan et al. (25) demonstrated the superior efficiency of irrigation solutions of 7% maleic acid and 10% citric acid compared to 1% NaOCl and 17% EDTA in the removal of calcium hydroxide mixed with 2% chlorhexidine (CHX) from the root canal. In the samples irrigated with 17% EDTA, 7% maleic acid, and 10% citric acid, the orange-brown precipitate was not observed.

Root Canal Irrigants Containing Citric Acid

a) MTAD

In 2003, Torabinejad et al. (26) introduced BioPure (Dentsply, Tulsa Dental, Tulsa, OK, USA), better known as MTAD, which consists of 3% doxycycline, 4.25% citric acid, and 0.5% Polysorbate 80 as a detergent.

Antimicrobial Activity

The effectiveness of MTAD against *Enterococcus faecalis* was demonstrated for the first time by Torabinejad et al. (26). Also, MTAD was reported to be substantially more effective against *Enterococcus faecalis*, when compared to the combination of NaOCl and EDTA. Shabahang et al. (27) found that MTAD was more effective than 5.25% NaOCl in endodontic disinfection, using a human tooth model. Kho and Baumgartner (28) showed consistent disinfection of the *Enterococcus faecalis* contaminated canals when NaOCl/EDTA mixture was used. However, when combined, 1.3% NaOCl/MTAD left close to 50% of the canals infected with *Enterococcus faecalis*. Krause et al. (29) compared the antimicrobial effects of NaOCl and MTAD against *Enterococcus faecalis*. In the agar diffusion test, NaOCl provided less bacterial inhibition than MTAD. Ghoddsi et al. (30) utilized MTAD as a final irrigation solution for the smear layer removal. Davis et al. (31) observed that MTAD was significantly more effective against *Enterococcus faecalis*, when compared to NaOCl and CHX. Newberry et al. (32) found that when MTAD was 1:8192 times diluted, most strains of *Enterococcus faecalis* were inhibited, although most were killed with 1:512 times dilution. Shabahang et al. (33) demonstrated that the effectiveness of MTAD was not adversely impacted by the addition or replacement of CHX with doxycycline. Portenier et al. (34) showed that there was a pronounced delay in the killing of bacteria due to the presence of dentin or bovine serum albumin.

Substantivity of MTAD

Tetracyclines can be easily bound to dentin and released while retaining their antibacterial activity (35). Therefore, the presence of doxycycline in MTAD may be an indication of some significant antimicrobial activity

(26). Mohammadi and Shahriari (36) demonstrated in an *in vitro* study that the substantivity of MTAD was substantially greater than that of NaOCl and CHX. In another analysis, the substantivity of 100% MTAD was evaluated to be significantly greater than that of the two other tested concentrations (37). When added to 1.3% NaOCl-irrigated dentin, MTAD showed a reduction in its antimicrobial substantivity, according to Tay et al. (38).

MTAD and Biofilms

Clegg et al. (39) indicated that the only irrigant capable of deactivating viable bacterial cells as well as the physical removal of the biofilm was 6% NaOCl. Dunavant et al. (40) reported that 16% of bacterial cells in *Enterococcus faecalis* biofilms were killed by MTAD, while the inability of MTAD to degenerate and remove bacterial biofilms was indicated by Giardino et al. (41).

Smear Layer Removal and Effect on Dentine

When canals were irrigated with NaOCl and rinsed with MTAD as the final irrigant, Torabinejad et al. (26) reported on the efficacy of MTAD in removing the smear layer, while no significant change in the structure of the dentinal tubules was observed. They found in another analysis that while much of the smear layer was removed when MTAD was used, the surface of the root canal walls still contained the scattered remaining remnants of the smear layer's organic portion (42). When used at low concentrations prior to using MTAD as the final irrigant, NaOCl enhanced the efficiency of MTAD in the complete removal of the smear layer. However, Lotfi et al. (43) showed that MTAD could not remove the smear layer. Tay et al. (44) found that a region of demineralized collagen matrices in eroded dentine and around the dentinal tubules was created by both MTAD and EDTA, while the demineralized dentin matrix created by MTAD was thicker than that of EDTA. It was observed by De-Deus et al. (45) that MTAD was capable of prompting significantly faster demineralization kinetics than 17% EDTA. There is only one study on the impact of MTAD on dentine, which was done by Machnick et al. (46). According to their findings, there was no significant difference in flexural strength and modulus of elasticity between the dentine exposed to MTAD and those that are not.

MTAD and Dentin Bonding (Anticollagenolytic Activity)

It was reported by Machnick et al. (47) that before the application of the dental adhesive, additional dentine conditioning might not be needed by endodontically treated teeth with the MTAD protocol for clinical use (20 min 1.3% NaOCl/5 min MTAD). Garcia-Godoy et al. (48) indicated that the hybrid layer of MTAD was thicker

than that of the 17% EDTA. There was a lower chance of nano-leakage in hybrid layers created in dentin covered with smear layer, than either of MTAD and EDTA hybrid layers (49). Yurdagüven et al. (50) demonstrated that after using MTAD as a root canal system irrigant, the bonding potential between Clearfil SE Bond and coronal dentine was significantly decreased.

Toxicity of MTAD

According to Zhang et al. (51), while showing more cytotoxicity than 2.6%, 1.3%, and 0.66% NaOCl, MTAD was less cytotoxic than H₂O₂, calcium hydroxide, 5.2% NaOCl, and EDTA. Yasuda et al. (52) showed the lower cytotoxic activity of MTAD as well as its inert behavior towards differentiation into osteoblasts, in comparison with other irrigation solutions such as NaOCl, EDTA, or CHX.

Tetraclean

Like MTAD, Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) is an acid-containing detergent with antibacterial properties. However, it differs from MTAD in the type of detergent (polypropylene glycol) as well as in antibiotic and doxycycline (50 mg/ml) concentrations (53). The surface tensions of 5.25% NaOCl, MTAD, Tetraclean, 17% EDTA, Cetrexidin, and Smear Clear were compared by Giardino et al. (54), concluding that NaOCl and EDTA showed the greatest surface tensions, compared with Cetrexidin and Tetraclean.

Antibacterial Activity

To date, there are minimal studies on Tetraclean antibacterial activity, including the one by Giardino et al. (41), which compared the antimicrobial effectiveness of NaOCl, MTAD, and Tetraclean against *Enterococcus faecalis*. Although, in comparison with MTAD, the biofilm exhibited a significant level of disaggregation at each time interval when treated with Tetraclean, only NaOCl was able to disaggregate and remove the biofilm at each tested time interval. The effectiveness of Tetraclean against *Enterococcus faecalis* was further demonstrated by Neglia et al (55).

Using the agar diffusion test, Ardizzoni et al. (56) demonstrated the effectiveness of Tetraclean against *Enterococcus faecalis*. Furthermore, Giardino et al. (57) reported on the superior efficiency of Tetraclean over CHX against common endodontic bacteria. According to Pappen et al. (58), compared to MTAD, Tetraclean was more effective against *Enterococcus faecalis*. An agar diffusion test was conducted by Poggio et al. (59) in order to show that Tetraclean's effectiveness against

Enterococcus faecalis, *Streptococcus mutans*, and *Staphylococcus aureus* was significantly higher than that of NaOCl. Mohammadi et al. (60) analyzed the efficacy of NaOCl, CHX, Tetraclean, and Hypoclean against *Enterococcus faecalis* and some other common endodontic bacterial strains, concluding that Hypoclean was the most effective irrigation solution.

Substantivity of Tetraclean

Among the minimal studies on the substantivity of Tetraclean, Mohammadi et al. (61) found that in addition to retaining in root canal dentin for a minimum duration of 28 days, the substantivity of Tetraclean was considerably higher than MTAD. In subsequent research, Mohammadi et al. (62) also revealed that the substantivity of Tetraclean was significantly higher than that of Hypoclean and NaOCl. They further reported on a direct relationship between Tetraclean substantivity and dentine exposure duration (63). Pretreatment of dentine with NaOCl may substantially decrease the substantivity of Tetraclean (64).

Smear Layer Removal Ability

Poggio et al. (65) compared the demineralizing potential of Tetraclean on dentin with that of EDTA and Tubuliclean. Results concluded that the greater release of Ca²⁺ in samples treated with Tetraclean was the important reason for Tetraclean's outstandingly its higher demineralization in comparison with other tested irrigation solutions.

Instrumentation must be carried out with active irrigation solutions in order to improve the efficiency of mechanical root canal preparation and bacterial removal. Future irrigant studies need to concentrate on identifying a single irrigant with tissue dissolving ability, smear layer removal property, and antibacterial efficacy (66-77).

Conclusion

Citric acid is a weak organic acid that can act as an intermediate in the metabolic pathways of aerobic organisms. MTAD and Tetraclean are two kinds of citric acid-containing root canal irrigants. Although some studies have confirmed their suitability as root canal irrigants, more research should be done on this topic in the near future.

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