นิพนธ์ต้นฉบับ

ฤทธิ์การต้านเชื้อแบคทีเรียของสเปรย์พ่นคอสูตรผสมโพวิโดน–ไอโอดีนและลิโดเคนต่อเชื้อ *Streptococcus pyogenes*; การศึกษาในหลอดทดลอง

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บทคัดย่อ

ความเป็นมา: ในเวชปฏิบัติทางคลินิกการแยกระหว่างการติดเชื้อคออักเสบจากเชื้อ *Streptococcus pyogenes* กับการติดเชื้อไวรัสที่หายได้เองนั้นเป็นเรื่องยาก ส่งผลให้มีการใช้ยาปฏิชีวนะเกินความจำเป็นในผู้ป่วยโรคคออักเสบเฉียบพลัน การใช้ผลิตภัณฑ์โพวิโดน–ไอโอดีนสำหรับช่องปากจึงอาจเป็นทางเลือกในการรักษาอาการเจ็บคอ โดยลดความเสี่ยงต่อการเกิดภาวะดื้อยาของจุลชีพในลำคอ อย่างไรก็ตาม งานวิจัยเกี่ยวกับปริมาณความเข้มข้นต่อฤทธิ์ต้านเชื้อแบคทีเรียต่อเชื้อ *S. pyogenes* ของสเปรย์พ่นคอที่มีโพวิโดน–ไอโอดีนยังมีอยู่อย่างจำกัด อีกทั้งผลิตภัณฑ์ส่วนใหญ่ยังไม่มีส่วนประกอบที่ออกฤทธิ์ระงับปวด จึงมีงานวิจัยเพียงไม่กี่ชิ้นที่ศึกษาผลของสารระงับปวดต่อฤทธิ์ต้านเชื้อแบคทีเรียของผลิตภัณฑ์โพวิโดน–ไอโอดีน

วัตถุประสงค์: งานวิจัยนี้มีวัตถุประสงค์เพื่อแสดงฤทธิ์ฆ่าเชื้อแบคทีเรียในหลอดทดลองของสเปรย์พ่นคอสูตรใหม่ที่ผสมระหว่างสารฆ่าเชื้อ povidone–iodine (PVP-I) และสารระงับปวด (lidocaine) ต่อเชื้อ *S. pyogenes*

วิธีการศึกษา: ทำการทดสอบฤทธิ์ต้านเชื้อแบคทีเรียของสเปรย์พ่นคอที่มีส่วนผสมของ PVP-I 0.45% ร่วมกับลิโดเคน 0.3% ต่อสายพันธุ์อ้างอิงของ *S. pyogenes* โดยใช้วิธีการทดสอบการฆ่าเชื้อแบบเชิงปริมาณตามมาตรฐาน EN13727:2012 + A2:2015 ผลิตภัณฑ์ทดสอบถูกเจือจางตามความเข้มข้นของ PVP-I ที่ 0.09%, 0.045%, 0.009%, 0.0045%, 0.00045% และ 0.000045% จากนั้นนำเชื้อแบคทีเรียไปผสมกับสารทดสอบเป็นเวลา 30 วินาทีภายใต้สภาวะสกปรกเพื่อจำลองสภาวะเสมือนช่องปาก แล้วนำไปเพาะบนจานเพาะเชื้อ (blood agar) จากนั้นทำการนับจำนวนโคโลนีที่เติบโตบนแต่ละจานและเปรียบเทียบกับกลุ่มควบคุมเพื่อประเมินฤทธิ์ต้านเชื้อแบคทีเรียของสารทดสอบ

ผลการศึกษา: บนจานเพาะเชื้อที่มีความเข้มข้นของ PVP-I ที่ 0.45%, 0.09%, 0.045% และ 0.009% ไม่พบโคโลนีของเชื้อ *S. pyogenes*หลังจากบ่มที่อุณหภูมิ 37°C เป็นเวลา 24 ชั่วโมง สำหรับจานที่พบการเจริญของเชื้อ พบว่าจำนวนโคโลนีมีความสัมพันธ์แบบผกผันกับความเข้มข้นของ PVP-I เมื่อเปรียบเทียบกับกลุ่มควบคุม จานที่มี PVP-I ความเข้มข้น 0.0045% และ 0.00045% พบว่ามีการลดจำนวนโคโลนีลง 97.45% และ 93.33% ตามลำดับ

สรุป: PVP-I ที่มีความเข้มข้นตั้งแต่ 0.009% เป็นต้นไป ร่วมกับลิโดเคน 0.3% แสดงฤทธิ์ต้านเชื้อแบคทีเรีย *S. pyogenes* ได้ดีในหลอดทดลอง ดังนั้นสเปรย์พ่นคอสูตรผสมความเข้มข้น PVP-I 0.45% ร่วมกับลิโดเคน 0.3% ซึ่งเข้มข้นกว่า PVP-I 0.009% ถึง 50 เท่า จึงสามารถยับยั้งการเจริญเติบโตของเชื้อได้ และสามารถลดความกังวลในการใช้จริงทางคลินิกจากความเข้มข้นของ PVP-I อาจเจือจางลงจากกระบวนการทางสรีรวิทยาต่าง ๆ ของร่างกาย

คำสำคัญ: ลิโดเคน, โพวิโดน–ไอโอดีน, สเปรย์พ่นคอ, *Streptococcus pyogenes*

In vitro antibacterial activity of newly combined povidone–iodine plus lidocaine throat spray against *Streptococcus pyogenes*

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Abstract

Background: In clinical practice, streptococcal pharyngitis is difficult to distinguish from self-limited viral pharyngitis, resulting in overuse of antibacterial agents in patients with acute pharyngitis. Povidone–iodine (PVP-I) oral preparation could be a treatment option for throat infection, sparing the risk of antibiotic resistance of throat microflora. However, research on the antibacterial effect of PVP-I throat sprays, which typically contain lower concentrations of PVP-I compared to other PVP-I oral preparations, against *Streptococcus pyogenes* is limited and most commercial PVP-I oral preparations contain no ingredients with analgesic activity. Thus, few studies have focused on the effect of analgesics on antibacterial activity of PVP-I oral preparations.

Objectives: This study aimed to demonstrate in vitro bactericidal activity of a newly developed, combined antiseptic–analgesic oral throat spray of PVP-I plus lidocaine against *S. pyogenes*.

Methods: Antibacterial activity of combined 0.45% PVP-I plus 0.3% lidocaine throat spray against a reference strain of *S. pyogenes* was demonstrated using the bactericidal quantitative suspension test EN13727:2012 + A2:2015. The test product was serially diluted to 0.09%, 0.045%, 0.009%, 0.0045%, 0.00045% and 0.000045% PVP-I solution. Suspensions of the reference strain were added to the PVP-I test solutions for 30 seconds under dirty conditions and then spread on blood agar plates. Colony growth on each plate was counted and compared with a negative control sample to evaluate the antibacterial effect of the tested solutions.

Results: On 0.45%, 0.09%, 0.045% and 0.009% PVP-I plates, no surviving *S. pyogenes* colonies were observed after 24 h of incubation at 37°C. On those plates with visible bacterial colonies, colony count was inversely correlated with concentration of PVP-I. Compared with negative control plates, 0.0045% and 0.00045% PVP-I plates showed 97.45% and 93.33% colony growth reduction, respectively.

Conclusion: PVP-I, at concentrations of at least 0.009%, has demonstrated effective antibacterial activity against *S. pyogenes* in vitro. Therefore, a throat spray formulation containing 0.45% PVP-I, which is 50 times more concentrated than 0.009% PVP-I, is capable of inhibiting bacterial growth. This concentration may also alleviate concerns regarding clinical use, as the PVP-I concentration could be reduced through physiological dilution or clearance mechanisms in vivo.

Keywords: Lidocaine, Povidone–iodine (PVP-I), Throat spray, *Streptococcus pyogenes*

Introduction

Acute throat infection is a common problem of upper respiratory traction infection. Sore throat can be uncomfortable and disruptive symptoms that can affect daily life. It is mostly caused by self-limited viral infection, which requires no specific treatment. Only 5%–15% in adults and 20%–30% in children with acute pharyngitis or pharyngotonsillitis has *Streptococcus pyogenes* as an etiology,[1-3] needed specific treatment with antibacterial agents intended for eradication of the bacteria itself and prevention of acute rheumatic fever and other possible suppurative complications.[4-6] Antiseptic throat sprays, particularly povidone-iodine (PVP-I) throat spray, have been demonstrated to be effective virucidal and bactericidal agents in various studies and should be considered a potential treatment option for patients with sore throat. Although the virucidal activity of PVP-I throat sprays against common respiratory viruses such as influenza, respiratory syncytial virus, and severe acute respiratory coronavirus has been recently studied,[7,8] research on the antibacterial effect of PVP-I throat sprays, which typically contain lower concentrations of PVP-I compared to other PVP-I oral preparations, against *S. pyogenes* is limited.[7-15] Furthermore, most commercial PVP-I oral preparations do not include ingredients with analgesic properties. Previous research has suggested that there may be an antagonistic interaction between lidocaine and PVP-I, which could potentially minimize the bactericidal activity of PVP-I.[16-19]

Objectives

The objective of this study is to assess the bactericidal effect against *S. pyogenes* of PVP-I throat spray at a concentration of 0.45% combined with lidocaine, which possesses both antibacterial and analgesic properties, for possible use in patients with acute pharyngitis. Our aim was to determine the minimum bactericidal concentration of PVP-I throat spray against this specific pathogen and to investigate whether the addition of lidocaine had any impact on the antibacterial activity of the spray.

Methods

The antibacterial activity of PVP-I with lidocaine was evaluated using the bactericidal quantitative suspension test according to European standard EN13727:2012 + A2:2015.[20] *S. pyogenes* ATCC 12344 was selected as a reference strain. This study did not involve human participants or animals. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (COA.MURA2021/572).

The test product was 0.45% PVP-I plus 0.3% lidocaine throat spray. Using sterile distilled water, the product was serially diluted to 0.09%, 0.045%, 0.009%, 0.0045%, 0.00045% and 0.000045% PVP-I solution. Normal saline was used as a negative control. Suspensions of the reference strain were added to the PVP-I test solutions and negative control under dirty conditions (3.0 g/L bovine serum albumin and 3.0 ml/L erythrocytes). After 30 seconds of contact between *S. pyogenes* and test solution, bactericidal activity was neutralized with 3% Tween 80, 0.1% histidine, 0.3% lecithin and 0.5% sodium thiosulfate. For each test suspension, a 1-mL sample was spread on a human blood agar plate. After 24 hours of incubation at 37°C, the number of bacterial colonies on each plate was counted to determine antibacterial efficacy.

Results

Figure 1 and Table 1 show bacterial counts of *S. pyogenes* on human blood agar plates after contact with different concentrations of PVP-I plus lidocaine solution. There were no *S. pyogenes* colonies on the 0.45%, 0.09%, 0.045% and 0.009% PVP-I plates. Bacterial growth was observed on the 0.0045%, 0.00045% and 0.000045% PVP-I plates. On those plates with visible bacterial colonies, colony count was inversely correlated with the concentration of the test solution. Compared with the control solution, the 0.0045% and 0.00045% PVP-I plates showed 97.45% and 93.33% colony growth reduction, respectively. Colony count on the 0.000045% PVP-I plates, the lowest concentration tested, was 2.0×10^7 CFU/mL, which was close to 1.65×10^7 CFU/mL observed with the negative control.

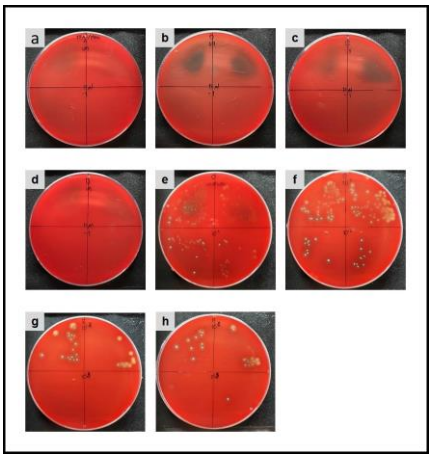

Figure 1 Bacterial counts of *Streptococcus pyogenes* on human blood agar plates after contact with different concentrations of povidone–iodine (PVP-I) plus lidocaine solution. **a**: 0.45% PVP-I. **b**: 0.09% PVP-I. **c**: 0.045% PVP-I. **d**: 0.009% PVP-I. **e**: 0.0045% PVP-I. **f**: 0.00045% PVP-I. **g**: 0.000045% PVP-I. **h**: normal saline solution (negative control).

Table 1 Colony counts of *Streptococcus pyogenes* on human blood agar plates after contact with different concentration of PVP-I plus lidocaine solution

PVP-I concentration (%)	Colony counts on blood agar (CFU/mL)
0.45	0
0.09	0
0.045	0
0.009	0
0.0045	4.2×10^5
0.00045	1.1×10^6
0.000045	2.0×10^7
NSS (negative control)	1.65×10^7

Abbreviation: CFU, colony forming unit; NSS, normal saline solution; PVP-I, povidone–iodine.

Discussion

PVP-I is a commonly used antiseptic in many medical situations worldwide. The antibacterial activities of PVP-I in many preparations have been extensively tested.[7-15,21-29] PVP-I gargles and throat sprays are effective against a wide range of bacteria, including both gram-positive and gram-negative species.[7-15] Although, other preparations of PVP-I have been found to be effective against *S. pyogenes,*[14,15,29] the antibacterial effect of PVP-I throat sprays, which typically contain lower concentrations of PVP-I compared to other PVP-I oral preparations, is limited. Our study showed that 0.45% PVP-I plus 0.3% lidocaine throat spray had a bactericidal effect against *S. pyogenes*, providing evidence of the antibacterial effect of PVP-I throat spray against this common pathogen. We showed that 0.009% PVP-I, which was a 50-fold dilution of the test product, was sufficient to totally inhibit growth of *S. pyogenes* under simulated *in vitro* dirty conditions. Lower concentrations down to 0.00045% PVP-I, a 1000-fold dilution of the test product, showed some antibacterial effect. These findings may reflect that PVP-I, at concentrations of at least 0.009%, has demonstrated effective antibacterial activity against *S. pyogenes in vitro*. Therefore, a throat spray formulation containing 0.45% PVP-I, which is 50 times more concentrated than 0.009% PVP-I, is capable of inhibiting bacterial growth. This concentration may also alleviate concerns regarding clinical use, as the PVP-I concentration could be reduced through physiological dilution or clearance mechanisms *in vivo*.

There is concern regarding the effect of lidocaine on the antibacterial activity of PVP-I in some clinical situations, especially in ocular surgery, where lidocaine gel is regularly used as a topical analgesic.[16-19] Application of lidocaine before PVP-I preparation decreases the antibacterial effect of PVP-I on standard agar plates and corneoscleral tissue. However, application of PVP-I preparation before lidocaine, or simultaneous application of both, does not affect antibacterial activity of PV-I preparations.[17-19] Our combined PVP-I plus lidocaine throat spray showed good antibacterial activity, confirming that simultaneous application of lidocaine and PVP-I does not cause antagonistic interaction.

PVP-I has been used worldwide for >50 years;[30] therefore, its safety profile has been thoroughly studied. Local side effects in the oral mucosa and systemic side effects, including those involving thyroid hormone production and function, are minimal with PVP-I oral preparations at concentrations commonly use in medical practice.[31-33] Thus, 0.45% PVP-I plus 0.3% lidocaine throat spray should be a safe treatment option for sore throat.

This *in vitro* study supports the antibacterial effect of PVP-I and lidocaine against *S. pyogenes*, an important cause of acute bacterial pharyngitis, and marks a step toward use of combined antiseptic–analgesic throat sprays in clinical practice. Future *in vivo* studies should be conducted to clarify the clinical significance of the product in patients with acute pharyngitis.

Conclusions

The newly developed 0.45% PVP-I plus 0.3% lidocaine throat spray has good *in vitro* bactericidal activity against *S. pyogenes*. This throat spray could be a potential candidate for treatment of patients with acute sore throat in the era of antibacterial resistance.

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Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author contributions

Conceptualization and design: Tienake Trisauvapak, Surapat Assawawiroonhakarn; Methodology: Tienake Trisauvapak, Chompunuch Klinmalai; Data collection and analysis: Tienake Trisauvapak, Chompunuch Klinmalai; Supervision: Tienake Trisauvapak, Surapat Assawawiroonhakarn; Writing – original draft: Tienake Trisauvapak, Surapat Assawawiroonhakarn; Writing – review & editing: All authors contributed to the review, editing, and approval of the final manuscript.

Disclosures

All of the authors declare no competing interests.

Compliance with ethics guidelines

This article does not contain any studies with human participants or animals performed by any of the authors. This study protocol was approved by the ethical committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (COA.MURA2021/572).

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