EFFICACY OF AMMONIACAL SILVER NITRATE
IN ROOT CANAL THERAPY

BY

Wan-Hong Lan

ABSTRACT

In order to find a simple and effective method for endodontic treatment of recalcitrant cases, the efficacy of ammoniacal silver nitrate was re-evaluated. The modified serial tube dilution method was conducted to determine the effective antimicrobial concentration of ammoniacal silver nitrate on selected test microorganisms and clinical isolates. The effective antimicrobial concentration for the most resistant microorganism, *Streptococcus faecalis*, was 5.0×10⁻¹⁰ g/ml and the effectiveness persisted for several days. The diffusion ability of 4% ammoniacal silver nitrate into the dentinal tubules from root canal wall was also tested with freshly extracted teeth treated as in routine procedure for root canal therapy. The diffusability was evident and the extent of infiltration was various, with more infiltration at cervical dentin than at apical one.

INTRODUCTION

Increased interest and attention have been given to the treatment and restoration of pulpless tooth, so as to re-establish its normal function. With improved prosthetic techniques, a well treated pulpless tooth will remain as an integral part of the dental apparatus. How disappointed it is to see a virtually important tooth being extracted only because the endodontic procedure was not properly executed or not even attempted to do so. Nevertheless, the control or the removal of infectious source from the root canal is a very troublesome problem sometimes. It is essential to solve this problem by a simple and effective method, while many sophisticated methods for dental prosthetic work are being developed.

As an aid to root canal treatment, ammoniacal silver nitrate is a favorite anti-

septic for sterilizing some recalcitrant cases. It has been used by a number of dentists to sterilize infected root canal[1,2]. Its effect is satisfactory, especially when used by “the open method”[2]. Being one of the heavy metal salts, its strong disinfecting action is by way of protoplasm poisoning in a very low concentration; a mechanism known as oligodynamic action. However, the actual effective antimicrobial concentration of ammoniacal silver nitrate has not yet been reported.

The purposes of this investigation are to study the effective antimicrobial concentration of ammoniacal silver nitrate on microorganisms which are encountered in infected root canal, to explore its persistent effective properties, and to study the diffusibility of ammoniacal silver nitrate into dentinal tubules.
Materials and Methods

In order to investigate the antimicrobial activity of ammoniacal silver nitrate as a medicament in root canal treatment, the effective antimicrobial concentration and its diffusibility into dentinal tubules were tested. For convenience, the experiment was divided into two parts.

1) Antimicrobial Effectiveness of 4% Ammoniacal Silver Nitrate

Preparation of Ammoniacal Silver Nitrate (4%)

A freshly prepared 4% ammoniacal silver nitrate was used as a stock solution for each experimental series. Four grams of silver nitrate was dissolved in 20 ml of sterile distilled water. Ten percent ammonia water was added to this solution drop by drop until the brown precipitation formed first disappeared. Then sterile distilled water was added to make the total volume 100 ml. The solution was stored in a dark brown bottle and kept away from light.

Test Microorganisms and Culture Medium

Microorganisms tested were stock cultures and clinical isolates. Stock cultures were obtained from the Division of Microbiology, Department of Clinical Pathology, National Taiwan University Hospital (NTUH). They were Alpha Streptococcus, Beta Streptococcus, Streptococcus faecalis, Pneumococcus, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas, Escherichia coli, Bacteroides, and Veillonella. Five clinical cultures isolated from infected root canals by the Division of Endodontics, Department of Dentistry, NTUH, were used as clinical isolates.

Thioglycollate broth was used for growing the above-mentioned test organisms.

Experimental Methods

A modified serial tube dilution method was used to determine the effective antimicrobial concentration of ammoniacal silver nitrate. In the test ammoniacal silver nitrate was diluted with sterile distilled water and a 24-growth of each test microorganism was used as an inoculum. Duplicate tests were made simultaneously in each experiment. To 2 ml of various concentrations of ammoniacal silver nitrate, 0.05 ml of undiluted or diluted suspension of the test organism was added and incubated at room temperature for 10, 30, or 60 min. Then 0.05 ml of the above thoroughly reacted mixture was transferred to a sterile thioglycollate broth and incubated at 37°C for 72 hr. When turbidity of the medium was noted, presence of the test organisms was confirmed by cultivating the turbid medium on blood agar and also by gram stain. The highest dilution of ammoniacal silver nitrate which inhibits growth of the test organism was considered as the minimum effective dose of the chemical. In each experiment two control tubes containing 2 ml each of sterile distilled water instead of ammoniacal silver nitrate were included. Each experiment was repeated at least once for confirmation of results. When the results from identical experiments did not agree, the entire series was repeated.

In order to determine the persistent effect of ammoniacal silver nitrate, a new actively growing test microorganism was added daily to the reacted serial diluted tubes of the chemical and then inoculated to the thioglycollate broth to determine the minimal effective concentration. The experiments were performed by the same procedures as those described above.

2) Diffusibility of 4% Ammoniacal Silver Nitrate into Dentinal Tubules
Ten human teeth without carious lesion or restoration were used for the experiment. A freshly extracted tooth was treated by routine endodontic procedures; namely, after opening the pulp chamber, the root canal was enlarged with a reamer or a file and irrigated with 3% H₂O₂ and 5% NaOCl. The prepared tooth was then irrigated with distilled water and completely dried. Four percent ammoniacal silver nitrate was dropped into the pulp chamber and root canal by the aid of a smooth broach until the pulp chamber was filled up with the agent. After 24 hr, the treated teeth were split longitudinally along the root canal. The extent of black stain into dentinal portion was determined and photographed.

**Results**

The antimicrobial activity of ammoniacal silver nitrate, determined by the modified serial tube dilution method with 10-fold dilution of 24-bacterial growth, seemed to be very strong. As shown in Table 1, its efficacy against all the test microorganisms ranged from 5.0×10⁻⁵ to 5.0×10⁻⁶ g/ml. *Streptococcus faecalis* was the most resistant among the tested microorganisms, while *Pneumococcus* exhibited a greater sensitivity to ammoniacal silver nitrate. The effective concentration of ammoniacal silver nitrate for laboratory stock cultures tested was essentially the same as that for the newly isolated strains obtained from clinical specimens.

Similar studies were made with *Streptococcus faecalis* at the concentration of 25×10⁶ cells/ml to observe the relationship between reaction time and effective antimicrobial concentration of ammoniacal silver nitrate. As shown in Fig. 1, the antimicrobial effectiveness was highly associated silver nitrate needed for killing the microorganism showed an inverse association with the exposure time. For example, in a 10-min exposure, the minimal effective concentration was 1.0×10⁻¹ g/ml and in a 100-min exposure it was 1.0×10⁻⁵ g/ml. In the experiment with a given reaction time, the concentration of silver nitrate had a marked effect on the number of microorganisms present.

**Table 1. Antimicrobial activities of ammoniacal silver nitrate (ASN) against various microorganisms**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Bacterial number (cell/ml)</th>
<th>Effective concentration of ASN ×10⁻⁵ (g/ml)</th>
<th>Effective antimicrobial dilution of ASN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus alpha</em></td>
<td>16×10⁹</td>
<td>2.0</td>
<td>1 : 2,000</td>
</tr>
<tr>
<td><em>Streptococcus alpha</em></td>
<td>14×10⁹</td>
<td>2.5*</td>
<td>1 : 1,600*</td>
</tr>
<tr>
<td><em>Streptococcus beta</em></td>
<td>12×10⁹</td>
<td>2.0</td>
<td>1 : 2,000</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>30×10⁹</td>
<td>5.0</td>
<td>1 : 800</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>29×10⁹</td>
<td>4.0</td>
<td>1 : 1,900</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>22×10⁹</td>
<td>4.0*</td>
<td>1 : 1,900*</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>15×10⁸</td>
<td>1.3</td>
<td>1 : 3,000</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>11×10⁶</td>
<td>0.5</td>
<td>1 : 8,000</td>
</tr>
<tr>
<td>E. coli</td>
<td>18×10⁸</td>
<td>2.0</td>
<td>1 : 2,000</td>
</tr>
<tr>
<td><em>Bacteroid</em></td>
<td>27×10⁹</td>
<td>2.0</td>
<td>1 : 2,000</td>
</tr>
<tr>
<td>Veillonella</td>
<td>12×10⁸</td>
<td>1.3</td>
<td>1 : 3,000</td>
</tr>
</tbody>
</table>

*Average concentration of antimicrobial dilution of ASN:

Exposure time: 30 min.

* 2 strains obtained from infected root canal
** 3 strains obtained from infected root canal

§ Average concentration
the effective concentration of ammoniacal silver nitrate depended on the number of the test microorganism, *Streptococcus faecalis* (Fig. 2). The larger the amount of bacteria present, the higher the concentration of ammoniacal silver nitrate was required. The persistent effective antimicrobial concentration of ammoniacal silver nitrate is shown in Fig. 3. Its efficacy was relatively stable during the first 7 days and diminished after that, especially after 10 days (Fig. 3).
Fig. 3. Persistent antimicrobial effect of 4% ammoniacal silver nitrate (ASN).

Fig. 4. Longitudinal section of a tooth along the root canal. The diffusibility of ammoniacal silver nitrate (4%) into dentinal tubules was evident. The extent of infiltration was various, with more infiltration at cervical dentin than at apical one.

Fig. 5. Cross section of the same tooth shown in Fig. 3. The diffusibility of ammoniacal silver nitrate (4%) into dentinal tubules was evident and homogeneous.
Black stain as a result of precipitated silver was observed in the longitudinal section of the teeth treated with ammoniacal silver nitrate, i.e., the diffusibility of ammoniacal silver nitrate into dentinal tubule was evident. The extent of infiltration was various, with more infiltration at cervical dentin than at apical one (Figs. 4 and 5).

**Discussion and Conclusion**

Appleton\(^5\) once noted that if bacteria were not involved, endodontics would be simple. The average general practitioner does not fear the technical difficulties of endodontics but rather the flare-up cases during the treatment of infected root canal. As a rule, elimination of infection from the root canal is accomplished by thorough chemomechanical preparation and the use of intracanal antimicrobial medications. However, Ingle\(^9\) demonstrated a high incidence of antibiotic-resistant bacteria on initial culture cases. The management of heavily infected root canal or retained root during endodontic treatment is challenging, particularly in view of the vexing problem of sterilization. If there is growing failure in routine method of debridement as well as sterilization and one considers the tooth to be treated as a strategic abutment for prosthetic work, the need for a sterilization aid to keep the treatment planning under control will become more apparent.

It has been well known that the various metallic ions have antibacterial activity and can be arranged in a series of decreasing order. Silver ion, one of the top on the list, is effective in a concentration less than 1 ppm\(^3\). It is known as an oligodynamic action that even in a very low concentration the antimicrobial activity is still effective.

For the sterilization of infected root canal, Howe had proposed a silver reduction method\(^10\), a solution of ammoniacal silver nitrate is dropped into the root canal and then a reducing agent such as formalin or eugenol is added to precipitate a silver layer on the canal wall. However, Suzuki\(^11\) had noticed that this reduced silver diminished the oligodynamic action of silver ion and, if without introducing the reducing agent, ammoniacal silver nitrate per se could keep a persistent antimicrobial activity. In the meantime, the concentration of ammoniacal silver nitrate for clinical application was decreased from 28% to 4% in Suzuki’s method\(^10\). This decrease in concentration lessens the irritation to tissues. However, there is no evidence to prove which concentration is the optimum.

The comprehensive and well-controlled microbiological study of the root canal flora had been reported by several investigators\(^4-7\). The microorganisms selected and tested were representative of various groups of organisms. Streptococcus faecalis was the most frequently isolated microorganism in pure culture and was also proved to be the most persistent one, as evidenced by its high frequency in subsequent cultures.

It was found in a pilot study that ammoniacal silver nitrate reacted with both culture medium and normal saline. The antimicrobial activity of ammoniacal silver nitrate might be influenced by such solutions, though it was not confirmed. Therefore, a modified method of serial tube dilution using sterilized distilled water as a diluent for 4% ammoniacal silver nitrate was used.

Our results indicated that ammoniacal silver nitrate was an extremely powerful antimicrobial agent and effective in a very low concentration against a variety of microorganisms commonly found in the root canal (Table 1). When bacteria was
Efficacy of Ammoniacal Silver Nitrate in Root Canal Therapy

Exposed to ammoniacal silver nitrate for 10 min, 100 ppm of the chemical killed more than 10^6 bacteria per ml (Fig. 1). This would be considered as a safety range of time for sterilization of root canal in practical use of 4% ammoniacal silver nitrate. The strong antimicrobial activity also persisted for several days (Fig. 3). However, in order to maintain the efficacy, the chemical should preferably be changed after 1 week. Although the sterilization of root canal depends on intracanal medication, instrumentation coupled with irrigation is still important because mechanical cleansing will remove the majority of debris and bacteria from the root canal. Thus the chemomechanical preparation will diminish the amount of bacteria and assist in enhancing the antimicrobial action of ammoniacal silver nitrate (Fig. 2).

Based on the results mentioned above, 4% ammoniacal silver nitrate was considered to have a safe and adequate antimicrobial activity in clinical practice. However, the effectiveness of ammoniacal silver nitrate after exposure to blood, saliva, necrotic tissue, or sulfur ion liberated from protein on decomposition, etc., which are encountered in clinical endodontic usage, should be evaluated.

To destroy the deep-seated microorganism a highly penetrating antiseptic is needed, especially for recalcitrant cases. Ammoniacal silver nitrate has a ready diffusibility into the dentinal tubules and, therefore, the oligodynamic action will be effective at a deep site inward to the wall of root canal. In addition to the oligodynamic action will be effective at a deep site inward to the wall of root canal. In addition to the oligodynamic action, it also contains a readily reducible silver. The fine silver grain which is laid down by this process may fill up the spaces in the dentinal tubules. With a sealing effect on the root canal wall, this process will make the procedure of root canal filling easy.

Ammoniacal silver nitrate is a silver diammonia nitrate in essence\(^\text{12}\)). It is a clear, colorless liquid, and sensitive to light. It is less caustic than silver nitrate \textit{per se}, but provides a more rapid reduction of the silver-containing ion. Its most important use in dental practice is based on its ready diffusibility into the dentin. If it did not cause a black stain in the tooth, it would undoubtedly have gained greater popularity for endodontic treatment. Because of its staining properties, ammoniacal silver nitrate is confined for treatment of retained roots difficult to carry out aseptic technique and some unmanageable non-vital posterior teeth. However, it can be used satisfactorily in anterior teeth if precautions are taken. Nevertheless, a stained tooth may be successfully crowned with a jacket or a porcelain or resin faced crown.

With the aid of ammoniacal silver nitrate, more teeth can be saved. Dentists and patients are frequently pleased by the outcome, especially if a full arch restoration becomes possible because of a successfully treated pulpless tooth.

In the long run, treatment and thus retaining of pulpless tooth is far more preferable than to extract the tooth, especially molar teeth, thus preventing dental arch collapse. Moreover, root canal treatment and restoration are usually less expensive than extraction, followed by a fixed replacement.

Ammoniacal silver nitrate is recommended to be used routinely for the treatment of heavily infected root canals, i.e., either those retaining roots or recalcitrant cases that had not responded to regular endodontic procedure.
REFERENCES


Errata

The Bulletin of
Tokyo Medical and Dental University
Vol. 24, No. 2

p. 169, Right, 9 lines from the top: action → action³
2 lines from the bottom: Republic → Republic

p. 170, Right, 22 l. f. t.: gram → Gram

p. 171, Left, 23 l. f. t.: 24-h bacterial growth → 24-hour growing bacteria
15 l. f. b.: Streptococci → Streptococcus

p. 175, Left, 7 l. f. b.: In addition ... root canal. → (delete)
Right, 11 l. f. b.: arch → arch from

p. 176, Right, 9 l. f. t.: p. 789 → p. 798