NEUTRALIZATION EFFECT OF SOME AGENTS ON 
THE ANTIMICROBIAL ACTIVITY OF 
AMMONIACAL SILVER NITRATE

BY

Wan-Hong Lan*1

ABSTRACT

The effect of some agents on the antimicrobial activity of ammoniacal silver nitrate, an endodontic medicament, was tested with *Streptococcus faecalis* by the serial tube dilution method. Its results indicated that sodium hypochlorite, hydrogen peroxide, and blood had a marked inhibitory effect. However, the presence of dentin, necrotic tissue, saliva, and hydrogen sulfide gas liberated from protein decomposition showed no or little effect on the antibacterial properties of this chemical. Since antiseptics or antibiotics generally may be decomposed by necrotic tissues, these findings suggested that the use of ammoniacal silver nitrate not only may resolve the problem of recalcitrant cases in endodontic treatment but also may simplify the disinfecting procedure for root canals.

INTRODUCTION

As many sophisticated methods for dental prosthetic work are being developed with great progress day by day, more infected root canals are being treated than ever before. Dental practitioners have a great interest in endodontics not only to save pulp-involved teeth for their own sakes but also to save them as abutments for supporting bridges or partial dentures. Unfortunately, in treating pulpless teeth, we may encounter many troublesome problems, such as persistent bacterial infection of lateral branches or ramifications of root canals, obstruction of root canals with periapical lesions or broken instruments in the canal, etc. Ammoniacal silver nitrate (ammoniacal AgNO₃) was recommended by many dentists*1-5* so as to keep the treatment planning under control. The root canal therapy may be divided arbitrarily into 3 phases: (1) Biomechanical preparation, (2) chemical preparation, and (3) disinfection of the root canal. Although disinfection follows only after the canal has been thoroughly cleansed and treated by biomechanical and chemical means, the canals are often found to be only superficially cleansed, and sometimes the pulp tissue has not been completely removed if the canal walls were examined microscopically. According to Davis et al.,*4* effectiveness of a disinfectant often depends strongly on the condition of the environment. Harrison and Madonis*5* also demonstrated that blood and necrotic tissue had a strong inhibitory effect on aqueous *p*-chlorophenol. *In vitro,**6* the minimum effective concentration of ammoniacal

---

*1 賀漢: Department of Endodontics (Chief: Prof. T. Senada), School of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku); Division of Endodontics, School of Dentistry (Dean: Prof. Y. C. Huong), College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.

Received for publication, November 14, 1977.
AgNO₃ for *Streptococcus faecalis*, the most resistant microorganism tested, was 5.0 × 10⁻⁸ g/ml. The dental diffusibility of 4% ammoniacal AgNO₃ was evident. However, in practical use, especially by the open method, ²) ammoniacal AgNO₃ is apt to be contaminated by saliva, blood, dentin, necrotic tissues, and/or hydrogen sulfide gas liberated from protein decomposition. During the chemomechanical preparation of the root canals, hydrogen peroxide and sodium hypochlorite are often used as adjuncts. The antimicrobial activity of 4% ammoniacal AgNO₃ might be greatly altered by the presence of these agents. Therefore, this study was undertaken to test the neutralization effect of the above-mentioned agents on the antimicrobial activity of ammoniacal AgNO₃.

**Materials and Methods**

The following solutions and substances were used for testing the neutralization effect on the antimicrobial activity of 4% ammoniacal AgNO₃: (1) Human saliva filtered through Millipore filters, (2) hydrogen sulfide saturated in sterile distilled water, (3) defibrinated whole goat blood, (4) 5% H₂O₂, (5) 5% NaOCl, (6) dentin fragments, and (7) necrotic tissue. Dentin fragments, which were crushed from freshly extracted teeth free of caries or restorations, were cleaned with running distilled water, dried with compressed air, and autoclaved at 120°C for 20 min. The necrotic tissue was prepared from gingival tissue obtained from periodontal surgery. The tissue was first cleaned with running distilled water, then suspended in distilled water in a capped glass container to prevent drying, and stored in a refrigerator. The necrotic tissue was cut into small debris, about 1×1 mm², to increase contact area. Each of these test preparations was checked for sterility by culture in thioglycollate broth and on blood-agar plate.

Serial tube dilution experiments were conducted to determine the neutralizing effect of the test preparation on the antimicrobial activity of 4% ammoniacal AgNO₃. Dilution of ammoniacal AgNO₃ in sterile distilled water was also used as the control. End-point determination was based on the smallest concentration of ammoniacal AgNO₃ inhibiting growth of the test microorganism, *Streptococcus faecalis*. Detailed procedures were described previously. ⁶) Briefly, the procedure was as follows. To 10 ml of 4% ammoniacal AgNO₃, 1, 2, 5, 10, or 20 ml of the test solution was added. After agitating thoroughly and standing at room temperature for 24 hr, the mixture was centrifuged and the supernatant was used to make the desired dilution for testing. As for the test substances such as tooth fragments and necrotic tissue, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, or 10.0 g of the sample was added to 10 ml of 4% ammoniacal AgNO₃. After thorough agitation and reaction for 1, 24, or 48 hr, the reacted ammoniacal AgNO₃ solution was pipetted to make the desired dilution.

**Results**

The control series gave uniform results showing antimicrobial effect at a minimum concentration of 5.0 × 10⁻⁶ or 4.0 × 10⁻⁵ g/ml (5.0 or 4.0 × 10⁻³ ppm) of ammoniacal AgNO₃. As shown in Fig. 1, NaOCl exerted the most profound inhibitory effect on the antimicrobial activity of ammoniacal AgNO₃. For example, 1.0 × 10⁻² g/ml (1.0 × 10⁴ ppm) of ammoniacal AgNO₃ was required to inhibit the growth of *Streptococcus faecalis* when mixed with 2 volumes of NaOCl. In a ratio of 2 volumes of H₂O₂ to 1 volume of 4% ammoniacal AgNO₃, 8.0 × 10⁻⁴ g/ml (8.0 × 10² ppm, 2.9 in the ordinate) of
Fig. 1. Effect of test solutions on the antimicrobial efficacy of ammoniacal AgNO₃
Test organism: *Streptococcus faecalis*, number of bacteria: \(78 \times 10^9/ml\)

* The concentration of ammoniacal AgNO₃ needed to inhibit the growth of *Streptococcus faecalis* in test solutions

** 4 indicates log \(10^4\) ppm \(1.0 \times 10^{-2}\) gm/ml

Fig. 2. Effect of dentin on the antimicrobial efficacy of ammoniacal AgNO₃ with different exposure time.
Test organism: *Streptococcus faecalis*, number of bacteria, \(98 \times 10^5/ml\)

* The concentration of ammoniacal AgNO₃ needed to inhibit the growth of *Streptococcus faecalis* in test substances.
ammoniacal AgNO₃ was required to suppress the bacterial growth.

Blood exerted a special reaction to ammoniacal AgNO₃. The larger the quantity of blood added, the stronger was the coagulation that took place. In a ratio of 2 volumes of blood to 1 volume of 4% ammoniacal AgNO₃, supernatant was not obtained. Blood showed an inhibitory effect indeed. However, the antimicrobial effect of ammoniacal AgNO₃ was less affected when a small amount of blood was present.

Saliva and hydrogen sulfide solution had only a small effect on the antimicrobial activity of ammoniacal AgNO₃.

Dentin and necrotic tissue showed no or little effect on the antimicrobial activity of ammoniacal AgNO₃, depending on the amount of tissue involved and exposure time, as shown in Figs. 2 and 3.

**DISCUSSION**

A well-treated pulpless tooth will remain as an integral part of the dental apparatus. In root canal therapy, although mechanical cleansing with a reamer or a file alone is reported to sterilize only 4.6% of the infected root canal, it is still the primary method used to remove majority of debris and bacteria from the canal. Instrumentation coupled with irrigation with H₂O₂ or NaOCl is particularly effective. With chemomechanical preparation of the root canal, thousands of dentinal tubules are shaved deeply enough to insure that few bacteria remain. However, complete canal sterilization depends on intracanal medicament with antibiotics or antiseptics. Nevertheless, the lateral branches of the root canal, ramifications at root apex, canal obstructions with periapical lesion, or broken instrument in the canal will hinder the outcome of chemomechanical preparation of the root canal. In the absence of deliberate and complete debridement of the entire root canal, it seems desirable that medicaments used be both powerful and penetrating. Ammoniacal AgNO₃ has been used as an aid for sterilization of these recalcitrant cases in different methods, such
as Howe's method, Suzuki's method, electrosterilization or ionization. With both sterilization and filling properties in lateral branch or ramification of a root canal, its effect is satisfactory; especially, Suzuki's open method is appreciated because the application procedure is so simple.

In a previous study, it was indicated that ammoniacal AgNO₃ was effective in a very low concentration against a variety of microorganisms commonly found in the infected root canal. However, the effectiveness of a disinfectant often depends strongly on the environmental conditions during topical application. Saliva, dentin, blood, necrotic tissue, hydrogen sulfide gas, and the irrigating solution, such as hydrogen peroxide and sodium hypochlorite, may affect the antimicrobial property of the chemical used in endodontic treatment.

Streptococcus faecalis was chosen as the test organism in this study because it was demonstrated to possess the greatest resistance in vitro to ammoniacal AgNO₃ among many microorganism tested. A marked inhibitory effect was exhibited by sodium hypochlorite on the antibacterial action of ammoniacal AgNO₃, possibly due to the formation of silver chloride precipitate which is difficult to ionize. Thus, the oligodynamic action of silver ion could not be expected. This is a common phenomenon when chlorine-containing substances, such as normal saline, react with silver ion. The antibacterial activity will be inhibited greatly, even though the chlorine-containing substance itself is an antiseptic. Hydrogen peroxide, an antiseptic as well as an irrigation solution, also inhibited the antimicrobial activity of ammoniacal AgNO₃.

The possible reason for it is that the chemical composition of ammoniacal AgNO₃ is altered by H₂O₂, and its effectiveness was reduced. Irrigation of the root canal is generally necessary in routine procedure of endodontic treatment in order to wash out debris, organisms, fragments of pulp tissue, and dentin shavings that have accumulated from reaming and filing of the canal. However, after irrigation with sodium hypochlorite or hydrogen peroxide, the root canal should be thoroughly flushed with distilled water or ethanol and dried to prevent the interaction of above agents with ammoniacal AgNO₃.

Blood cells took up a relatively large amount of silver ion and coagulation occurred. Thus, the quantity of silver ion in ammoniacal AgNO₃ solution was markedly reduced and the residual silver ion was not sufficient to show the expected effect. For this obvious reason, blood should not be allowed to remain in the root canal before topical application of ammoniacal AgNO₃. Therefore, thorough irrigation in routine endodontic treatment should be carried out to obtain maximal antimicrobial effect. Nevertheless, it seems that a small amount of blood at the apical foramen would not interfere the bactericidal effect of ammoniacal AgNO₃ too much. If the root canal was flooded with ammoniacal AgNO₃, the increased quantity of the chemical would restore the original bactericidal effect.

When infection occurs, the pulp becomes purulent. As one of the end products, hydrogen sulfide gas might be liberated due to the decomposition of pulp. It was demonstrated that hydrogen sulfide affected slightly the antimicrobial property of ammoniacal AgNO₃ (Fig. 1).

In this study, it is apparent that ammoniacal AgNO₃ can maintain a high degree of antimicrobial effectiveness in the presence of saliva. Thus, the antimicrobial activity of 4% ammoniacal AgNO₃ would be maintained for several days, even in
topical application by the open method.

Necrotic tissue and dentin showed little neutralization effect on the action of ammoniacal AgNO₃. The reacted product, silver protein, will slowly release silver ions again for maintaining the level of antimicrobial activity.⁹⁰ In clinical experience, especially in dealing with the recalcitrant cases, the presence of necrotic tissue or pulp tissue fragments in irregular spaces of the canal may serve as a growth medium for multiplication of microorganisms. With high diffusibility into dentinal tubules, ammoniacal AgNO₃ not only kills the microorganism immediately, but also interacts with the necrotic tissue to form silver protein which would release silver ions slowly to keep a persistent bactericidal activity. However, the pulp tissue in the main canal should be removed as completely as possible by thorough chemomechanical preparation in order to prevent reduction in the quantity of ammoniacal AgNO₃. At the same time, complete cleansing procedure assists the penetration of ammoniacal AgNO₃.

In a general sense, root canal medicaments, antiseptics or antibiotics, will be decomposed by the necrotic tissue. The above findings showed that ammoniacal AgNO₃ can maintain its high potency of antimicrobial activity even in the presence of a necrotic tissue. Therefore, the use of ammoniacal AgNO₃ not only resolves the problem of recalcitrant cases, but also simplifies the disinfecting procedure for root canals.

Acknowledgements
The author is greatly indebted to Prof. I. Sunada of the Department of Endodontics, Tokyo Medical and Dental University, Prof. K. Suzuki of the Department of Endodontology, School of Dentistry, Showa University, Prof. Y. C. Hong of the School of Dentistry, National Taiwan University, and Prof. C. S. Yang of the Department of Bacteriology College of Medicine, National Taiwan University for their invaluable advice, and to Dr. H. Horiiuchi of the Department of Endodontics, Tokyo Medical and Dental University and Mr. S. W. Ho, School of Medical Technology, College of Medicine, National Taiwan University for their fruitful discussions while this work was in progress.

References