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A comparative evaluation of calcium ion release and pH change using calcium hydroxide nanoparticles as intracanal medicament with different vehicles – An in vitro study.

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Abstract

Introduction: It has been demonstrated that nanocalcium hydroxide (NCH) has a number of advantages over regular calcium hydroxide, indicating the possibility of using it as an intracanal medication.

Aim: The objective is to assess and contrast calcium ion release and pH modification when NCH is used as an intracanal medication in various vehicles.

Subjects and Methods: 30 recently excised premolars were length-standardized and decoronated at the cementoenamel junction level. By means of the protaper rotary mechanism, canals were extended to F2 size. Three groups of the specimens (n = 10) were created: NCH + distilled water (Group A), NCH + propylene glycol (Group B), and NCH + chitosan (CT) (Group C). Only the apical third of the roots of each specimen, which was floated in 6 cc of deionized water using silicone putty, were submerged. Using a pH meter and a UV spectrophotometer, the pH and calcium ions were measured at 24 hours, 7 days, 14 days, and 28 days respectively.

Statistical Analysis: The Kruskal-Wallis ANOVA with post-hoc Dunn's test was used to compare the mean release of calcium ions and pH shift. 5% was chosen as the degree of significance.

Results: In 24 h, 7 days, 14 days, and 28 days, there was a discernible difference during comparison of the three groups in the discharge of calcium ions and pH alteration.

Conclusions: NCH combined with propylene glycol demonstrated an alkaline pH and sufficient calcium ion discharge for 28 days. At the conclusion of 28 days, NCH combined with CT demonstrated an elevated pH.

Both mixtures demonstrated their effectiveness as intracanal medications.

Keywords: Chitosan, Nanocalcium Hydroxide, Propylene Glycol, And Calcium Ion Discharge.

Introduction

The use of intracanal medication (ICM) is advised when curing infected root canals for a number of causes. After root canal preparation, it eliminates any remaining bacteria, lessens inflammation in the pulpal and periapical tissues, neutralizes tissue debris and makes the components of the canal inert, inhibits leakage by being a barrier alongside the temporary filling, and helps the drying of obstinately wet canals. [1] In hopes of achieving full healing of the periapical tissues, a microbe-free environment must be created prior to the obturation of the root canal. [2,3]

In endodontics, calcium hydroxide (CH) is indeed a popular material. The substance has been applied during a variety of clinical treatments, such as cleansing root canals, stopping inflamed root dissolution, and fostering apexification.[4] By removing fatty acids, CH acts as bactericidal while decreasing the potency of the residual lipopolysaccharides and other cell wall constituents.[5] Its tendency to split in calcium and hydroxyl ions, raising pH, is intimately associated to the manner of action.[6] The pH stays elevated after setting because CH reacts quickly in an aqueous phase releasing hydroxyl ions. The elevated pH encourages the tooth to heal naturally even in the absence of bacterium. The method of delivery of CH is critical because it impacts the rate of ionic disintegration, that determines how quickly the periapical tissues in the canal can dissolve & reabsorb the mixture. [7]

The carriers could be oil-based (eugenol, silicone oil, olive oil, metacresyl acetate, camphor), viscous (glycerin, propylene glycol (PG), polyethylene glycol (PEG), or water-soluble (normal saline, distilled water (DW), local anaesthetic, Ringer's solution). [4,8,9] Whilst viscous vehicles would release ions over time, the watery vehicle promotes dispersibility. They reduce the amount of replacement periods needed by slow releasing calcium ions (Ca) over extended periods of time. [9] According to reports, the carrier used makes it easier for Ca ions to pass and through apex & change the pH. [10]

PG is a white fluid containing dihydric alcohol. It possesses a sweet flavour and a light odour. In contrast to other ICM delivery methods, PG is a little less cytotoxic & possesses antibacterial qualities that really are helpful in endodontic treatment. [12] A polysaccharide called chitosan (CT) is employed as a medicinal additive. It is produced by partially deacetylating chitin, a component of the exoskeleton of arthropods. This is composed of copolymers of glucosamine & N-acetylglucosamine. [13] It exhibits a range of biologic attributes, comprising prolonged release, mucoadhesive, wound repair, hypercholesterolemic, & antimicrobial activities. [14]

There in the realm of Endodontics, nano-calcium hydroxide (NCH) has only just lately been developed. Being antibacterial agents, these are becoming more and more common in dentistry and medicine. Nanoparticles are minuscule objects with a size not more than 100 nm. Those compounds communicate with their surroundings more due to their higher surface-to-volume ratios and charge densities, which increases their antibacterial effect. Nanoparticles of calcium hydroxide eliminate the endodontic bacteria that are present in the dentinal tubules. NCH has benefits over CH, including less surface modification, improved access into greater depths of dentin, and improved antibacterial action towards high pH-resistant Enterococcus faecalis, that have been the subject of past investigations.[15] As a result, these are able to serve as an intracanal medicament during root canal treatment. The long-term release of calcium (Ca) ions from CH coupled with different carriers has been studied. Yet, hardly researchers compared persistent Ca ion release with NCH coupled with different vehicles have been published in the endodontic field (DW, PG, CT). So, this study was conducted to assess Ca ion discharge and pH modification by mixing NCH with different vehicles (DW, PG, CT).

Subjects and methods

Assortment and processing of samples: The projected size of the sample was 30, the study's power was 95%, and the significance level was P 0.05. Newly extracted maxillary premolars with equivalent root length, diameter, and taper were procured, manually cleansed with an ultrasonic scaler, and stored in normal saline at room temp. Using water spray, diamond discs with a 15 mm length were utilised to decoronate the teeth at the cement-enamel interface. Using a 10-k reamer, the canal's patency was established, as well as the working length got determined by subtracting 1.0 mm out from file's exit at the apex.

Biomechanical Preparation

Protaper rotary files up to size F2 were used for standardised biomechanical preparation. A bevelled 27gauge stainless steel needle that was placed 1 mm short of the working length was utilised for passive irrigation using 2 mL of 2.5% NaOCl over 1 minute after every instrumentation.

The canal was rinsed with DW (5 ml) to remove residual precipitation after a last irrigation of 2 mL of a 17% ethylene diamine tetraacetic acid solution lasted for 1 minute. The canals were dried with sterile paper points. The samples were assigned to three groups at random: DW and NCH (Group A; n = 10), PG and NCH (Group B; n = 10), & CT & NCH (Group C; n = 10).

Nano-Calcium Hydroxide Paste Preparation

For the initial two groups, a paste was created by combining 150 mg of NCH powder with 0.15 ml of each of the various vehicles in accordance with the manufacturer's instructions. For the third group, lentulo spirals were used to insert a thick paste made of 150 mg of NCH powder and 0.20 ml of 3%(w/v) CT solution into the root canal. The paste was compacted into the canals using hand pluggers. The medication was positioned with enough care to guarantee that no residue from the substance stuck to the surrounding surface. Glass ionomer cement was used to seal the opening.

Just the apical portion of the tooth was suspended in glass vials with 6 ml of DW using silicone putty was submerged. The pH and calcium ion concentrations then were determined.

Measurement of Calcium Ion Concentration

After 24 hours, 7 days, 14 days, and 28 days out of each group, one millilitre of DW from the glass vials was subsequently removed, and each time, it was replaced with new DW.

The Ca ion concentration then was determined using a standard calibration curve made with water after the mixture was examined using an ultraviolet (UV) spectrophotometer (1601 PC, Shimadzu, Japan) at 220 nm.

pH Measurement

A pH meter (Systronics Limited, Mumbai) was used to quantify the pH shift in DW at periods of 24 hours, seven days,14 days and 28 days.

The solution's pH value was measured after each 5 seconds of whirling the mixture to disperse the hydroxyl ions evenly in order to determine how much each formulation's pH changed. Between observations, the electrode was cleaned with DW and wiped with sterilized tissue paper.

Statistical Analysis

Version 20 of the SPSS (Statistical Program for the Social Sciences) programme was used to conduct the statistical analysis (IBM SPSS corp. Released 2011). The Shapiro-Wilk test was used to determine whether the statistics were normal. The dataset had an atypical pattern. The Kruskal-Wallis ANOVA with post hoc Dunns test was used to compare the mean calcium release and pH. The threshold for statistical significance was P 0.05.

Results

In each of the four time points, there was a statistically significant difference in the mean calcium ion concentration between the three groups (P 0.001, 0.001, and 0.001, respectively). At 24 hours, 7, 14, and 28 days, respectively, the post-hoc test revealed that group B had higher mean calcium ions compared to all other groups. At 14 and 28 days, group C had the least calcium ion concentration [Table 1].

In 24 h, 7, and 14 days, there wasn't a statistically relevant disparity in the mean pH between the 3 groups (P = 0.113, 0.881, and 0.651, resp).

The mean pH at 28 days, however, varied significantly between the three groups. Group C had the greatest pH, trailed by Groups B and A according to the post-hoc analysis [Table 1].

Table 1: Intergroup comparative analysis of mean calcium-ion release & pH alterations at variable time period

	Mean±SD			P -value	Post-hoc test
	Group A	Group B	Group C		
Calcium-ion Released					
24 h	9.85±0.6	13.62±0.88	11.36±0.8	<0.001	2>3>1
1 week	22.82±1.08	30.85±1.86	23.95±1.03	<0.001	2>1,3
14 days	36.12±1.56	53.55±1.57	33.45±1.61	<0.001	2>1>3
28 days	61.63±1.08	73.21±1.67	52.62±1.78	<0.001	2>1>3
p^H Altered					
24 h	7.43±0.17	7.78±0.71	7.4±0.13	0.113	-
1 week	7.71±0.53	7.79±0.67	7.86±0.61	0.881	-
14 days	8.44±0.42	8.31±0.51	8.35±0.53	0.651	-
28 days	10.21±0.29	10.23±0.18	10.88±0.55	<0.001	3>1,2

SD: Standard deviation. P-value <0.05 was considered statistically relevant

Discussion

Due to its compact size and large surface area, NCH can efficiently kill endodontic bacteria in the dentinal tubules by penetrating the deep dentin layers. [15] Whenever taken as an intracanal drug for 4 weeks, NCH has similarly been demonstrated to result in fewer alterations in the dentin microhardness than CH. [16] Numerous research have assessed the relationship between CH and different carriers. [8-10] When utilised as a carrier with CH, prior investigations have demonstrated the controlled releasing properties of PG and CT. [10,14,17] And therefore, in this research, PG, CT, and DW were chosen as a carrier for NCH to assess its efficacy as a substitute intracanal medication.

Formerly, the pH of radicular dentin was investigated over a span of four weeks, which was determined to be an adequate time for CH-based materials to offer therapeutic benefits. [18]

However, certain CH formulations simply exhibit a brief discharge of Ca ions. Out of a clinical perspective, this indicates that the root canal will need to be redone several times before the desired result is realised, increasing the number of consultations. [19] Hence, throughout the course of 28 days, different time durations of medication release and pH change were tested.

According to a prior study, human removed teeth were used in this one. [14,17] By immersing only the apical portion of the tooth in DW to simulate a clinical scenario, NCH diffusion from the canal opening was reduced.

A calorimetric technique, flame photometry, atomic absorption spectrophotometry, and a UV spectrophotometer can all be used to precisely quantify the release of Ca ions.

[20] An UV spectrophotometer was used in the current investigation since the assessment was fast and inexpensive compared to other techniques. [14]

There at completion of 28 days, Group B had the largest Ca ion release when compared across groups. This is consistent with other studies[14] and may be attributable to PG's hygroscopic nature, which enables absorption of water and offers a better, more sustained release of CH for a longer time frame than a paste comprising DW. Other advantage is that the consistency makes the paste easier to handle. In comparison with other vehicles, it causes the highest hydroxyl and Ca ion discharge. [21] The watery character of DW, which fostered a faster ion release and ought to be employed in clinical settings like dental replantation and severe exudative instances, can be blamed for the abrupt Ca ion release by Group A after 7 days. Fast release is advantageous in healthcare settings where inter-appointment disinfection is needed for a brief period of time. [10]

With an initial rush of release, followed by a steady release over time, CT revealed a biphasic release pattern. An early surge effect is typical with CT-based administration techniques since they contain free amino groups. The medicine that is on the polymer's surface is promptly dispersed into the surrounding fluid upon mixing. Prior to the active component being released and the following drug diffusion, the polymer first swells. Release of drugs is influenced by ionic interactions between CT chains depending on the cross-linking density established during matrix network development. [14,22] In clinical situations like inflammatory root resorption, the recovery of substantial periapical lesions, and lowering the frequency of canal re-dressing, extended discharge is helpful. [18,23]

All 3 experimental groups showed a significant elevation in the surrounding medium's pH (pH > 10) that was similar to earlier research. [14] After 28 days, Group C's pH was found to be high. The same findings were obtained with Chitosan-based accelerated Portland cement that employed different doses of CT solution in previous research,[24] which found that it had a high alkaline pH of 11. The amino groups in CT undergo protonation, which raises pH and contributes to its enhanced alkalinity. [24]

Further research can be done to assess the antibacterial efficacy of various NCH preparations. To further support NCH's effectiveness as an intracanal medication, researchers can examine and evaluate the period of action and dentinal tubular penetration depth of different NCH preparations.

Conclusions

It was possible to draw the conclusion, within the constraints of the research, that NCH with PG demonstrated an alkaline pH and an acceptable calcium ion release for 28 days. After 28 days, CT displayed a high pH when combined with NCH. NCH might be used as a substitute for calcium hydroxide as an intracanal medicament.

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