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Cover page: A case of oligodontia receiving orthodontic treatment to improve function and aesthetics. Picture courtesy of Dr. Wey Mang Chek.
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The Malaysian Dental Journal covers all aspects of work in Dentistry and supporting aspects of Medicine. Interaction with other disciplines is encouraged. The contents of the journal will include invited editorials, original scientific articles, case reports, technical innovations. A section on back to the basics which will contain articles covering basic sciences, book reviews, product review from time to time, letter to the editors and calendar of events. The mission is to promote and elevate the quality of patient care and to promote the advancement of practice, education and scientific research in Malaysia.

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EDITORIAL: PUBLICATIONS AND KEY PERFORMANCE INDEX

Welcome to this issue of Malaysian Dental Journal.

As the official publication for Malaysian Dental Association, the Malaysian Dental Journal is slowly gaining popularity in this region. The electronic versions of the current and previous issues of MDJ have been made available at the secured e-journal section of the MDA website. We have received contribution of articles from authors in neighbouring countries i.e. Indonesia, India, Mongolia and Singapore. With more dental schools mushrooming in this country, contributions from local authors have been encouraging. There are ten dental schools in Malaysia at present i.e. six in public university and four in private university/college. Dental officers from the government sector have also been actively contributing as government has encouraged the dental officers to get involved in research, scientific paper presentation and publication.

Key performance index has been introduced to improve the efficiency and productivity of an organisation. Various layers in an organisation ranging from the top management to the supporting staff have to lay down measurable indices to bring the performance of the organisation to a greater height. Amongst the indices relevant to dentistry were improvement in patient charter, organisation and participation in community projects, creativity and innovations in teaching and learning activities, scientific paper presentations and publications.

With increasing emphasis on evidence-based practice to provide the best to the patients, scientific research, scientific presentations and publications have become an important triad to improve the provision of healthcare. This is especially so for academic institutions that train and produce the next generation of healthcare workers, much emphasis has been placed for staff, postgraduate students and also undergraduate students to conduct research and publish the scientific findings. The MDJ is currently a peer-reviewed, indexed journal and provide a good avenue for disseminating knowledge in the dental literature. It is hope that the academic institutions and government sector will encourage staffs to continue contributing articles to MDJ. It is also hope that academic staffs and specialists are willing to impart their expertise and spare their precious time in the manuscript reviewing process.

As the renewal of annual practicing certificate will be tied with CPD points in the near future, MDJ has provided another source for obtaining the CPD points. After reading through the articles in the journal, there is a short section of quizzes pertaining to the articles, upon answering the quiz, CPD points can be obtained from the MDA secretariat.

Thank you kindly for your warm support.

Associate Professor Dr. Seow Liang Lin
Editor
Malaysian Dental Journal
Inhibitory Effect of Mixed Paste of Ca(OH)\textsubscript{2} And Baso\textsubscript{4} against Bacteria In Infected Root Canal.

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Liesan, DDS.
Faculty of Dentistry, Trisakti University, Indonesia

ABSTRACT

The aim of this study was to evaluate the inhibitory effect of mixed paste of Ca(OH)\textsubscript{2} and BaSO\textsubscript{4} in various ratios against bacteria isolated from infected root canal. The isolated bacteria sample was taken from the lower first molar diagnosed with necrotic pulp. The sample of bacteria was selected by gram staining and further cultured in the brain heart infusion solution. Bacteria suspension in blood gel was given 5 holes with diameter of 5 mm to accommodate Ca(OH)\textsubscript{2} and BaSO\textsubscript{4} mixture with the ratio of 6:1, 7:1, 8:1, 9:1 and 10:1 to observe the inhibitory zone with intervals of 24 hours, 48 hours, 72 hours, and 10 days. The addition of BaSO\textsubscript{4} to Ca(OH)\textsubscript{2} decreased the inhibitory effect against bacteria growth. Two-way analysis of variants test between the inhibitory diameter with the ratio of Ca(OH)\textsubscript{2} and BaSO\textsubscript{4} and time of incubation showed significant difference (p < 0.05). There was a significant difference between ratio of 6:1 to the rest of other ratios in the inhibition of bacterial growth. There was also a significant difference between the incubation periods- 10 days incubation period was different from other incubation periods. Calcium hydroxide was more effective in eliminating aerobic bacteria compared to the anaerobic. Within the limitations of the present study, it is concluded that addition of barium sulfate affected the efficacy of antibacterial effect and the time period of contact between calcium hydroxide with the micro-organisms also has an effect on the bacterial growth.

Key words
bacteria inhibition, mixture of Ca(OH)\textsubscript{2} and BaSO\textsubscript{4}, aerobe and anaerobe bacteria, infected root canal

INTRODUCTION

Microorganisms are the main cause of endodontic infection. Many attempts for endodontic success are devoted to eliminate as many as possible microorganisms that exist in the root canal. In addition to the appropriate preparation of the root canals, medicament of root canals is required to achieve the objective.

The medicaments for root canals include chlorophenol camphor menthol, formocresol and cresatin. These medicaments have adverse impacts due to their antigenic and cytotoxic nature and short effectiveness. Apart from these medicament, calcium hydroxide Ca(OH)\textsubscript{2} have been proven its efficacy clinically and possessed beneficial characteristics to be used as ideal medicament for root canal. If the use of Ca(OH)\textsubscript{2} for maintaining pulp capping and pulpotomy is considered as a biological wound dressing, Ca(OH)\textsubscript{2} also serves as biological root healing at apex and periapical tissue. Besides triggering lipopolysaccharide degradation, Ca(OH)\textsubscript{2} with pH of 11.5 plays the role as powerful alkaline substance and is recommended by some researchers to be used as medicament for root canal and contains anti bacterial properties against most bacteria species found in endodontic infection.

The efficacy of Ca(OH)\textsubscript{2} as medicament for root canal will be maximum if injected during the process and make sure it fills the root canal space thoroughly. Radiograph may be taken to confirm that the entire root canal has been filled with the medicament. Pure calcium hydroxide is not visible under any radiograph, and to make this material radio-opaque, it must be added with a contrastive material, namely BaSO\textsubscript{4}.

The aim of this experiment was to find out the optimal ratio between Ca(OH)\textsubscript{2} and BaSO\textsubscript{4} in minimizing inhibiting the growth of various bacteria that trigger infection in the root canal.

Safavi & Nakayama (2000)\textsuperscript{1} have shown that pH of Ca(OH)\textsubscript{2} is 11.5 and to increase its pH , saline or anesthesia solution may be added and mixed with Ca(OH)\textsubscript{2} powder. Hydroxyl ions penetrate the dentinal tubule when smear
layer is cleaned using ethyl diamine tetra acid (EDTA) solution. The high pH of calcium hydroxide is effective to eliminate bacteria, as most bacteria cannot withstand pH ≥ 9.5, and only a few bacteria can live in a condition with pH=11 or higher. Consequently, Ca(OH)₂ may also be used as the “dressing” for root canal by killing the bacteria. I The most resistant bacterial within root canal and dentinal tubule are Enterococcus faecalis.

As some of the bacteria may survive in an environment with pH of 11.5, Stock (1995) argued that if the root canal contains much exudates, thick Ca(OH)₂ may be applied for 1-2 weeks and repeated until the root canal is completely dry. On the other hand, Rivera and Williams (1994) have mentioned that a redundant filling may trigger minor but acute inflammation and the excessive Ca(OH)₂ will be quickly reabsorbed due to the activity of macrophage cells.

Nerwich (1993) suggested that calcium hydroxide has bactericidal and disinfectant characteristics. High concentration of hydroxyl ion may eliminate microorganisms in the root canal that was not reachable during the biomechanical preparation. Hydroxyl ion may denature protein and hydrolyze fat in lipopolysaccharide of microorganisms. Consequently, the bacteria cell walls will be damaged and the bacteria are killed. Lipopolysaccharide are found on the surface of negative gram bacteria and own biological effect which may trigger periapical diseases.

Cohen and Burns (2002a) showed that Ca(OH)₂ was able to denaturalize protein and hydrolyse necrotic tissue, in both aerobe or anaerobe conditions. Safavi and Nichols (1993) suggested that the hydroxyl ion bound to lipopolysaccharide (LPS) will damage the ester bond of hydroxide acid fat as characterized with the loss of hydroxide fat. The use of Ca(OH)₂ in endodontics will lead to the detoxification of lipopolysaccharide residue within the root canal, and it was said that such might prevent bone resorption. Aside from that, Ca(OH)₂ is not a good heat conductor, it is easily manipulated and fairly stable and does not cause any tooth colouration. Cohen and Burns (2002b) also pointed out that the application of Ca(OH)₂ may alleviate dentinal sensitiveness from the external and internal stimulations as Ca(OH)₂ and CO₂ from the air will form CaCO₃ to protect against the pain. It was said that the application of Ca(OH)₂ causes only minor irritation to the tissue.

Calcium hydroxide may inhibit macrophage phagocytes and it therefore reduces the inflammation reaction in the periapical area. Calcium hydroxide paste will disperse into calcium ion and hydroxide ion. Calcium ion will mix with O₂ (air) and form calcium carbonate. The formation process of calcium carbonate (CaCO₃) in the root canal will be overly sluggish and clinically the amount will be insignificant.

**MATERIALS AND METHODS**

The tooth with necrotic pulp tissue was isolated using cotton roll and saliva injector. The pulp roof was opened with sterile round drill. The extirpation of the pulp tissue was conducted using sterile extirpation syringe and then directly put into the seedling tube containing Thioglycolate. In this study, the sample was taken from the first right lower molar diagnose with necrotic pulp. The sample was taken from mesiobuccal, mesiolingual and distal roots. The incubation was carried out at the temperature of 37°C for 24 hours.

After 24-hour, all bacteria were Gram stained to differentiate the types of bacteria. The bacteria were planted into the blood medium and incubated at the temperature of 37°C for 24 hours. The growing bacteria were further identified. These types of acquired bacteria were made as the sample with the exception of hypha fungi and fungi because it had to employ a special media such as Sabourand dextrose gel.

Then each sample was planted in BHI (Brain Heart Infusion) solution and left in room temperature for 6-8 hours. The bacteria sample planted to BHI was called “suspension”. After 6-8 hours the bacteria suspension was poured into the blood bel, spread evenly until homogenous, with the ratio of one bacteria suspension = one blood gel and in each blood gel was given 5 holes with the diameter of 5 mm to accommodate the Ca(OH)₂ and BaSO₄ solution of various ratio. When inhibitory zone occurs, it will be visible around such holes. The ratio of Ca(OH)₂ and BaSO₄ in 6:1, 7:1, 8:1, 9:1 and 10:1 were investigated. The inhibitory zone was observed at intervals of 24 hours, 48 hours, 72 hours, and 10 days.

**RESULTS**

The bacteria that have been isolated from the necrotic pulpal tissue were as shown below in Table 1.

**Table 1. Types of bacteria found in necrotic pulp**

<table>
<thead>
<tr>
<th>Location</th>
<th>Types of bacteria Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal Root</td>
<td>Gram + Bacillus ++</td>
</tr>
<tr>
<td></td>
<td>Gram + Coccobacil ++</td>
</tr>
<tr>
<td></td>
<td>Gram + Staphylococcus anaerobe +</td>
</tr>
<tr>
<td>Mesiobuccal Root</td>
<td>Gram + Bacillus +++</td>
</tr>
<tr>
<td></td>
<td>Gram + Coccobacil ++</td>
</tr>
<tr>
<td></td>
<td>Gram + Staphylococcus +</td>
</tr>
<tr>
<td></td>
<td>Fungi +</td>
</tr>
<tr>
<td>Mesiolingual Root</td>
<td>Gram + Bacillus anaerobe ++</td>
</tr>
<tr>
<td></td>
<td>Fungi +</td>
</tr>
</tbody>
</table>

**Notes:**

+ = 100 bacteria per specimen with 100 x magnification
++ = 101-250 bacteria per specimen with 100 x magnification
+++ = 251-400 bacteria per specimen with 100 x magnification
Table 2 showed that the inhibitory zone of all types of bacteria observed become larger with increased observation period of 24 hours, 48 hours, 72 hours and 10 days. In general, higher ratio of calcium hydroxide to BaSO₄ has larger inhibitory effect to all types of bacteria.

Based on the diameter inhibitory zone measurement, it was shown that the largest inhibitory effect was in the ratio of Ca(OH)₂ : BaSO₄ = 10:1 and the incubation time was 10 days as shown in Table 3. However, based on the result of multiple comparison test with HSD Tukey test, it was shown that such result was not statistically significant different at the ratio of Ca(OH)₂ : BaSO₄ at 7:1, 8:1 and 9:1 within the 10-day incubation.

The result of statistical test with two-way analysis of variants between the diameter of blocking and ratio of the average magnitude of inhibitory zone during the 4 periods in all types of anaerobe bacteria observed and in various ratios of Ca(OH)₂ : BaSO₄ during incubation, indicated a significant difference (p < 0.05). Accordingly, a multiple posterior comparative test was administered using the honestly significant difference (HSD) from Tukey.

Prior to the test using HSD Tukey, a one-way variant analysis was administered among the treatment groups to obtain the critical value that will be used as the multiplier. Based on one-way anova, the multiplier of 5.07 was obtained with average quadrate (Msc) of 2.68. With such value, HSD value as the differential figure of 2.369 was acquired from among the average groups.

There was a statistical significant difference between the ratio of 6:1 and 7:1, 6:1 and 8:1, 6:1 and 9:1, 6:1 and 10:1. The addition of BaSO₄ to Ca(OH)₂ affected its inhibitory effect against bacteria that trigger endodontic infection (Table 3). There was also a significant difference between incubation period of 72 hours and 10 days with all various ratios of Ca(OH)₂ : BaSO₄. There was no significant difference between incubation period of 24 hours, 48 hours and 72 hours all various ratios of Ca(OH)₂ : BaSO₄ except 9:1 and 10:1 at 48 and 72 hours.

Table 2 The average and standard deviation of the observation results of root canal bacteria inhibitory zone based on the ratio of Ca(OH)₂ : BaSO₄ and the seeding time.

<table>
<thead>
<tr>
<th>Ratio of Ca(OH)₂ : BaSO₄</th>
<th>Seeding time</th>
<th>Average ± standard deviation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6:1</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>1.208 ± 0.334</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>1.625 ± 0.606</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>2.625 ± 0.882</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>4.083 ± 2.275</td>
</tr>
<tr>
<td></td>
<td>7:1</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>1.917 ± 0.900</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>3.000 ± 1.243</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>4.417 ± 2.592</td>
</tr>
<tr>
<td></td>
<td>8:1</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>2.208 ± 1.076</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>3.125 ± 1.316</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>4.625 ± 2.595</td>
</tr>
<tr>
<td></td>
<td>9:1</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>2.458 ± 1.322</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>3.500 ± 1.784</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>4.917 ± 2.721</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>24 hours</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>2.542 ± 1.322</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>3.625 ± 1.785</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>5.292 ± 3.056</td>
</tr>
</tbody>
</table>

Table 3 The average diameter of inhibitory zone during the 4 observed intervals and various ratios of Ca(OH)₂ and BaSO₄.

<table>
<thead>
<tr>
<th>Ratio of Ca(OH)₂ : BaSO₄</th>
<th>Inhibitory zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours 48 Hours 72 Hours 10 days</td>
</tr>
<tr>
<td></td>
<td>6:1</td>
</tr>
<tr>
<td></td>
<td>7:1</td>
</tr>
<tr>
<td></td>
<td>8:1</td>
</tr>
<tr>
<td></td>
<td>9:1</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
</tr>
</tbody>
</table>

Tables 4 and 5 showed that in general the inhibitory zones were smaller in anaerobic bacteria compared to the aerobic bacteria. This meant that Ca(OH)₂ was more effective in eliminating aerobic bacteria compared to anaerobes.

Table 4 The average diameter of inhibitory zone for anaerobic bacteria during the 4 observed intervals and various ratios of Ca(OH)₂ and BaSO₄.

<table>
<thead>
<tr>
<th>Ratio of Ca(OH)₂ : BaSO₄</th>
<th>Inhibitory zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours 48 Hours 72 Hours 10 days</td>
</tr>
<tr>
<td></td>
<td>6:1</td>
</tr>
<tr>
<td></td>
<td>7:1</td>
</tr>
<tr>
<td></td>
<td>8:1</td>
</tr>
<tr>
<td></td>
<td>9:1</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
</tr>
</tbody>
</table>
Table 5 The average diameter of inhibitory zone for aerobic bacteria during the 4 observed intervals and various ratios of Ca(OH)₂ and BaSO₄

<table>
<thead>
<tr>
<th>Ratio of Ca(OH)₂ : BaSO₄</th>
<th>Inhibitory zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>6:1</td>
<td>2.1</td>
</tr>
<tr>
<td>7:1</td>
<td>1.7</td>
</tr>
<tr>
<td>8:1</td>
<td>2.1</td>
</tr>
<tr>
<td>9:1</td>
<td>2.4</td>
</tr>
<tr>
<td>10:1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

DISCUSSION

This study has isolated 6 types of bacteria that trigger endodontic infection. The bacteria were taken from the tooth pulp sample diagnosed with pulp necrosis. The result six types of bacteria were:

A. Aerobic:
   1. Bacillus Gram (+)
   2. Staphylococcus Gram (+)
   3. Coccobacil Gram (-)

B. Anaerobic:
   1. Coccobacil Gram (-)
   2. Staphylococcus Gram (+)
   3. Bacillus Gram (+)

The various ratios of Ca(OH)₂ and BaSO₄ paste exhibited inhibitory effect to all types of isolated bacteria. According to the study of Safavi and Nakayama (2000), the most resistant bacteria found in the root canal that will survive within the dentine tubule with pH = 11.5 are Enterococcus faecalis.

Based on the study conducted by Siqueira and Uzed (1998), calcium hydroxide does not prove to be effective against Enterococcus faecalis. To increase the effectiveness of calcium hydroxide against Enterococcus faecalis, this material must be combined with camphorated parachlorphenol (CMCP) and glycerin. Such mixture will produce calcium paraphenolchlorphenol and hydroxyl ion as bactericide agent. However, there were also suggestions that the addition of camphorated parachlorphenol will increase irritation. The effective use of Ca(OH)₂ as medicament during the endodontic care is excellent, addition of other materials such as BaSO₄ may effect its effectiveness. It is suggested to use pure Ca(OH)₂ or with the addition of other materials in least amount. However, to ensure that the filling material looks radio-opaque, it may be added with barium sulfate with the ratio of 6:8:1.⁹

In the present study, addition of more BaSO₄ influenced the effectiveness of the inhibitory effect of Ca(OH)₂ against bacterial growth. The ratio of 7:1 or more proved to have no difference in terms of the antibacterial efficacy.

The main factor that leads to the failure of endodontic care is the bacteria that remain in the periapical region and within the root canal. Such bacteria entered the periapical area through the lateral canal or additional canal, due to low oxygen pressure, existence of debris and necrotic tissue that serve as nutrition for the bacteria, particularly in the teeth of which their pulp was necrose. As a result, the bacteria form colonization, multiply themselves and cause infection to the root canal system including the periapical tissue.¹⁰

The types of bacteria found in the roots are mainly gram + bacillus and gram negative Coccobacillus. The findings of this study concurred to that conducted by Silva and colleagues (2002)¹¹ whereby in the necrotic root canal and chronic periapical condition, anaerobic bacteria were abound, especially negative gram bacteria. It is also said that not only negative gram bacteria has different virulence but it produces toxic in the periapical tissue and contains endotoxin in the cell walls.

Endotoxin comprises lipopolysaccharide (LPS) which is released when the bacteria are dead and this resulted in inflammation reactions and bone re-absorption in the apical areas, and according to Silva and colleagues (2002)¹¹, calcium hydroxide may neutralize the toxins of endotoxin bacteria and inhibit lipopolysaccharides (LPS). In the present study, the Ca(OH)₂ and BaSO₄ paste was able to provide inhibitory effect against the growth of bacteria that trigger endodontic infection. The inhibitory zone increased in line with the length of the contact with such paste (24 hours, 48 hours, 72 hours, 10 days). Calcium hydroxide cannot diffuse through dentine tubule in a short period of time. Calcium hydroxide can eliminate bacteria via a direct contact but it takes at least a week to increase the dentinal pH to 9.4. According to the study by Suzuki and colleagues (1999)¹³, calcium hydroxide will become more effective when its pH is higher. High pH level of calcium hydroxide stimulates the formation of calcification tissue. Apart from that, calcium hydroxide may reduce the toxicity and stimulate the mechanism of local cure.¹⁴

According to Ingle (2002)¹⁵, calcium hydroxide may inhibit the bacteria growth in the root canal, although it works slowly and must be in direct contact with the tissue. Calcium hydroxide is also recommended for root canal that is unlikely or difficult to dry (weeping canals). Its use as inter-canal cure between visits gives a favorable result. However prolonged period of contact may be needed to impart maximum efficacy to eliminate the bacteria as shown in this study. The contact time has significant effect on the inhibition of bacterial growth.
CONCLUSION

Within the limitations of the present study, it is concluded that:

1. The Ca(OH)$_2$ and BaSO$_4$ mixture paste showed the inhibitory effect to the growth of bacteria in infected canal. The addition of BaSO$_4$ affected the effectiveness of Ca(OH)$_2$ at a ratio of 6:1, higher ratio of Ca(OH)$_2$ : BaSO$_4$ at 7:1, 8:1, 9:1 and 10:1 appear to have similar efficacy.

2. Calcium hydroxide was more effective in eliminating aerobic bacteria compared to anaerobics.

3. The time frame of contact for Ca(OH)$_2$ is also important, as Ca(OH)$_2$ needs sufficient time to diffuse in order to impart its efficacy.

REFERENCES


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