รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยเรื่องกลไกอาการปวดฟันในภาวะที่เนื้อเยื่อในโพรงฟันปกติและอักเสบในมนุษย์

โดย

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คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

กรกฎาคม 2552

สัญญาเลขที่ RMU4980049

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ภาควิชาสรีรวิทยาและชีวเคมี

คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและ

สำนักงานคณะกรรมการการอุดมศึกษา

(ความเห็นในรายงานนี้ สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิติกรรมประกาศ

ผู้วิจัยใคร่ขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัยและสำนักงานคณะกรรมการการ-อุดมศึกษาที่ให้ทุนสนับสนุนโครงการวิจัยนี้ ขอกราบขอบพระคุณ ศาสตราจารย์เกียรติคุณนายแพทย์วิจารณ์ พานิช ที่แนะนำและให้โอกาสผู้วิจัยได้รับทุนจากสำนักงานกองทุนสนับสนุนการวิจัยเป็นครั้งแรก ขอกราบขอบพระคุณศาสตราจารย์เกียรติคุณ ดร. วิชัย บุญแสง ที่เมตตาและให้การสนับสนุนผู้วิจัยได้ทำงานวิจัยด้านกลไกความเจ็บปวดจากฟันได้ให้โอกาสผู้วิจัยได้ทำงานต่อเนื่องมาถึง 20 ปีแล้ว ขอกราบขอบพระคุณ ศาสตราจารย์เกียรติคุณ ทันตแพทย์ ดร. สถิตย์ สิริสิงห ที่เมตตาและให้การแนะนำด้านการวิจัยและวิชาการ ขอกราบขอบพระคุณ ศาสตราจารย์เกียรติคุณ ทันตแพทย์สมศักดิ์ จักรไพวงศ์ อดีตคณบดีคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล ที่เมตตาและให้การสนับสนุนการวิจัยและสนับสนุนอย่างมากด้านเครื่องมือและอุปกรณ์การวิจัย ขอกราบขอบพระคุณรองศาสตราจารย์ ทันตแพทย์ ดร. สุขุม ธีรดิลก อดีตหัวหน้าภาควิชาสรีรวิทยาและชีวเคมีและอดีตคณบดีคณะทันต-แพทยศาสตร์ มหาวิทยาลัยมหิดล ที่เมตตาและให้การแนะนำด้านการวิจัยและวิชาการตั้งแต่ผู้วิจัยยังเป็นนักศึกษาทันตแพทยศาสตร์ปีที่4 เมื่อปีพ.ศ. 2520 และเรื่อยมาจนถึงปัจจุบัน ขอกราบขอบพระคุณ รองศาสตราจารย์ ทันตแพทย์หญิงวรรณะ อภัย รองศาสตราจารย์ทันตแพทย์ชูโชติ ธนะภูมิ ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิงอิงบุญ เทียนศิริ อดีตหัวหน้าภาควิชาสรีรวิทยาและชีวเคมี ที่เมตตาและให้ให้การสนับสนุน ขอกราบขอบพระคุณ รองศาสตราจารย์ ทันตแพทย์หญิง ดร. ธีรลักษณ์ สุทธเสถียร คณบดีคณะทันตแพทยศาสตร์มหาวิทยาลัยมหิดล และรองศาสตราจารย์ ทันตแพทย์สุรินทร์ สูอำพัน รองคณบดีฝ่ายโครงการพิเศษที่ให้การสนับสนุน

ในระดับนานาชาติขอกราบขอบพระคุณ Professor Bruce Matthews แห่ง University of Bristol UK อาจารย์ที่ปรึกษาระดับปริญญาเอกที่ยังคงร่วมมือในการศึกษาวิจัยด้านกลไกความเจ็บปวดจากฟัน ต่อเนื่องมากว่า 20 ปี ขอกราบขอบพระคุณ Professor Rex Holland แห่ง University of Michigan Ann Arbor USA, Professor Hideaki Suda แห่ง Tokyo Medical and Dental University, Japan ที่ให้การสนับสนุนด้านต่างๆ

ขอขอบคุณอดีตนักศึกษาปริญญาเอกและนักศึกษาปริญญาเอกปัจจุบันสาขาชีววิทยาช่อง-ปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล รองศาสตราจารย์ ทันตแพทย์ ดร. สิทธิชัย วนจันทรารักษ์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร. ปานตา เจริญลาภ ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร. วรางคณา ชิดช่วงชัย อาจารย์ทันตแพทย์หญิง กนิษฐา กิจสมานมิตร คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล อาจารย์ทันตแพทย์หญิง ดร. อรพินทร์ อัจฉรานุกูล คณะทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ และขอขอบคุณ คุณศิริรัตน์ วัชรอุดมปัญญา คุณฉลองรัตน์ หมื่นขวา คุณศิรินทิพย์ ชูเนตร นักวิทยาศาสตร์ของภาควิชาฯ

ท้ายที่สุดขอขอบคุณ ภรรยาของผู้วิจัย รองศาสตราจารย์ ทันตแพทย์หญิงคัดเค้า วงษ์สวรรค์ ที่เป็นกำลังใจ และช่วยเหลือในทุกๆเรื่องให้โอกาสผู้วิจัยได้ศึกษาวิจัยด้านกลไกความเจ็บปวดจากฟันทั้งต่างประเทศและในประเทศต่อเนื่องมากว่า 20 ปี

บทคัดย่อ

รหัสโครงการ: RMU4980049

ชื่อโครงการ: กลไกอาการปวดฟันในภาวะที่เนื้อเยื่อในโพรงฟันปกติและอักเสบในมนุษย์

ชื่อนักวิจัย: รองศาสตราจารย์ ทพ. ดร. นพคุณ วงษ์สวรรค์

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การศึกษาวิจัยในการศึกษาแรกเพื่อหาความสัมพันธ์ระหว่างอาการเสียวฟันและอัตราการไหลของของไหลในท่อเนื้อฟัน หรืออาจกล่าวอีกนัยหนึ่งคือเพื่อพิสูจน์กลไกของการเสียวฟันที่เรียกว่า “สมมุติฐานไฮโดรไดนามิก” นั่นเอง การศึกษากระทำในฟันกรามน้อยที่จะต้องถอนเพื่อจัดฟัน จำนวน 16 ซี่ในผู้ป่วย 13 คน กรอฟันที่ปุ่มฟันใกล้แก้มจนถึงระดับเนื้อฟัน (ระดับนี้ยังห่างจากเนื้อในโพรงฟัน 1.5 มม) กำจัดชั้นสเมียร์ด้วยกรดฟอสฟอริคแล้วล้างด้วยน้ำเกลือสเตอไรด์ กระตุ้นเนื้อฟันด้วยความดัน 400 มม ของปรอท สูงกว่าและต่ำกว่าความดันบรรยากาศเป็นเวลา 5 วินาที ให้ผู้ป่วยประเมินความรู้สึกเสียวฟันด้วยวิชวยอนาลอกสเกล (วีเอเอส) วัดอัตราการไหลของของไหลในท่อเนื้อฟันเมื่อกระตุ้นเนื้อฟันด้วยความดันในลักษณะที่ทำในคลินิกโดยกระทำในห้องปฏิบัติการหลังจากที่ถอนฟันแล้ว ผลการทดลองพบว่าค่ามัธยฐานของความดันที่น้อยที่สุดที่กระตุ้นให้เกิดอาการเสียวฟันคือ 125 มม ของปรอทต่ำกว่าบรรยากาศ และ 200 มม ของปรอทสูงกว่าบรรยากาศ ซึ่งจะทำให้เกิดการไหลของของไหลในท่อเนื้อฟันเท่ากับ 3.29 นาโนลิตร/วินาที/มม^2 และ 5.75 นาโนลิตร/วินาที/มม^2 ตามลำดับ โดยสรุบ กลไกของอาการเสียวฟันในมนุษย์ เป็นไปตาม“สมมุติฐานไฮโดรไดนามิก”และจะไวต่ออัตราการไหลของของไหลในท่อเนื้อฟันที่ออกนอกตัวฟันมากกว่ามุ่งสู่ตัวฟันเช่นเดียวกับที่พบในฟันแมว การศึกษาวิจัยในการศึกษาที่สองเพื่อศึกษากลไกของความเย็นในการทำให้เกิดอาการเสียวฟันในมนุษย์ การศึกษากระทำในฟันกรามน้อยที่จะต้องถอนเพื่อจัดฟันเช่นเดียวกัน การศึกษากระทำในฟันกรามน้อยจำนวน 24 ซี่ ในผู้ป่วย 17 คน กรอฟันที่ปุ่มฟันใกล้แก้มจนถึงระดับเนื้อฟัน กระตุ้นเนื้อฟันด้วยหลอดน้ำแข็งขนาดเส้นผ่าศูนย์กลาง 2 มม เป็นเวลา 5 วินาที ให้ผู้ป่วยประเมินความรู้สึกเสียวฟันด้วยวิชวยอนาลอกสเกล จากนั้นกำจัดชั้น สเมียร์ด้วยกรดฟอสฟอริคแล้วล้างด้วยน้ำเกลือสเตอไรด์ ให้ผู้ป่วยประเมินความรู้สึกเสียวฟันด้วยวิชวยอนาลอกสเกลอีก จากนั้นใส่สารออกซาเลท 3 เปอร์เซ็น เป็นเวลา 2 นาที เพื่อให้เกิดผลึกปิดท่อเนื้อฟัน กระตุ้นเนื้อฟันด้วยหลอดน้ำแข็งอีกครั้ง ให้ผู้ป่วยประเมินความรู้สึกเสียวฟันด้วยวิชวยอนาลอกสเกล ในฟัน 8 ซี่ได้วัดอัตราการไหลของของไหลในท่อเนื้อฟันเมื่อกระตุ้นเนื้อฟันด้วยความเย็นในลักษณะที่ทำในคลินิก โดยกระทำในห้องปฏิบัติการหลังจากที่ถอนฟันแล้ว ผลการทดลอง พบว่า วีเอเอส ก่อนกำจัดชั้นสเมียร์ หลังกำจัดชั้นสเมียร์ และ หลังการใส่ออกซาเลท เท่ากับ 21.3 ± 19.5 มม 85.4 ± 15.6 และ 8.5 ± 13.3 ตามลำดับ ซึ่งมีผลอัตราการไหลของของไหลในท่อเนื้อฟันเท่ากับ 2.15 ± 1.02 1.55 ± 0.84 และ 2.29 ± 1.28 นาโนลิตร/วินาที/มม^2 โดยสรุบ กลไกของอาการเสียวฟันในมนุษย์ต่อความเย็นไม่

เป็นไปตาม“สมมุติฐานไฮโดรไดนามิก” แนะว่าน่าจะมีใยประสาทที่รับความเย็นโดยตรงในเนื้อใน โพรงฟัน

คำสำคัญ: อาการเจ็บปวด กลไกเสียวฟัน การไหลของของไหลในท่อเนื้อฟัน ความเย็น ฟันมนุษย์

Abstract

Project Code: RMU4980049

Project Title: Mechanisms of dental pain in normal and inflamed pulp in man

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Project Peroid: 20th July 2006- 19th July 2009

The first series of experiments was aim to determine the relationship between pain intensity and the rate of fluid flow through dentine in human subjects. Alternatively, the objective of these experiments was to proof so called “Hydrodynamic hypothesis of dentine sensitivity” in man. The experiments were carried out on 16 premolars in 13 human subjects (aged 15-25 yrs.). Dentine was exposed at the tip of the buccal cusp, etched with acid and covered with saline. A series of 5 s hydrostatic pressure stimuli between 400mmHg above and 400mmHg below atmospheric were applied to the dentine, in steps of 50mmHg. The subject indicated the intensity of any pain produced on a visual analogue scale (VAS). The fluid flow through dentine during application of the same stimuli was measured in vitro within 3 hours after tooth extraction. *Results:* The median pain threshold with negative (subatmospheric) stimuli was -125mmHg and, with positive pressure stimuli, 200mmHg, which corresponded to dentinal fluid flow rates of 3.29nL/s/mm^2 and 5.75nL/s/mm^2, respectively. Both the median pressure and the mean rate of flow at threshold with negative pressures were significantly lower than with positive pressures. The curves relating VAS score to stimulus intensity were similar with both negative and positive pressures. *Conclusion:* The sensory transduction mechanism for pain in human teeth is more sensitive to outward than inward flow through dentinal tubules. The difference in sensitivity was however much less than that of the hydrodynamic receptors in the cat, which respond very much more strongly to negative than positive pressure stimuli. The second series of experiments was aim to determine the effects on the sensitivity of exposed dentine to cold that are produced when dentine is etched to remove the smear layer and when the tubules are blocked again with calcium oxalate. Separate in vitro observations were made on the effects of these procedures on fluid flow through the dentine. The experiments were carried out on 24 premolars in 17 subjects. Dentine was exposed at the tip of the buccal cusp and cold stimuli were applied by placing the tip of an ice stick on the cavity floor for 5 seconds under the following conditions: before etching the dentine, after etching, and after oxalate treatment. The subject indicated the intensity of any pain produced on a visual analogue scale (VAS). Fluid flow through the dentine was recorded under similar conditions in 8 of the teeth in vitro. *Results:* The mean VAS score produced by the ice before etching was 21.3±19.5 (s.d.) mm. This increased significantly to 85.4±15.6 mm after etching ($P<0.01$). After oxalate treatment, it decreased significantly to 8.5±13.3 mm. The corresponding mean rates of fluid flow through dentine were 2.15±1.02, 1.55±0.84, and 2.29±1.28 nL/s/mm^2 exposed dentine, respectively. The mean after etching was significantly less than the other two values ($P<0.05$).*Conclusion:* If the pain was due to hydrodynamic receptors, their sensitivity to dentinal fluid flow changed when the tubules were opened or closed. Alternatively the pain was produced by receptors sensitive to some other change produced by the cold stimuli, such as specific cold receptors.

Key Words: Pain, Mechanisms of Dentine Sensitivity, Fluid Flow through Dentine, Cold Stimulation, Human Teeth

Research project
Mechanisms of dental pain in normaland inflamed pulp in man
Project no. RMU4980049

This project was composed of three series of experiments to determine the mechanisms of dental pain in man. In series I experiments entitled "Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man" The objective of this experiments was to test the hypothesis that fluid movement through dentinal tubules is responsible for pain sensation in man. This was the first paper that be able to proof the hydrodynamic hypothesis in man after Vongsavan and Matthews proof this hypothesis in the cats in 1994 (Vongsavan and Matthews, 1994). This paper was published in *Archives of Oral Biology* in 2007; (cited up to 2009 = 3) with the note from the editor with "an outstanding manuscript". In series II experiments entitled "Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man" The objective of this experiments was to determine the mechanism of cold stimulation to exposed dentine in human subjects. This was also the first paper that demonstrated that cold stimuli did not not evoke dental pain via hydrodynamic hypothesis. This paper was also published in *Archives of Oral Biology* in 2007 (cited up to 2009 = 6). In series III experiments entitled "The effect of applying potassium chloride solutions at atmospheric pressure on the sensitivity of dentine in man". The objective of this experiments was to determine the effects of applying KCl solutions to exposed dentine at atmospheric pressure on dentinal pain in human subjects. This paper was also published in *Archives of Oral Biology* in 2009 (cited up to 2009 = 0). This paper was also commented as the good scientific paper on the effect of potassium ions on dental pain sensation or dentine hypersensitivity.

Series I experiments

Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man

Abstract

Objective: To determine the relationship between pain intensity and the rate of fluid flow through dentine in human subjects.

Design: The experiments were carried out on 16 premolars in 13 human subjects (aged 15-25 yrs.). Dentine was exposed at the tip of the buccal cusp, etched with acid and covered with saline. A series of 5 s hydrostatic pressure stimuli between 400mmHg above and 400mmHg below atmospheric were applied to the dentine, in steps of 50mmHg. The subject indicated the intensity of any pain produced on a visual analogue scale (VAS). The fluid flow through dentine during application of the same stimuli was measured in vitro within 3 hours after tooth extraction.

Results: The median pain threshold with negative (subatmospheric) stimuli was -125mmHg and, with positive pressure stimuli, 200mmHg, which corresponded to dentinal fluid flow rates of 3.29nL/s/mm^2 exposed dentine and 5.75nL/s/mm^2, respectively. Both the median pressure and the mean rate of flow at threshold with negative pressures were significantly lower than with positive pressures. The curves relating VAS score to stimulus intensity were similar with both negative and positive pressures.

Conclusion: The sensory transduction mechanism for pain in human teeth is more sensitive to outward than inward flow through dentinal tubules. The difference in sensitivity was however much less than that of the hydrodynamic receptors in the cat, which respond very much more strongly to negative than positive pressure stimuli.

Introduction

The sensory transduction mechanism by which impulses are generated in intradental nerves when potentially painful stimuli are applied to enamel or dentine appears to involve displacement of the contents of dentinal tubules; the tubules acting as hydraulic links between the enamel or an exposed dentine surface and the nerve endings located either in the pulpal ends of the tubules or in the underlying pulp. This is the so-called hydrodynamic mechanism (Brännström 1963, Pashley 1990, Matthews *et al*. 1996, Orchardson and Cadden 2001). Much of the evidence for this is based on data obtained by recording from intradental afferents in experimental animals (Matthews 1977, Kollmann and Matthews 1982, Närhi *et al* 1982a, Närhi *et al* 1982b, Närhi and Haegerstam 1983). Vongsavan and Matthews (1992) developed a method for recording fluid flow through dentine in vivo in cats and this method has been used to relate dentinal flow to the impulse discharge evoked in intradental afferents during the application to exposed dentine of hydrostatic pressure stimuli between 500 mmHg above, and 500 mmHg below atmospheric pressure (Matthews and Vongsavan 1994, Vongsavan and Matthews 1994, Andrew and Matthews 2000). In those studies it was found that the afferents that responded to dentinal flow (hydrodynamic afferents) were much more sensitive to the outward flow through the dentine produced by negative (subatmoshpheric) pressure stimuli than to the inward flow produced by positive pressure stimuli (Matthews and Vongsavan 1994, Vongsavan and Matthews 1994, Andrew and Matthews 2000).

The aim of the present experiments was to determine the relationship between pain intensity and the rate of fluid flow through the dentine in human subjects, and to compare this relationship with the properties of hydrodynamic receptors determined earlier. Positive and negative pressure stimuli over a range similar to those used in the cat were applied to exposed dentine in man and the intensity of any pain evoked was recorded. For technical reasons, it was not possible to record simultaneously the flow through dentine produced by the stimuli. This was recorded later, in vitro. The number and diameters of the tubules through which the fluid flowed were estimated from SEM images of the cavity floor.

Materials and methods

The experiments were carried out on 16 premolar teeth in 13 subjects (mean age 20 years, range 15-25). All the teeth were scheduled to be extracted for orthodontic reasons. They were fully erupted, vital, and free of caries and restorations. The study was approved by The Committee on Human Rights Related to Human Experimentation of Mahidol University.

Without local anaesthesia, dentine was exposed at the tip of the buccal cusp of each tooth by cutting a cavity (diameter approx. 2 mm., depth approx. 3 mm.) with round and cylinder diamond burs (No. 201 and 204, Intensive®, Viganello-Lugano, Switzerland) under a constant stream of water. The cavity was then etched with 35% phosphoric acid for 30 seconds and rinsed with water for 1 minute. A short length of stainless steel tube (14G, o.d. 2.11 mm, i.d.1.56 mm) was sealed to the wall of the cavity with a mixture of light-cure composite resin (Z100™ Restorative; 3M Dental Products Division, St. Paul, USA) and fissure sealant (Concise; 3M Dental Products Division, St. Paul, USA). The cavity was filled with normal saline and the tube was connected to a syringe (for setting the stimulus pressure) and a manometer via a 3-way tap and polyethylene tube (Fig. 1). The syringe was disconnected from the tap to return the pressure in the system to atmospheric.

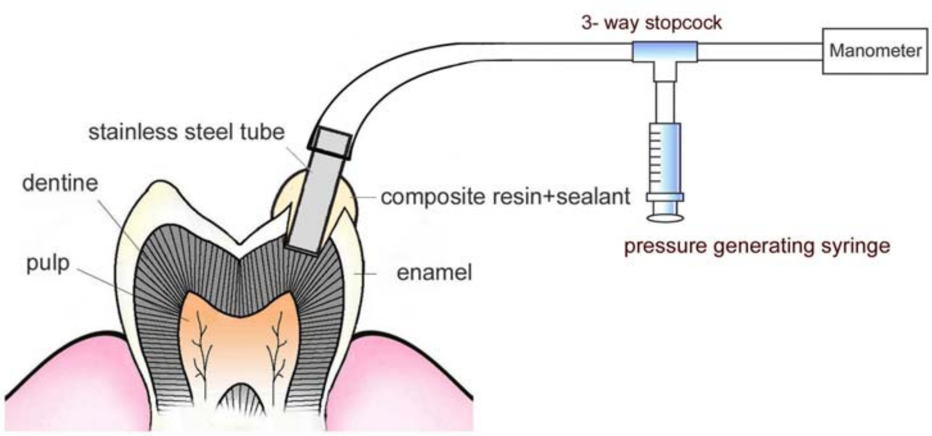

Figure 1. Diagram of the experimental set up for recording responses to hydrostatic pressure stimulation of exposed dentine in man.

Hydrostatic pressure stimuli between +400 and –400 mmHg were used. The stimuli were applied as pairs of alternate positive and negative pressures of the same absolute magnitude, starting at 50 mmHg and increasing in steps of 50 mmHg. The duration of each stimulus was 5 s and the interval between the stimuli in each pair was approx. 2 s. The interval between the pairs of stimuli was 2 minutes. Each pressure was applied once to each tooth. After each pair of stimuli, the subject indicated the intensity of any pain produced by the positive and by the negative pressure by placing marks on a simple visual analogue scale (VAS) (Clark and Troullos 1990, Holland et al 1997, Gillam et al 2000) that had been calibrated from 0 (no sensation) to 100 mm (the most severe pain one can imagine).

Once this series of stimuli had been completed, the tooth was carefully extracted under local anaesthesia (2% lidocaine HCl with 1:100,000 epinephrine) and stored in a sterile normal saline solution at 5°C. The stainless steel tube was left, sealed into the cavity.

Within 3 hours of extraction, each tooth was sectioned transversely at the cemento-enamel junction with a diamond disc and water coolant. Any coronal pulp tissue remaining was removed with fine tweezers, care being taken to avoid touching the wall of the pulp chamber. The pulp chamber was then irrigated with water from a triple syringe for 10 minutes to remove any remaining tissue. The crown was set up for recording fluid flow through the dentine as shown in Fig. 2. The cut surface of cervical side of the crown was glued (Superglue Gel Extra; Locktite, Welwyn Garden City, UK) to an acrylic block into which had been sealed a stainless-steel tube (18G, o.d. 1.27 mm, i.d. 0.84 mm). This tube was connected to a glass capillary with a uniform internal diameter of 300 µm. The pulp chamber, tube and capillary were filled with Ringer's solution and kept on the same horizontal plane. The Ringer's was connected to a manometer and maintained at a pressure of 15 mmHg to represent the normal tissue fluid pressure of the pulp. Fluid movement between the pulp and dentine was measured by recording with a microscope (overall magnification: X200) the movement of a small air bubble that was introduced into the capillary. The volume flow through the capillary that occurred during each stimulus was calculated from the distance moved by the bubble.

The flow through dentine was recorded in this way during the application to the cavity of the same series of pressure stimuli that had been used in vivo to investigate pain. During each pressure stimulus, at least three flow measurements were made.

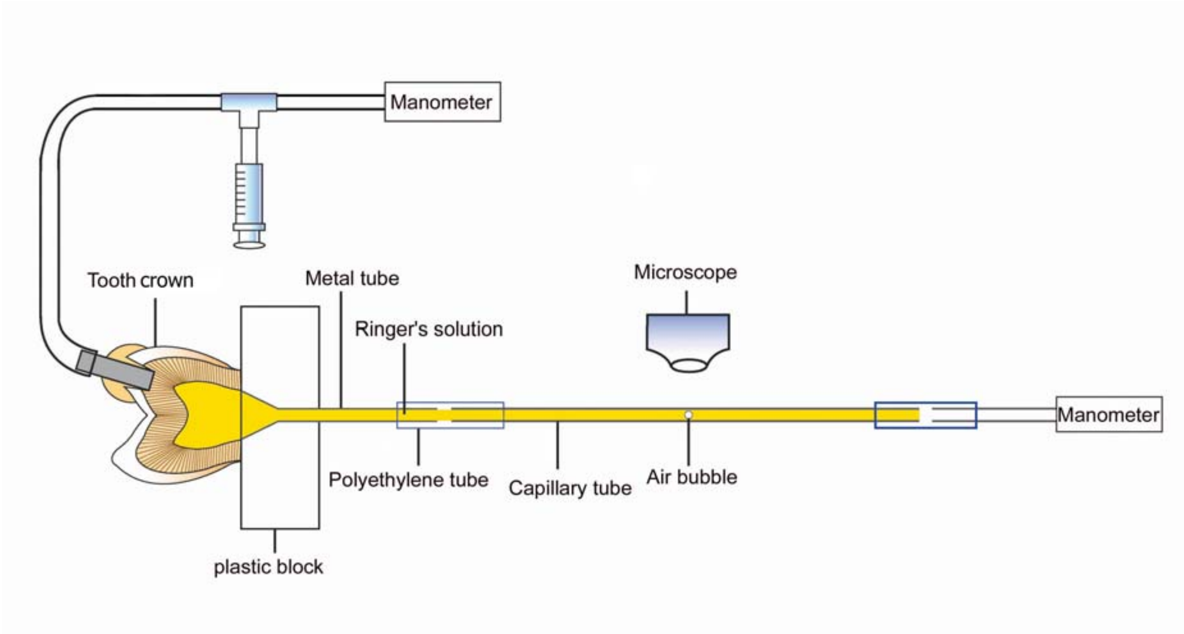

Figure 2. Diagram of the experimental set up for recording the rate of fluid flow through dentine produced by hydrostatic pressure stimuli in vitro.

After the flow measurements were completed, the tube was removed and the crown was detached from the acrylic block and dried at room temperature for 2 days. The crown was then attached to a stub with conductive adhesive tape (SPI Supplies, USA) and sputter-coated with gold-palladium under vacuum (SPI-Module™: SPI Supplies Division of Structure Probe, Inc., USA). The exposed dentine in the floor of the cavity of each tooth was examined under a scanning electron microscope (Jeol® JSM-5410LV, Jeol Ltd., Tokyo, Japan) and a low-power (X35) scan made for measuring the total area of exposed dentine. The total number and the diameters of the tubules exposed on the cavity floor were estimated from higher power scans of selected areas using a method based on that described by Vongsavan and Matthews (1992). Because there was a tendency for the tubules to be larger in diameter and more closely spaced in the centre of the cavity over the pulp horn, several different areas were sampled. The cavity floor was divided into three concentric, circular zones of equal width, with the radius of the central zone one third that of the whole. These zones were labelled A (inner), B (middle) and C (outer). The tubules were measured and counted in five rectangular sample areas: one in the inner zone (A) and two in each of the outer zones (B & C). The areas were selected so that they lay across a diameter of the cavity floor. The area of each rectangle was approximately 12,740 μm^2, giving a total of about 0.064 mm^2. Each of these areas was scanned at a magnification of X1000 for counting the tubules. Within each of these areas, five sub-areas were scanned at a magnification of X3500 for measuring the tubule diameters. Each sub-area covered 970

μm^2. In this way, the diameters of approx. 40% of the tubules counted in the X1000 scans were measured.

After these measurements were complete, each tooth was sectioned bucco-lingually through the cusps with a diamond disc and water coolant. The remaining dentine thickness was measured between the floor of the cavity and the closest point of the pulp chamber along the dentinal tubules in the scanning electron microscope.

Results are summarized as mean ± 1 s.d. or, for non-parametric data, median and range. The significance of differences between means was determined using the paired t-test and between medians, with the Wilcoxon Signed Rank test for paired data. P values of less than 0.05 were considered significant.

Results

Fig. 3 shows the VAS scores recorded at each stimulus intensity by two subjects and Fig. 4 shows the pooled data for all 16 teeth. The pain thresholds for negative pressure stimuli ranged from -50 to -200 mmHg (median -125 mmHg) and, for positive pressure stimuli, from 150 to 400 mmHg (median 200 mmHg). Considering only the absolute values of the thresholds, irrespective of whether they were above or below atmospheric, the median threshold for negative pressure stimuli was significantly lower than that for positive pressure stimuli ($p<0.001$, Wilcoxon Signed Rank test). The mean VAS score produced by stimuli of -400 mmHg was 48.3 ± 29.3 mm and by +400 mmHg, 33.2 ± 27.2 mm. The difference between these means was significant ($p<0.001$, paired t-test).

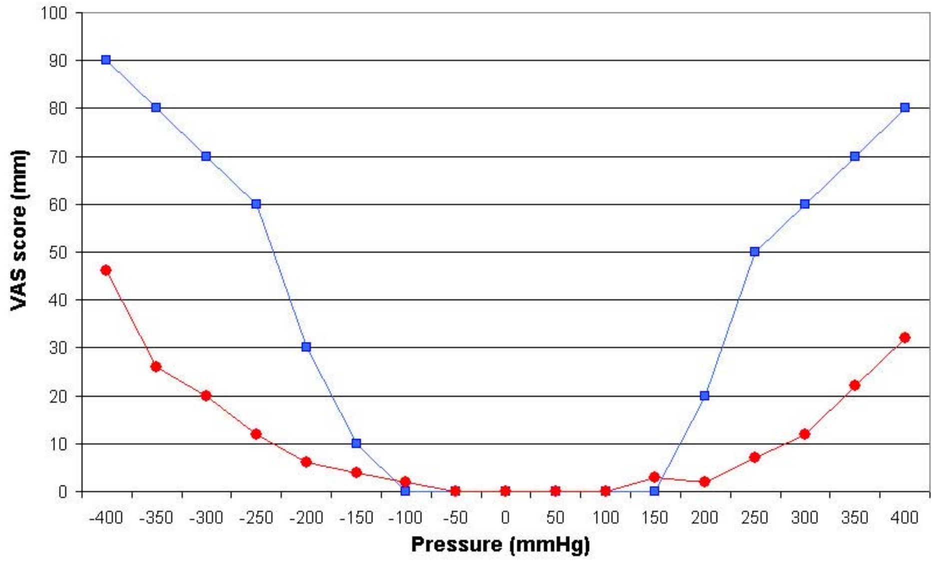

Figure 3. VAS scores, representing the intensity of the pain, recorded at each stimulus Intensity by two subjects. The pressures are relative to atmospheric. The circular symbols represent data from one subject, and the squares, those from the other.

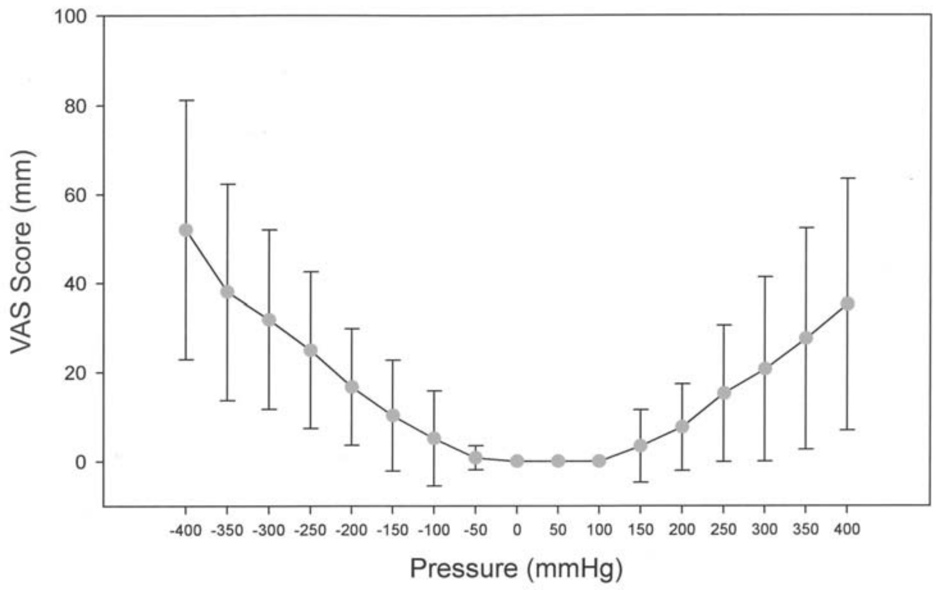

Figure 4. Mean VAS scores recorded at each stimulus intensity. Pooled data for all 16 teeth. The pressures are relative to atmospheric. The error bars represent ± 1 s.d.

Examples of the pressure/flow relationship in vitro in individual teeth are shown in Fig. 5. The flow rates have been normalized to account for differences between teeth in the area of exposed dentine. This area was measured from the SEM images (see below). The data shown in Fig. 5 are from the same teeth as those shown in Fig. 3. The pooled pressure/flow results from all 16 teeth are shown in Fig. 6. The median hydraulic conductance with inward flow (0.0397 nL/s/mm^2/mmHg) was significantly greater than that with outward flow (0.0202 nL/s/mm^2/mmHg). The mean conductance over the whole range rested (i.e. the slope of the line fitted to the data in Fig. 6) was 0.0302 nL/s/mm^2/mmHg. The mean flow rate at the threshold for pain in individual teeth was 3.29 ±2.36 nL/s/mm^2 outwards for negative pressures and 5.75 ±3.62 nL/s/mm^2 inwards for positive pressures. The absolute values of these mean flow rates are significantly different ($p<0.05$, Paired t-test). Differences between teeth in the VAS score recorded with a particular stimulus could not be accounted for by differences in the corresponding fluid flow rates. This is illustrated by the examples shown in Figs. 3 & 5, in which the flow rates were generally greater in the tooth that gave the lower VAS scores.

Based on the tubule counts from the SEM study (see below), these mean thresholds are equivalent to 120 ± 90 and 240 ± 150 fL/s/tubule for outward and inward flow respectively.

With atmospheric pressure in the cavity and the pulpal pressure maintained at 15 mmHg, the average outward flow of fluid through the dentine was 0.38 ± 0.42 nL/s/mm^2.

The SEM results are summarized in Table 1. The average remaining dentine thickness was 1.10 ± 0.34 mm.

There was no correlation between remaining dentine thickness and the pain thresholds with either positive or negative pressures ($p>0.05$, Pearson correlation).

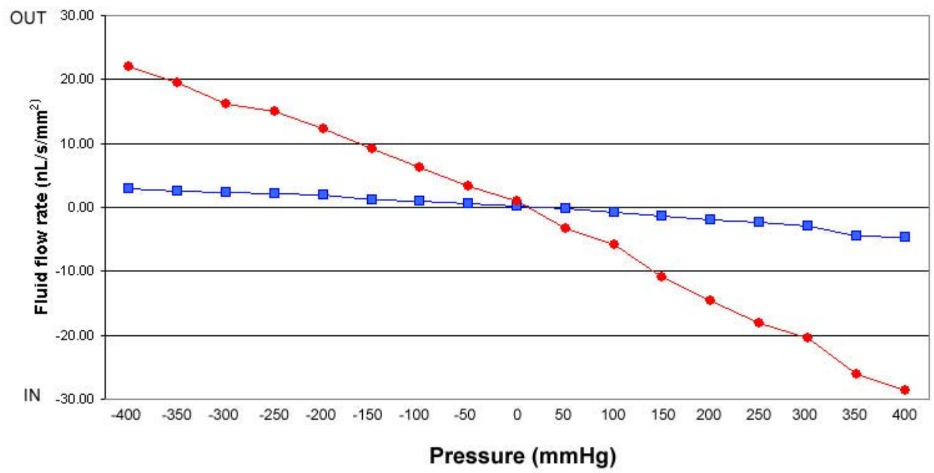

Figure 5. Examples of the pressure/flow relationship recorded in individual teeth in vitro. The flow rates have been expressed per square mm of exposed dentine. Positive flow rates represent flow from the pulp into dentine (outward) and negative values, flow from dentine into pulp (inward). The data are from the same teeth as those shown in Figure 3.

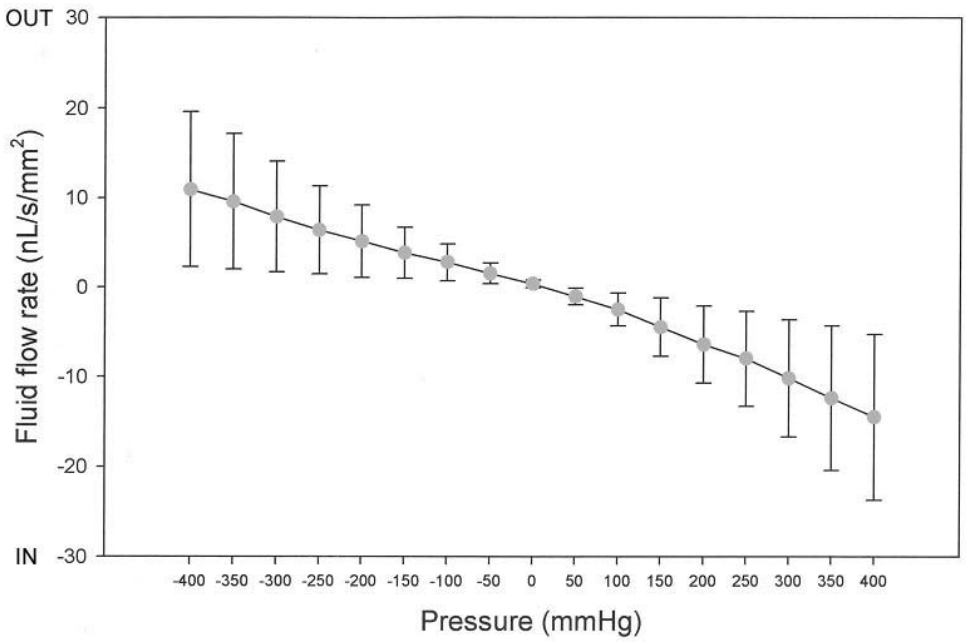

Figure 6. Mean flow rates recorded at different pressures from all 16 teeth. The flow rates have been expressed per square mm of exposed dentine. Positive flow rates represent flow from the pulp into dentine (outward) and negative values, flow from dentine into pulp (inward). The error bars represented ± 1 s.d.

Discussion

Hydrostatic pressure stimulation was used in this experiment because it could be well controlled and, unlike thermal stimuli for example, will selectively excite hydrodynamic receptors as a result of the flow it produces in the contents of the dentinal tubules. In an attempt to minimize damage to the odontoblasts and underlying pulp, the stimuli were kept brief and negative and positive pressure pulses were applied in pairs of matched intensity, as in the cat in previous studies (Matthews and Vongsavan 1994, Vongsavan and Matthews 1994). This is the first study to have recorded pain thresholds during the application of positive and negative hydrostatic pressure stimuli to etched dentine in man. A few previous studies have been carried out using such stimuli, but they have all been made on unetched dentine and much more intense stimuli were used.

In 1960, Brännström showed that a pressure of 757 mmHg below atmospheric caused pain in all 24 teeth he tested. No attempt was made to determine the pain threshold. As Brännström pointed out, the pressure he used was below the saturated vapour pressure of water at 37°C and as a result the stimulus would have caused water to evaporate from the tubules. The total pressure gradient producing outward is therefore difficult to estimate accurately, although it would have been very large and much greater than the stimuli used in the present experiments.

Brännström (1961) investigated positive pressure stimuli that were applied to unetched dentine in buccal cavities. When pressures were applied that increased at a rate of approximately 440 mmHg/s, the pain threshold was usually between 1470 and 2200 mmHg. Using a similar procedure, Brännström et al. (1969) found the thresholds to be between 440 and 2200 mmHg. One very interesting finding in that experiment was that, in several of the teeth, filling the cavity with gutta percha for a week, which causes inflammation of the pulp, resulted in desensitisation of the dentine to positive pressure stimuli while the sensitivity to other forms of stimulus appeared to increase. Later, Ahlquist et al. (1994) investigated the pain produced by hydrostatic pressure stimuli in 4 teeth. They found that no pain was elicited in the presence of a smear layer or after oxalate treatment; but without a smear layer, either negative or positive (range: 1551- 4654 mmHg) hydrostatic pressure evoked pain. However, no attempt was made to determine the pain thresholds under any of these conditions.

The stimulus response curve relating stimulus pressure to VAS score (Fig. 4) was much more symmetrical than the corresponding curve relating pressure to evoked impulse discharge in the cat (Vongsavan and Matthews 1994, Andrew and Matthews 2000). In man, the increase in VAS score with increase in pressure was almost the same with both positive and negative pressures; but in the cat, a much greater discharge was evoked by negative than positive pressure stimuli across the whole range of stimuli tested (see Fig. 7). Although the sensitivities to both forms of stimulus were similar in man, that to negative pressures was greater; as shown by the findings that the median negative pressure threshold (-125 mmHg) was significantly lower than the median positive pressure threshold (200 mmHg), and the mean VAS score with stimuli of -400 mmHg was significantly higher than that with stimuli of +400 mmHg. The thresholds in man were similar to those in the cat, both for negative and positive pressure stimuli (Vongsavan and Matthews 1994, Andrew and Matthews 2000).

The difference between the stimulus/response curves in man and cat referred to above cannot be attributed to differences between the hydraulic conductances of the dentine with inward and outward flow. In the cat, the conductances were similar in both directions (Vongsavan and Matthews 1994), and in man that to inward flow was greater than that to outward flow. The difference between the stimulus/response curves suggests that the hydrodynamic receptors in man are less affected by the direction of fluid flow in the dentinal tubules than those in the cat.

The sensitivity of dentine is generally believed to increase as the exposed surface approaches the pulp, and evidence for this was obtained with positive pressure stimuli by Brännström et al. (1969) No such effect was detected in the present experiments. The lack of any correlation between RDT and the threshold to either positive or negative pressure stimuli was probably because the range of the RDTs was too small.

The mean hydraulic conductance of the dentine in the teeth used in the present experiments was 0.0302 $nL/s/mm^2/mmHg$. For comparison with data from other preparations[21,22], this is equivalent to 0.133 $\mu L/cm^2/min/cmH_2O$ or $22.7x10^{-8}$ m/s/kPa. Pashley et al.[22] made measurements from slices of dentine from the crown of human third molars in vitro and obtained the value range from 0.068 to 0.495 $\mu L/cm^2/min/cmH_2O$, which in the range obtained from the present study. The value obtained from buccal cavities in vivo in man[23] was lower, approx. 0.03 $\mu L/cm^2/min/cmH_2O$. The hydraulic conductance in vivo would be expected to be lower than in vitro on account of the presence of vital odontoblast processes in the tubules. Also, the conductance of the dentine over the pulp horns will tend to be greater than that buccally because the total cross-sectional area of the tubules per unit area of exposed dentine is much larger there (Ciucchi et al. 1995, Fogel et al. 1988).

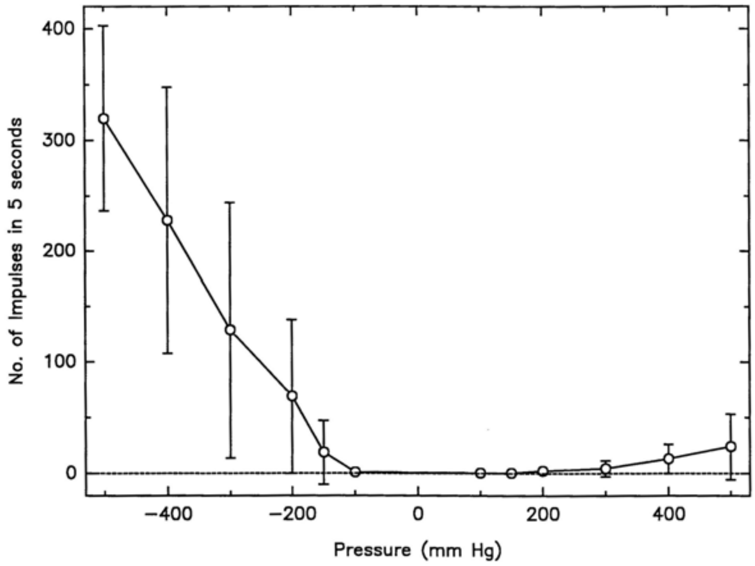

Figure 7. The relationship between the rate of flow of fluid through dentine produced by the application of pressure stimuli and the number of action potentials in the multi-unit record obtained from the cats' tooth

When the sensory thresholds in man were expressed in terms of the flow produced at the same pressures in vitro, the mean threshold for outward flow (3.29 nL/s/mm^2) was significantly less than that for inward flow (5.75 nL/s/mm^2). These values are greater than the corresponding data obtained for the evoked discharge in the cat. These were 0.8 nL/s/mm^2 for outward, and 1.3 nL/s/mm^2 for inward flow (Vongsavan and Matthews 1994),

The mean threshold with negative pressure stimuli was equivalent to a flow rate of 120 fL/s/tubule, outside the range for the thresholds of the evoked discharge in the cat, which was 21 to 53 fL/s/tubule.

The numbers of tubules/ mm^2 (Table 1) on the exposed dentine surface in the present study are within the range found at a similar distance from the pulp cornu in human teeth by Garberoglio and Brännström (1976). These tubule counts were also almost the same as the average figures in the dentine over the pulp horn of the cat canine stimuli (Vongsavan and Matthews 1994, Andrew and Matthews 2000), the area stimulated in the experiments in which recordings were made of the neural discharge evoked by hydrostatic pressure stimuli. The total area of exposed dentine in the floor of the cavity in the present experiments was much greater than in the cat studies (mean 3.33 compared with 0.82 mm^2) although the variation in the number of tubules/mm^2, and in the diameters of the tubules, between zones was much less in the human than the cat teeth. In the cat, there were many more tubules in the central zone A over the pulp horn (mean: 70,144/mm^2) than peripherally in zone C (mean: 10,196/mm^2), and the mean diameter of the tubules was 0.92 µm in zone A and 0.49 in zone C. Also, because of the difference in tooth size, the variation in length of the tubules under the exposed dentine surface would have been much greater in the cat than in the human teeth. It is to be expected therefore that the rate of flow in individual tubules would have been fairly uniform across the dentine surface in the human teeth, but much high in the centre than peripherally in the cat.

The diameters of the tubules in the present study were about double those recorded by Garberoglio and Brännström (1976). This difference can be accounted for by the fact that the dentine was not etched in the earlier study. Using Garberoglio and Brännström's figure of 1.2 µm for the mean diameter of the tubules of unetched dentine 1.1 mm from the pulp horn, the mean velocity of the tubule contents at the median sensory threshold for negative pressure stimuli is estimated to have been 106 µm/s, which is similar to the estimate of 64 µm/s for the evoked discharge in the cat stimuli (Vongsavan and Matthews 1994).

In conclusion, the sensory transduction mechanism for pain in human teeth is more sensitive to outward than inward flow through dentinal tubules. The difference in sensitivity was however much less than that of the hydrodynamic receptors in the cat, which respond very much more to negative than positive pressure stimuli.

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Series II experiments

Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man

Abstract

***Objective*:** To determine the effects on the sensitivity of exposed dentine to cold that are produced when dentine is etched to remove the smear layer and when the tubules are blocked again with calcium oxalate. Separate in vitro observations were made on the effects of these procedures on fluid flow through the dentine.

Design: The experiments were carried out on 24 premolars in 17 subjects. Dentine was exposed at the tip of the buccal cusp and cold stimuli were applied by placing the tip of an ice stick on the cavity floor for 5 seconds under the following conditions: before etching the dentine, after etching, and after oxalate treatment. The subject indicated the intensity of any pain produced on a visual analogue scale (VAS). Fluid flow through the dentine was recorded under similar conditions in 8 of the teeth in vitro.

Results: The mean VAS score produced by the ice before etching was 21.3±19.5 (s.d.) mm. This increased significantly to 85.4±15.6 mm after etching ($P<0.01$). After oxalate treatment, it decreased significantly to 8.5±13.3 mm. The corresponding mean rates of fluid flow through dentine were 2.15±1.02, 1.55±0.84, and 2.29±1.28 $nL/s/mm^2$ exposed dentine, respectively. The mean after etching was significantly less than the other two values ($P<0.05$).

Conclusion: If the pain was due to hydrodynamic receptors, their sensitivity to dentinal fluid flow changed when the tubules were opened or closed. Alternatively the pain was produced by receptors sensitive to some other change produced by the cold stimuli, such as specific cold receptors.

Introduction

The sensory transduction mechanism by which impulses are generated in intradental nerves when potentially painful stimuli are applied to enamel or dentine appears to involve displacement of the contents of dentinal tubules through a hydrodynamic mechanism. It seem that the tubules act as hydraulic links between the site of stimulations and the nerve endings which are located either in the pulpal ends of the tubules or in the underlying pulp (Matthews 1985, Matthews and Sessle 2001, Pashley 1990). Recently, the direct evidences (Vongsavan and Matthews 1994, Andrew and Matthews 2000) demonstrated that the transduction mechanism is more responsive to outward than inward flow through the dentinal tubules during the application to exposed dentine of hydrostatic pressure stimuli and the impulses may be generated simply as a result of damage to the nerve endings as they are stretched or compressed between the odontoblasts and the tubules walls.

In cold stimulation, there is evidence to support the hydrodynamic theory. This includes the finding that the latencies of sensory responses to cold stimulation in humans Naylor (1968) and of neural response in the cat (Kollmann and Matthews 1982), which are less than a second, are too short to be accounted for by a mechanism that involves a temperature change in the pulp or at the pulp/dentine interface. It is possible that the responses are due to a transduction mechanism that involves the displacement of the contents of the dentinal tubules at the moment the stimulus is applied and produce negative intrapulpal pressures (Matthews and Hughes 1988). In addition, cooling the exposed dentine teeth produced outward flow of the fluid in dentinal tubule *in vitro* study in human (Horiuchi and Matthews 1973) and *in vivo* study in cat (Vongsavan and Matthews 1992). Further support is provided by the observation that the pain responses to cold stimulation decreased immediately after oxalate treatment by obstruction the tubules (Kontturi-Närhi and Närhi 1993, Muzzin and Johnson 1989).

Previous studies have demonstrated that cold stimulation of teeth in dogs (Matthews 1977) and in cats (Kollmann and Matthews 1982) evoked responses in intradental nerve and the decline in responses with repeated testing were similar. The study in cat demonstrated that removal of the enamel and outer dentine of the teeth in cat decreased substantially the response to cold stimulation (Kollmann and Matthews 1982). This suggested that the progressive decrease in response with repeated cooling may have been due to structural damage and permanent displacement of tubule contents occurred.

However, the transduction mechanism is not fully understood and no data is available in well-control study in human teeth. Thus, the aims of the present experiments were (1) to investigate whether the response to cold stimulation decrease when the cusp tip removed in human teeth, (2) to evaluate the effect of smear layer and oxalate treatment on the sensitivity to cold stimulus in human teeth, and (3) to test that fluid flow through dentinal tubules is part of the mechanism involved in the transduction of cold stimulus throughout this experiment.

Materials and methods

Subjects

The experiments were carried out on 24 healthy premolars in 17 human subjects (aged 16-29 years, mean 19.2 ± 3.9 years). These teeth were scheduled for extraction as part of orthodontic treatment. All the teeth were fully erupted, free of caries, without restorations and intact. Radiographic and clinical examinations confirmed that they were vital and healthy.

The experiments were carried out in the Advanced Clinic, Faculty of Dentistry, Mahidol University. The study was approved by The University Ethics Committee. Prior to the experiment, informed consent was obtained from each subject, for those under 18 years, a parent or guardian.

Experimental design and procedure

Series 1 experiments (In vivo)

This series of experiments was carried out on 24 premolars in 17 subjects for measurement the response to cold stimulation under different conditions.

Without local anesthesia, dentine is exposed at the tip of the buccal cusp by cutting a small cylindrical cavity (approximately 3 mm in diameter and 3 mm in depth) with 2 diamond burs (round and cylinder diamond burs; Intensive® No. 201 and No. 204, Viganello-Lugano, Switzerland) in an airotor handpiece under an adequate water coolant (Figure 1). The base of the cavity was prevented from drying with distilled water.

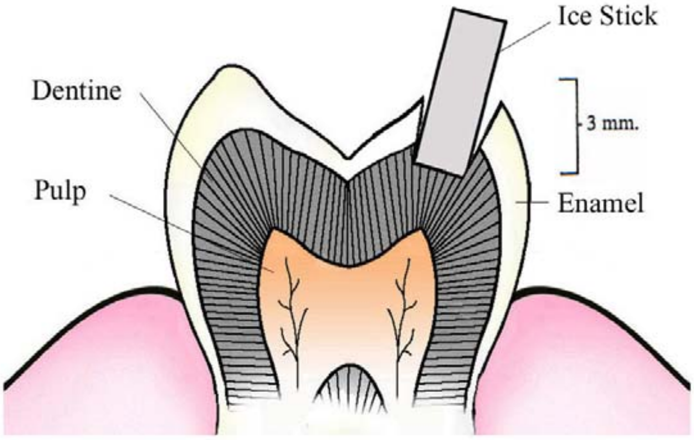


Figure 1. Diagram of tooth preparation and cold stimulation of exposed dentine in human premolar teeth in series 1 experiment.

Cavity was prepared at the tip of buccal cusp (approximately 3 mm in diameter and 3 mm in depth). Cold stimulus was applied by placing the tip of an ice stick (3 mm in diameter) gently to the cavity for 5 seconds.

Cold stimuli are applied by placing the tip of an ice stick (3 mm in diameter) gently to each tooth for 5 seconds. The subject indicated the intensity of any pain produced by placing a mark on a 100-mm visual analog scale (VAS). This method of testing dentine sensitivity was repeated under 5 different conditions: (1) before the tooth preparation (tooth intact) by stimulation the premolar tooth with ice stick on the tip of buccal cusp, (2) after the tooth preparation by repeating the cold stimulation on the exposed dentine, (3) after etching the exposed dentine with 35% phosphoric acid (Scotchbond™, 3M Dental Products, ST. Paul, MN, USA) for 30 seconds and rinsing with water for 1 minute, (4) after filling distilled water in the cavity for 2 minutes, and (5) after treating the etched dentine with 3% potassium tetraoxalate for 2 minutes and rinsing with water for 1 minute. Throughout this experiment, the exposed dentine was prevented from drying with distilled water droppings. An interval of 2 minutes elapsed between the stimuli.

Series 2 experiments (In vivo and In vitro)

This series of experiments was carried out on 8 of the teeth in 6 subjects for measurement rate of fluid flow through dentine.

After completion of the series 1 experiment, eight of the teeth were gently removed of oxalate layer with a cylinder diamond bur and stream of water and produced smear layer on the floor of the cavity. The subject indicated the intensity of any pain produced by placing ice stick into the cavity again. Then, the premolar teeth were carefully extracted with a forceps and elevators under local anesthesia (2% lidocaine HCl with 1 : 100,000 epinephrine) and stored in a sterile normal saline solution at 5^{o}C until the time they were used, which was within 2-3 hours after extraction.

The teeth were sectioned transversely lower than the cemento-enamel junction 1-2 mm using a diamond disc under adequate water coolant. The coronal pulp was removed with fine tweezers and then irrigated with water from a triple syringe for 10 minutes to remove the remaining tissues. The crown was glued by its cut edge to a plastic block (Perspex, ICI Plastics Division, Welwyn Garden City, U.K.) into which had been sealed a stainless-steel tube (external diameter 2 mm; internal diameter 1.75 mm). The tube, then, was connected to a glass capillary (uniform internal diameter 300 µm) to accommodate the rate of flow that occurs during the cold stimulation. The pulp chamber and capillary were on the same horizontal plane and filled with Ringer's solution, which was maintained at a pressure of 15 mm Hg to represent the normal tissue fluid pressure of the pulp by connecting to a manometer. The air bubble in the capillary (Pashley et al 1981) was observed using an operating microscope (x10 eyepiece, x20 objective lens) (Figure 2).

The fluid flow through dentine produced by the cold stimuli were recorded under similar conditions as describe in series 1 experiment (*in vivo* study): (1) after tooth preparation for removal of oxalate and producing smear layer (before etching); (2) after etching the exposed dentine with 35% phosphoric acid for 30 seconds and then rinsing with water for 1 minute to remove smear layer; and (3) after treating the etched dentine with 3% potassium tetraoxalate for 2 minutes and then rinsing with water for 1 minute. The temperature of the tooth was maintained at 37°C through the *in vitro* experiment by continuous dropping warm water (37°C) except when the ice stick was applied.

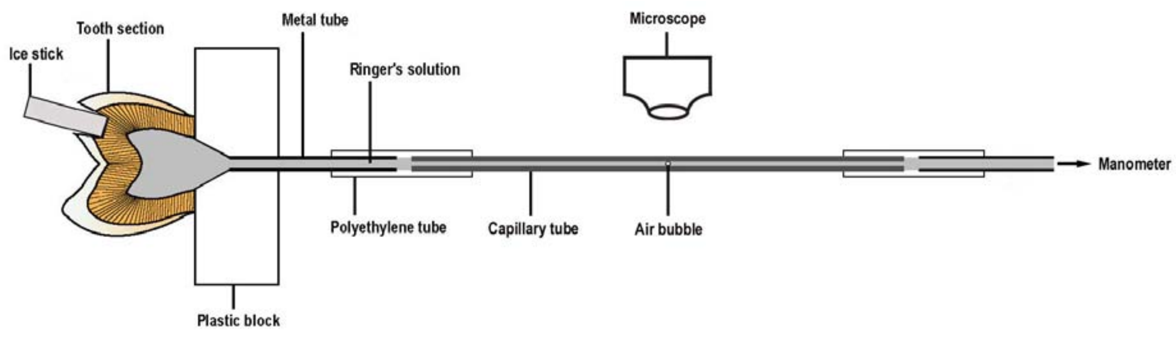


Figure 2. Diagram of the experimental set up in series 2 experiment for measurement of the fluid flow through dentine.

The teeth were sectioned transversely lower than the cemento-enamel junction 1-2 mm. The coronal pulp was removed and then the crown was glued by its cut edge to a plastic block into which had been sealed a stainless-steel tube (external diameter 2 mm; internal diameter 1.75 mm). The tube, then, was connected to a glass capillary (uniform internal diameter 300 μm) to accommodate the rate of flow that occurs during the cold stimulation. The pulp chamber and capillary were on the same horizontal plane and filled with Ringer's solution, which was maintained at a pressure of 15 mm Hg to represent the normal tissue fluid pressure of the pulp by connecting to a manometer. The air bubble in the capillary was observed using an operating microscope (x10 eyepiece, x20 objective lens).

The volume flow through the capillary, and hence the dentine, can be calculated by measuring the distance of the bubble movement in capillary within 5 seconds. In each condition, at least three such measurements were made.

The volume flow through the capillary was calculated from the formula:

$$F = \pi r^2 l$$

Where F = volume flow through dentine within 5 seconds, r = radius of the capillary (150 μm), and l = average distance of a bubble movement in capillary within 5 seconds

In order to normalize the fluid flow rate per 1 mm^2 of exposed dentine, the area of exposed dentine was measured. Before measurement of exposed dentine, teeth were dried under room temperature for 2 days, fixed to a stub with conductive adhesive tape (SPI Supplies, USA), and sputter-coated with gold-palladium under vacuum (SPI-Module™: SPI Supplies Division of Structure Probe, Inc., USA). The cut dentine surface was then examined in a scanning electron microscope (SEM; Jeol® JSM - 5410LV; Jeol Ltd., Tokyo, Japan). Photomicrograph was taken at a magnification of x1000 to measure the area of exposed dentine in each tooth using the Image ProPlus software programme.

Then, the velocity of fluid flow through dentine was calculated form the formula:

$$V = F/5A$$

Where V = fluid flow rate per 1 mm^2, F = volume flow through dentine within 5 seconds.

Measurement of remaining dentine thickness

At the end of the experiments, all teeth were sectioned longitudinally in such a way that the plane of section passed through the buccal and lingual pulp horns with a diamond disc (Komet®, Gebr. Brasseler, Lemgo, West Germany, Type 943, thickness 0.15 mm) under adequate water coolant. Then, the remaining dentine thickness (RDT) was measured between the highest of pulpal horn and floor of the cavity along the dentinal tubules by using a measuring microscope.

Statistical Analysis

Means VAS and rate of fluid flow through dentine in responses to cold stimulations under different conditions were calculated. Differences of means between the data were analyzed statistically using one-way repeated-measures analysis of variance (One-way RM ANOVA). Where there were significant differences between the means, the Tukey test was used to make multiple comparisons between them. Data were reported as means ± 1 S.D. and P values of less than 0.05 were considered significant. The correlation between VAS and RDT, and rate of fluid flow and RDT were also evaluated.

Results

Series 1 experiments (*In Vivo *Study)

In 24 premolar teeth from 17 in human subjects, the pain sensations produced by placing the ice stick (0°C) were obtained with the five different conditions. No pain response was reported before tooth preparation (tooth intact) but the mean VAS scores produced by ice stick after tooth preparation (dentine exposed in the presence of smear layer) was 21.25 mm (SD 19.46 mm). When the smear layer was removed by acid-etching, the mean VAS score increased significantly to 85.42 ± 15.60 mm, and 90.13 ± 13.17 mm, after etching and after filling distilled water in the etched cavity for 2 minutes, respectively.

After oxalate treatment, it decreased significantly to 8.54 ± 13.31 (Figure 3) ($P<0.01$, One-way RM ANOVA and Tukey test).

Series 2 experiment (*In Vivo and In Vitro* Study)

In 8 of the teeth, pain sensation *in vivo* and fluid flow through dentine *in vitro* produced by the cold stimuli under different conditions was recorded to compare the values between the two parameters obtained on the same conditions. The mean VAS scores under different conditions in this series of experiment were similar to those in series I experiment (Figure 4A). In addition, the mean VAS scores after cavity preparation and after removal of oxalate to produce smear layer were 16.25 ± 10.94 and 18.75 ± 11.26 mm, respectively. They were no significantly different ($P>0.05$).

Ice stick produced an outward fluid movement from the pulp chamber toward the exposed dentine. The mean rates of fluid flow through dentine induced by the cold stimulation before etching, after etching and after oxalate treatment were 2.15 ± 1.02, 1.55 ± 0.84, 2.29 ± 1.28 nL/s/mm^2 respectively. The values before etching and after oxalate treatment were not significantly different ($P>0.05$). There were significantly different between the mean rates of fluid flow through dentine before etching and after etching, and between after etching and after oxalate treatment (Figure 4B) ($P<0.05$, 1-way RM ANOVA and Tukey test).

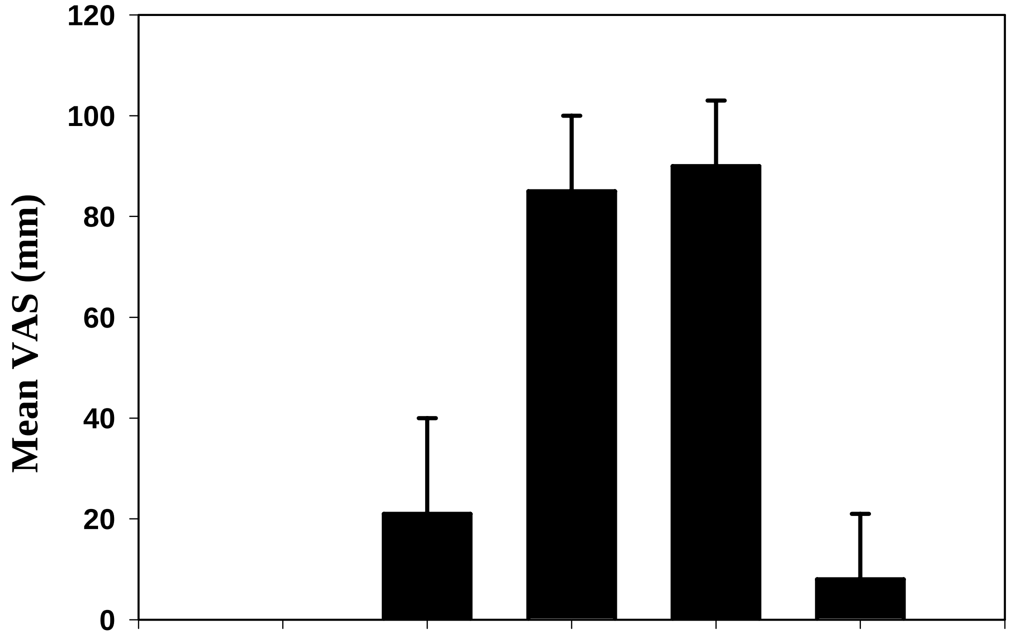


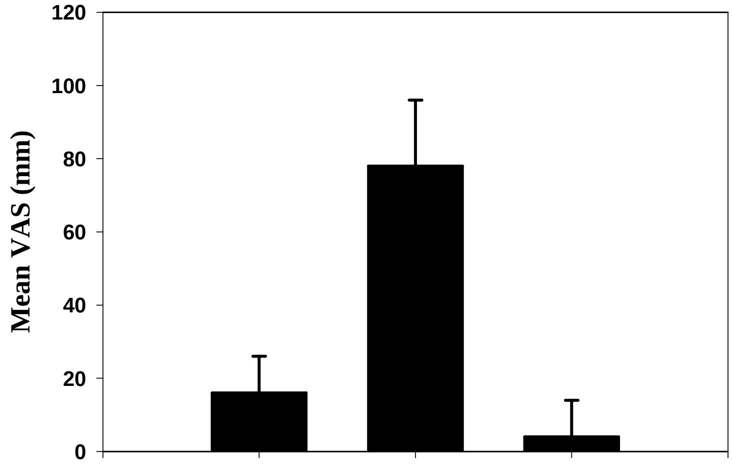
Figure 3. Effect of cold stimulation of exposed dentine on pain sensation under different conditions in series 1 experiment (*in vivo*).

The summary of the mean VAS score produced by the ice stick under 5 different conditions *in vivo* from 24 teeth in 17 subjects was shown. The error bars represent +1 SD. No pain response was reported before tooth preparation (intact) but the means produced by ice stick after tooth preparation. After etching and treatment with distilled water (DW), the mean VAS scores increased significantly at P<0.01 (*). After oxalate treatment, the mean decreased significantly (P<0.01).

Remaining Dentine Thickness

The RDT had an average of 1.15 ± 0.66 mm (n = 24). There were no significant relationships between RDT and any pain responses produced by the cold stimulation of exposed dentine under different conditions in series 1 experiment (*in vivo*) (P>0.05). Moreover, there were also no correlation between the RDT and the fluid flow rate through dentine produced by the cold stimuli under similar conditions in series 2 experiment (*in vitro*).

A



B

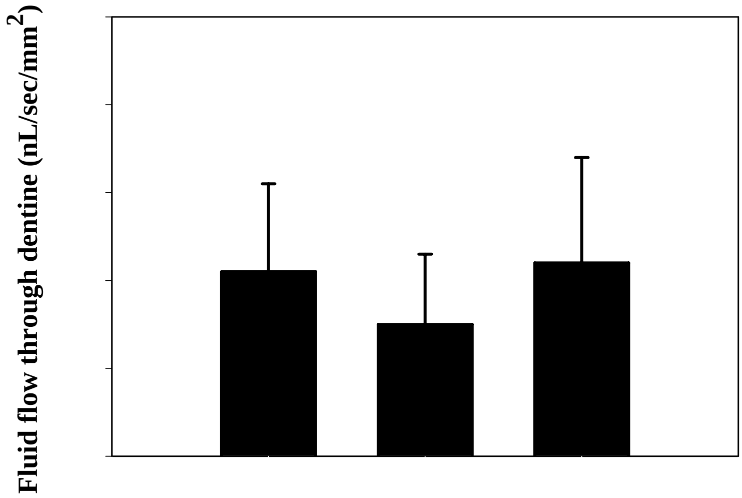


Figure 4. Effect of cold stimulation of exposed dentine on pain sensation and the rate of fluid flow through dentine under different conditions in series 2 experiment (*in vivo* and *in vitro*)

(A) The summary of the mean VAS score produced by the ice stick under 3 different conditions *in vivo* from 8 teeth in 6 subjects was shown. The error bars represent +1 SD. After etching the mean VAS scores increased significantly when compare to the other means at $P<0.01$ (*).

(B) The summary of the mean fluid flow rates through dentine produced by the ice stick under similar conditions from the same teeth as shown the results in (A) was shown. The error bars represent +1 SD. After etching the mean fluid flow rates decreased significantly when compare to the other means at $P<0.05$ (*).

Discussion

In the present experiments, the relationship observed between fluid flow through dentine *in vitro* and pain sensation produced by cold stimulation of exposed dentine *in vivo* in the same teeth under different conditions in human subjects do not follow with the hydrodynamic hypothesis. It contrasts with the previous studies, which demonstrated that the responses to cold stimulation were due to the transduction mechanism which involved the displacement of the contents of the dentinal tubules (Kollmann and Matthews 1982, Matthews 1977), especially the outward flow direction (Vongsavan and Matthews 1994, Andrew and Matthews 2000, Horiuchi and Matthews 1973, Vongsavan and Matthews 1992b).

Clinically, patients suffering from dentine sensitivity or dental pain complaint most to cold stimuli, such as cold drinks or foods (Kontturi-Närhi and Närhi 1993, Orchardson and Collins 1987) and patients reported more painful when the enamel and outer dentine were removed by attrition. In addition, exposed dentine covered with a smear layer has been reported to be relatively insensitive (Hirvonen et al 1984). Blocking of dentinal tubules in hypersensitive dentine with potassium oxalate reduced the pain sensation to cold stimulation tubules (Kontturi-Närhi and Närhi 1993, Muzzin and Johnson 1989). This sensitivity in man is very different from the results of the experiments obtained from the cats (Kollmann and Matthews 1982) and dogs (Matthews 1977). The intradental nerve responses recorded during cooling a cat tooth from 30°C to 10°C had propertied similar to those recorded from isolated single nerve fibers in the earlier experiments on dogs. The units in the cats responded initially when the stimulus was applied to a nearly intact tooth but removal of the enamel and outer dentine decreased substantially the response to cooling. They suggested that the responses were due to a transduction mechanism, which involved the displacement of the contents of the dentinal tubules as the result of different dimensional changes in the matrix and the tubule contents when the outer layer of the tooth were cooled suddenly. Removing the outer dentine and opening this end of some tubules could reduce the displacement of tubule contents at their pulpal ends, where the nerve endings and the final stage in the transduction process could be located.

In well-controlled study of this experiment in series 1, the pain sensations produced by ice stick (0°C) under different conditions in human subjects were recorded to compare with the results of the clinical studies in man and the experiment studies in animals. In addition, to investigate the effect of smear layer and oxalate treatment on dentine sensitivity to cold stimulation of exposed dentine was also done.

It is clearly that the nerve responses recorded after cooling a tooth in the previous studies in dogs (Matthews 1977) and cats (Kollmann and Matthews 1982) are different from the behaviors in mans. The *in vivo* results in the present study confirm the earlier clinical surveys (Kontturi-Närhi and Närhi 1993, Orchardson and Collins 1987) that the application of cold stimuli caused pain sensation in man *in vivo*. When the enamel and outer dentine were removed by tooth preparation, pain sensation increased. Moreover, it increased to the ceiling when the smear layer was removed by acid-etching. However, the reduction of pain response after treatment of exposed dentine with oxalate was observed. Pain response was decreased dramatically when comparing with the ceiling response in positive controlled tooth of which dentinal tubules treated with distilled water. This desensitizing agent was very effective to treatment dentine hypersensitivity because 76.5% of subjects reported no pain sensations to 0°C of the cold stimulations. The oxalate can also decrease pain sensation about 2.5 times of smear layer. In human premolar teeth were treated with oxalate demonstrated that oxalate crystal penetrated into the dentinal tubules about 6-7 μm (Hongpakmanoon et al 1999), which is thicker than the thickness of smear layer covering on exposed dentine produced during the cavity preparation, 0.5-1.0 μm (Pashley 1996). The more thickness of oxalate crystals seems to result of the better insulators to cold stimulation than the smear layer. Thus, temperature stimulus used was not sufficient intensity to excite the receptors inside the pulps of some subjects (Naylor 1963).

In the series 2 experiment, the results *in vivo* were consistent and similar to *in vivo* study in series I experiment. Pain sensations to cold stimulation under 3 conditions were used to compare with the fluid flow through dentine under similar conditions *in vitro* – before etching (in presence of smear layer), after etching (in absence of smear layer) and after oxalate treatment. After complete the *in vivo* study in series 2 experiment, the oxalate layer was gently removed with a cylinder diamond bur and stream of water and smear layer on the floor of the cavity was produced again. The sensitivity of exposed dentine to cold stimulation after removal of oxalate and producing smear layer (before etching II) did recover to the same intensity as before etching I. It confirmed that oxalate crystal was removed.

The *in vitro* results showed that the fluid flow rates recorded during cold stimulation under the 3 conditions were comparable (range 1.55 - 2.29 $nL/s/mm^2$). But the fluid flow rate of etched exposed dentine was significantly less than that of the other conditions. The fluid flow rates recorded during cold stimulation of exposed dentine before etching and after etching in man in the present experiment (2.15 ± 1.02 and 1.55 ± 0.84 nL/s/mm^2, respectively) were greater than those recorded previously (a peak of 1.0 nL/s/mm^2; (Horiuchi and Matthews 1973) and a peak of 0.42 nL/s/mm^2; (Vongsavan and Matthews 1992b), respectively. But these are to be expected because in those studies, they applied 20 – 25 °C of cold Ringer's solution while we applied 0°C of water ice stick. However, the results were similar trend that after etching exposed dentine the flow rates were decreased.

The RDT in our study was not correlated with the pain sensation produced by cold stimulation under different conditions. The pain response of the subjects depend on their pain experiences and it is subjective, not depend on the thickness of the dentine left. The RDT was also not correlated with the fluid flow rate during our cold stimulation. Unlike the previous studies (Kollmann and Matthews 1982, Vongsavan and Matthews 1992b), the cold stimulus used in the present study is ice stick, which can be placed only on dentine exposed.

Throughout the present experiment both *in vivo* and *in vitro*, the exposed dentine was prevented from drying with distilled water droppings during the 2-minute interval of cold stimulations. Because there is outward movement of dentinal fluid due to evaporative waters loss (Pashley 1994), which is about 1 µl/cm^2/min (Matthews et al 1993). Thereby this fluid loss activates mechanoreceptors and causes pain *in vivo* or can produce the outward movement of the bubble during the *in vitro* study. This can cause pain sensation during the 2-minute interval of cold stimulations *in vivo* or affect the system *in vitro*.

Interestingly, after compare the pain sensation produced by cold stimulation of exposed dentine under the 3 conditions *in vivo* and the fluid flow through dentine under similar conditions, the results were against to hydrodynamic mechanism. The fluid flow rate of etched exposed dentine was the least value, while pain sensation of etched exposed dentine to cold stimulation was the greatest value when compare to those of the other two conditions. Thus, the transduction mechanism of dental pain sensation produced by cold stimulation dose not depended on the displacement of the content of dentinal tubule. The possible explanations for sensory transduction of cold stimulation in man are following. (1) It seems likely to have cold fibers in dental pulp responding for reduction of temperature. Belmonte and his colleague studied about sensory nerves terminal in cornea, which is supplied by ophthalmic division of trigeminal nerve. This group identified the nerve impulses originating polymodal receptors, mechanoreceptors and cold receptors in guinea-pig cornea (Brock et al 1998) and demonstrated that there was 29% of cold receptors in the sensory fiber of cornea in cat (Gallar et al 1993). They also studied about the sensory experiences in human cornea and concluded that cold stimuli selectively activated cold-sensitive fibers (Acosta et al 2001). It is possible that there are cold fibers in intradental nerves which are the nerve terminal of maxillary and mandibular divisions of the same cranial nerve (Matthews and Sessle 2001). During the acid etching process, there may be damaging the pulpal tissues under the dentinal tubules including odontoblasts, fibroblasts and nerve fibers. The substances released form these cells may change the nerve environment and sensitize these nerve endings. Thus, cold stimulus causes more painful after etching exposed dentine but it is not sufficient intensity to activate or excite the nerve receptors after oxalate treatment. It was emphasized that the properties of individual units are required in interpreting the response to cooling (Kollmann and Matthews 1982) and not all intradental afferent nerves respond to such hydrodynamic stimuli (Matthews et al 1996).

However, there are many evidences supporting that the hydrodynamic mechanism involves to the transduction mechanism of cold stimulation but the precise transduction mechanism of cold stimulation remains unknown. So far, the present experiments demonstrated that sensory transduction of cold stimuli in human teeth does not depend only on a hydrodynamic mechanism.

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Series III experiments

The effect of applying potassium chloride solutions at atmospheric pressure on the sensitivity of dentine in man

Abstract

Objective: To determine the effects of applying KCl solutions to exposed dentine at atmospheric pressure on pain evoked by probing and air blast stimuli in human subjects.

Design: The experiments were carried out on 24 premolars in 16 subjects (aged 16-30 years). A cavity (diam. 3mm, depth 3mm) was cut at the tip of the buccal cusp and etched with 35% orthophosphoric acid. The cavity was filled with 500 mmol/l KCl, 250 mmol/l KCl or 500 mmol/l NaCl for 10 minutes, after which it was rinsed with normal saline. Each solution was tested in 8 teeth. Air blast and probing stimuli were applied to the exposed dentine prior to dentine treatment and at 0, 2, 5, 10, and 20 min after treatment. After each stimulus the subject indicated the intensity of the pain evoked on a visual analogue scale (VAS).

Results: In teeth treated with 250 or 500 mmol/l KCl, the mean VAS response to air blast stimuli was significantly decreased at 5 and 10 min. after treatment. The mean VAS response to probing was significantly decreased 10 min. after treatment with 500 mmol/l KCl. Otherwise there were no significant changes.

Conclusion: Topical application to exposed dentine of solutions containing a high concentration of potassium ions at atmospheric pressure produce a temporary reduction in the sensitivity of dentine to air blast and probing stimuli.

Introduction

It is generally accepted that toothpastes containing high concentrations of potassium ions are effective in reducing dentine hypersensitivity in man (Orchardson and Gillam 2000, Orchardson and Gillam 2006). The evidence for the use of potassium salts as desensitizing agents was based in part on observations of their effects on intradental nerves in cats (Markowitz et al 1991). Other evidence of both inhibitory and excitatory effects of potassium ions on the sensitivity of exposed dentine in cats has been reported (Ajcharanukul et al 2007).

More recently, Ajcharanukul et al. (2007) demonstrated that, in man, the application of 500 mmol/l KCl to exposed dentine at a pressure of 150 mm Hg above atmospheric for 4 min. produced a temporary reduction in the pain evoked both by probing and by air blast stimulation of dentine. The reason for applying the KCl solution at a raised pressure was to reverse the normal outward flow in the dentinal tubules and produce a rapid increase in the K^+ concentration in the inner dentine. In the present experiments the effect of applying KCl solutions at atmospheric pressure has been investigated, when K^+ ions will have entered the tubules only by simple diffusion, as with topical applications in the form of toothpaste.

Materials & methods

Subjects

The experiments were carried out on 24 healthy premolars in 16 human subjects (age range 16 - 30 years, mean 20.8, S.D. 4.1). All teeth were scheduled for extraction as part of orthodontic treatment. Radiographic and clinical examinations confirmed that all teeth were fully erupted, vital, free of caries, and without restorations.

The experiments were carried out in the Advanced Clinic, Faculty of Dentistry, Mahidol University and conformed with the standards set by the Declaration of Helsinki. The study was approved by the Ethics Committee on the Use of Human Rights Related to Human Experimentation of the Mahidol University. An informed consent was obtained from each subject, or for those under 18 years, a parent or guardian.

Outline of experimental procedures

Several of the methods were similar to those used in the earlier experiments.[4]

Dentine was exposed by cutting a cavity at the tip of the buccal cusp of the test tooth. The exposed dentine was etched, and control responses to air blast and probing stimuli recorded. One of three test solutions was then applied for 10 minutes, after which the cavity rinsed with normal saline for 1 min. and the stimuli repeated. The test solutions were 500 mmol/l KCl, 250 mmol/l KCl and 500 mmol/l NaCl. Each tooth was treated with only one solution and the solutions were allocated randomly. Each solution was tested in 8 teeth.

The experiment was designed on a double blind basis, in which one researcher selected the dentine treatment of each experimental tooth and cut the cavity; and another applied the treatment and the test stimuli and carried out the pain assessments, without knowing which solution was being tested.

Tooth preparation

Fig. 1 shows a diagram of the preparation. Without local anesthesia, dentine was exposed at the tip of the buccal cusp by cutting a small cylindrical cavity (approximately 3 mm in diameter and 3 mm in depth) with diamond burs (No. 201, round and No. 204, cylindrical; Intensive®, Viganello-Lugano, Switzerland) in an air-rotor handpiece under a constant stream of water. The smear layer was removed from the exposed dentine by etching with 35% phosphoric acid for 30 s, after which the dentine was rinsed with water for 1 minute. The margin of the cavity was built up with composite resin by approx. 2 mm to increase the volume of test solution that could be accommodated. The dentine surface was kept moist throughout the experimental period.

Application of pain-producing stimuli and pain assessment

Two forms of stimulus were used to test the sensitivity of the exposed dentine: air blast followed by probing. The air blast stimulus consisted of a 3 s jet of air at room temperature directed onto the exposed dentine from a triple syringe approximate 2 mm from cavity's edge. The air flow rate was 6.51 l/min from a 1.1 mm diameter orifice. The probing stimuli were applied using a ball-ended (0.5 mm diameter), stainless steel probe (exd. 5, Hu-Friedy®, Chicago, IL, USA). Forces in the range 20-30 g, each of 0.5-1 s duration, were applied to the floor of cavity.[4] The interval between stimuli was 2 minutes.

Pairs of stimuli were applied prior to dentine treatment and at 0, 2, 5, 10, and 20 min after dentine treatment.

After the application of each stimulus, the subjects indicated the intensity of any pain produced by placing a mark on a simple visual analogue scale (VAS) (Holland et al

1977) that had been calibrated from 0 (no sensation) to 100 mm. (the most severe pain one can imagine) immediately prior to the experiment.

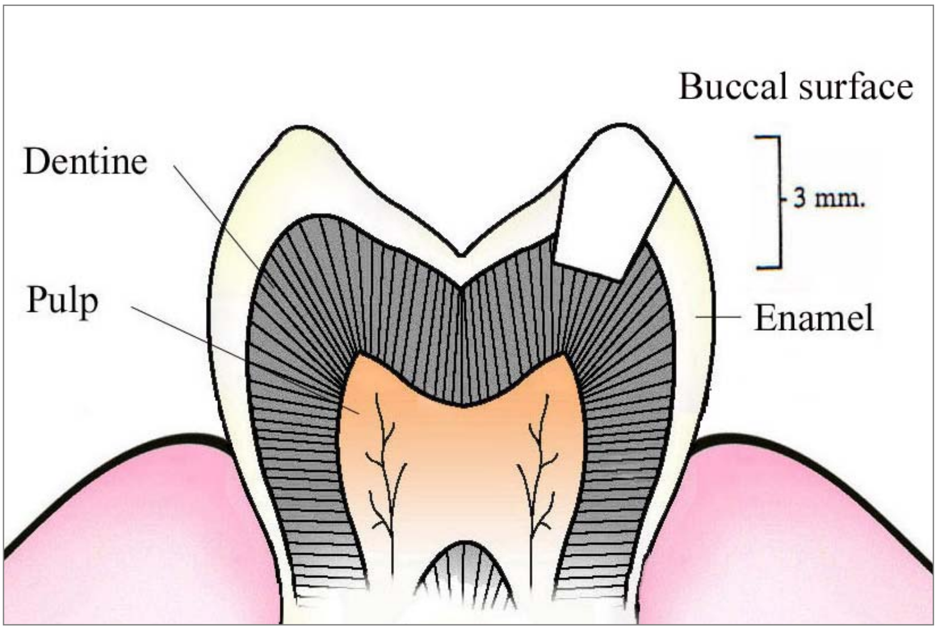

Figure 1. - Diagram of the preparation.

Cavity area and remaining dentine thickness

At the end of the experiment, the teeth were carefully extracted under local anesthetic (2% lignocaine HCl with 1:100,000 epinephrine) and processed for the measurement of the exposed dentine area and remaining dentine thickness. The exposed dentine area was measured from photomicrographs taken at a magnification of X25 using the *Image ProPlus®* software program. After measuring the area of exposed dentine, each tooth was sectioned longitudinally through the cavity with a diamond disc and water coolant. The remaining dentine thickness was measured with a calibrated microscope as the shortest, straight-line distance along the dentinal tubules between the floor of the cavity and the pulp horn. The apices of the roots were examined to confirm that root formation was complete.

Statistical Analysis

The VAS scores are reported as means ± 1 standard deviation (S.D.). The significance of differences between mean baseline VAS scores obtained with different forms of stimulus prior to treatment was determined using Student's paired t-test. The significance of differences between baseline VAS scores and those obtained after different treatments was determined using repeated-measures analysis of variance where the data were normally distributed, as determined by the Kolmogorov-Smirnov normality test; otherwise the Friedman repeated-measures ANOVA on ranks was used. Where the appropriate test showed a significant change, multiple comparisons between individual means or medians were made using the Tukey test.

Correlations between the baseline VAS scores for each form of stimulus and both the remaining dentine thickness and the exposed dentine area were determined by calculating Pearson product moment correlation coefficients.

P values of less than 0.05 were considered significant.

Results

The topical application of 500, 250 mmol/l KCl or 500 mmol/l NaCl in Ringer's solution for 10 min at atmospheric pressure did not produced pain in any of the subjects.

Before treatment, both air blast and probing stimuli produced pain from all the teeth. The mean VAS score with air blast stimuli (88.0, S.D. 14.8, n=24) was significantly greater than that with probing (59.1, S.D. 20.1) (paired t-test, $P<0.001$).

The effects of the 3 forms of treatment on the VAS scores are summarized in Fig. 2. In each case, there was a tendency for the mean responses to decrease from the baseline values for up to 10 min. after treatment, and then to recover during the subsequent 10 min. The only treatments that produced significant changes were the 500 mmol/l KCl solution, after both 5 and 10 min. with air blast stimuli, and after 10 min. with probing; and the 250 mmol/l KCl solution, after both 5 and 10 min. with air blast stimuli. Five min. after treatment with 500 mmol/l KCl, the fall in the mean VAS score with air blast stimuli was from a baseline of 88.1 mm (S.D. 9.1) to 54.6 mm (S.D. 26.9) (one way repeated-measures ANOVA, Tukey test, $p<0.05$); and after 10 min. it had fallen further to 44.3 mm (S.D. 32.0) ($p<0.05$). The corresponding mean values for 250 mmol/l KCl were a fall from a baseline of 79.1 mm (S.D. 21.1) to 63.9 mm (S.D. 26.3) ($p<0.05$) after 5 min. and to 57.4 mm (S.D. 28.0) ($p<0.05$) after 10 min. With probing, the mean VAS score 10 min. after treatment with 500 mmol/l KCl had fallen from a baseline of 64.4 mm (S.D. 19.0) to 41.6 mm (S.D. 21.0) ($p<0.05$). The effect on responses to probing 5 min. after 500 mmol/l KCl was not significant (one way repeated-measures ANOVA, Tukey test, $p>0.05$), nor were the responses at either 5 or 10 min. after 250 mmol/l KCl (Friedman repeated-measures ANOVA on ranks , $p>0.05$). With 250 mmol/l KCl, the median VAS score to probing was 54.5 mm before treatment and 45.5 and 37.5 mm, respectively, 5 and 10 min. after treatment. None of the changes from baseline after 500 mmol/l NaCl were significant.

The overall mean area of dentine exposed in the floor of the cavity was 7.30 mm^2 (S.D. 1.06), and the mean remaining dentine thickness was 1.19 mm (S.D. 0.25). In neither case were there significant differences between the mean values for the groups of teeth used for each of the different test solutions (one way ANOVA, $p>0.05$). There was no

significant correlation between the baseline VAS scores with air blast or probing stimuli, and either the remaining dentine thickness or the exposed dentine area (Pearson product moment correlation coefficient). The same results were obtained when the correlations were calculated using the changes in VAS score after 10 min. instead of the baseline VAS scores.

There were no significant differences between the median ages of the subjects used to test the different solutions (19.5, 21 and 18.5 years; Kruskal-Wallis one way ANOVA on ranks). The root apices of all the teeth were closed.

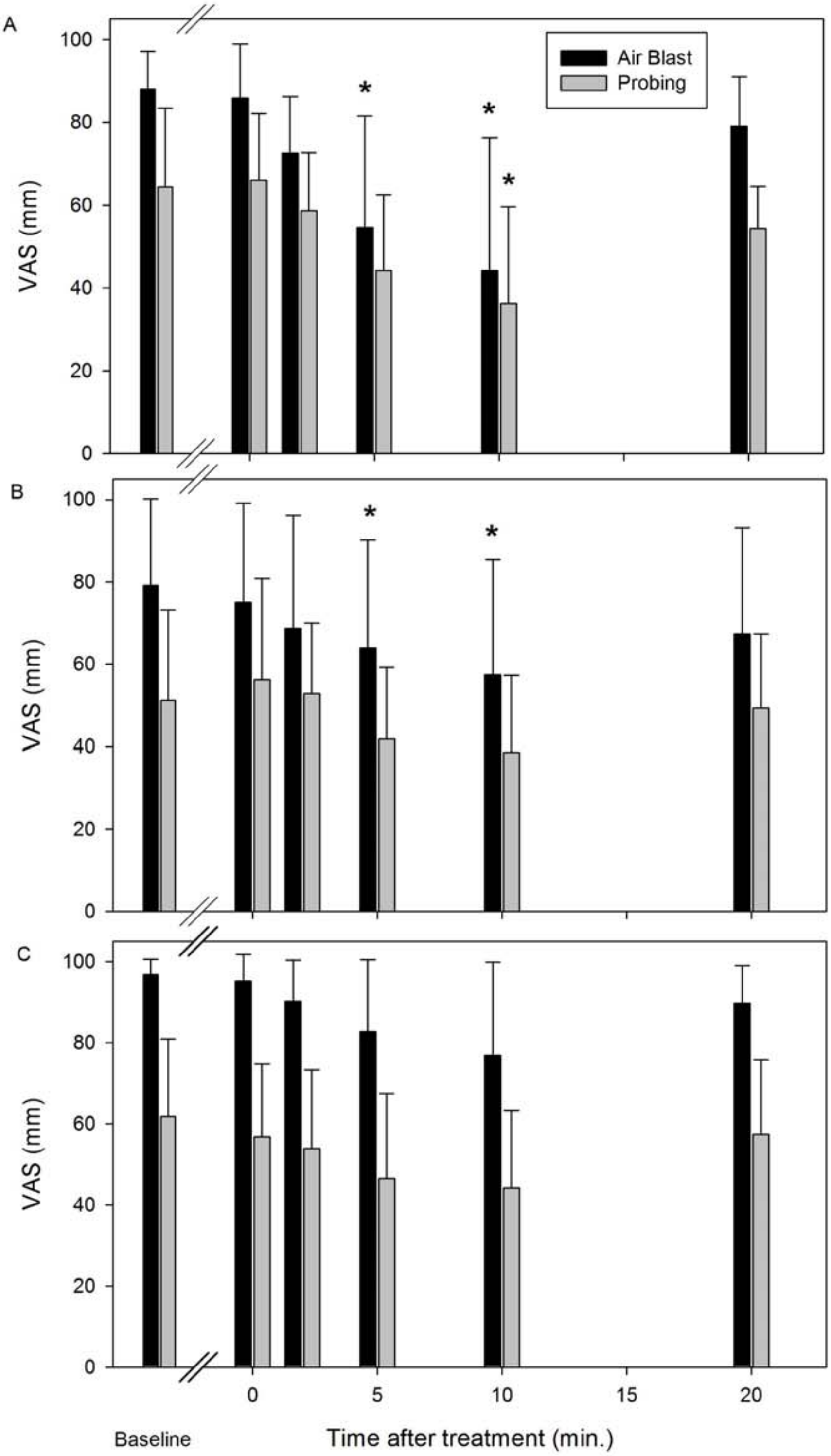

Figure 2. - Effects of applying solutions of KCl or NaCl to exposed dentine on the VAS responses to air blast (black) and probing (dot pattern) stimuli. Each histogram shows baseline data before dentine treatment, and data obtained at different times after treatment. Each column represents the mean VAS score from 8 teeth, with the corresponding S.D. The solutions applied to the dentine were 500 mmol/l KCl in A, 250 mmol/l KCl in B, and 500 mmol/l NaCl in C.

Discussion

These experiments are the first to demonstrate that the topical application of a solution of potassium to exposed dentine in man at atmospheric pressure desensitizes the dentine to pain-producing stimuli. The mean VAS scores were reduced by up to 50% 10 min after a 10 min application of 500 mmol/l KCl solution, but the desensitization was short-lived; the sensitivity of the dentine was not significantly different from baseline values 20 min after treatment.

A decrease in the sensitivity of dentine has been observed in other experiments during repeated stimulation, including the application of osmotic stimuli in man (Anderson and Matthews 1967) and cold, mechanical and hydrostatic pressure stimuli in the cat (Kollmann and Matthews 1982, Andrew D, Matthews 2000). The mechanisms of these effects are not known. The tendency for the VAS scores to fall with repeated stimulation following treatment with 500 mmol/l NaCl in the present experiments may have been a related phenomenon. Such an effect could have contributed to the desensitization of the dentine observed after KCl treatment.

Transient desensitization of dentine by potassium ions is consistent with observations in the cat by Markowitz et al (1991) and particularly with those of Wanachantararak and Matthews (1999, 2000). Also, Ajcharanukul et al (2007) found that applying a 500 mmol/l KCl solution to exposed dentine in man at a pressure of 150 mm Hg above atmospheric reduced markedly the pain produced by air blast and probing stimuli for up to 10 minutes.

In the latter experiments the KCl was added to Ringer's solution in an attempt to avoid changing the concentration of other ions in the dentinal fluid as the test solution was forced into the tubules under pressure. In the present experiments, in which the test solutions were applied at atmospheric pressure, they were made up in distilled water to more closely replicate the conditions when toothpaste is applied to hypersensitive dentine.

A raised concentration of potassium ions in the extracellular fluid at the pulpal ends of the tubules and in the adjacent pulp under the treated dentine is likely to have contributed to the desensitization of the dentine by depolarizing nerve terminals (Ajcharanukul et al 2007). The time-course of the changes in dentine sensitivity indicate that the maximum concentration in the extracellular fluid around the nerve terminals was reached 10 min. after the KCl solution was washed off. This could have occurred if there was little change in the K^+ concentration in the inner ends of the tubules during the 10 min. treatment period, but inward diffusion of K^+ towards the inner ends of the tubules continued for a short while after the KCl solution was washed off. Outward flow of fluid in the tubules would have affected the rate of inward diffusion of K^+ from the exposed dentine surface (Vongsavan and Matthews 1991, 1992, Vongsavan et al 2000) and would have resulted in the K^+ concentration around the nerve terminals being progressively restored to that of normal extra-cellular fluid of approx. 4 mmol/l as the tubular fluid was replaced with tissue fluid from the pulp.

Hypersensitive dentine in man is similar to the preparation used in the present experiments in that the outer ends of the dentinal tubules are patent in both cases (Yoshiyama et al 1989) and the factors affecting the rate of inward diffusion of K^+ into the tubules from the tooth surface are likely to be similar. These experiments therefore fail to explain how the topical application of substances such as toothpastes containing a high concentration of potassium ions can have a lasting effect on hypersensitive dentine.

In conclusion, solutions containing a high concentration of potassium ions, when applied to exposed dentine in human subjects at atmospheric pressure, produce a significant but temporary reduction in the sensitivity of dentine to air blast and probing stimuli.

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Outputs จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. **ผลงานที่ตีพิมพ์ในวารสารระดับนานาชาติ** Archives of Oral Biology.

   (impact factor = 1.554, From Journal Citation Reports, 2008)

1. Charoenlarp P, Wanachantararak W, Vongsavan N, Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man, *Archives of Oral Biology*; 2007; 52:625-631.

2. Chidchuangchai, W., Vongsavan, N. and Matthews, B. Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man. *Archives of Oral Biology*; 2007; 52: 154-1607.

3. Noparatkailas, S, Wanachantararak W, Vongsavan N, Matthews B. The effect of applying potassium chloride solutions at atmospheric pressure on the sensitivity of dentine in man. *Archives of Oral Biology*; 2009; 54: 50-4.

2. **การนำผลงานวิจัยไปใช้ประโยชน์**

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Charoenlarp P, Wanachantararak W, Vongsavan N, Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man, *Archives of Oral Biology*; 2007; 52:625-631.

ได้รับการอ้างอิงใน ISI Web of Science® (cited) = 3 ครั้ง

Chidchuangchai, W., Vongsavan, N. and Matthews, B. Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man. *Archives of Oral Biology*; 2007; 52: 154-1607.

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ได้รับเชิญให้เป็น Key note speaker ในการประชุม “The International Symposium on Molecular Destruction and Reconstruction of the Dentin/Pulp Complex and Its Surrounding Tissues” Tokyo, Japan, 5 มีนาคม พ.ศ. 2550)(ตามเอกสารแนบ)

ภาคผนวก

1. Reprints ของ

Charoenlarp P, Wanachantararak W, Vongsavan N' Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man, *Archives of Oral Biology*; 2007; 52:625-631.

Chidchuangchai, W., Vongsavan, N. and Matthews, B. Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man. *Archives of Oral Biology*; 2007; 52: 154-1607.

Noparatkailas, S, Wanachantararak W, Vongsavan N' Matthews B. The effect of applying potassium chloride solutions at atmospheric pressure on the sensitivity of dentine in man. *Archives of Oral Biology*; 2009; 54: 50-4.

ภาคผนวก

2. ได้รับการอ้างอิงใน ISI Web of Science® (cited)

Charoenlarp P, Wanachantararak W, Vongsavan N, Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man, *Archives of Oral Biology*; 2007; 52:625-631.

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Chidchuangchai, W., Vongsavan, N. and Matthews, B. Sensory transduction mechanisms responsible for pain caused by cold stimulation of dentine in man. *Archives of Oral Biology*; 2007; 52: 154-1607.

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ภาคผนวก

3. เอกสารที่ได้รับการเชิญให้ไปบรรยายในที่ประชุมวิชาการระดับนานาชาติ

ได้รับเชิญให้เป็น Key note speaker ในการประชุม “The International Symposium on Molecular Destruction and Reconstruction of the Dentin/Pulp Complex and Its Surrounding Tissues” Tokyo, Japan, 5 มีนาคม พ.ศ. 2550)

Editorial comment:.

Paper:

Charoenlarp P, Wanachantararak W, Vongsavan N, Matthews B. Pain and the rate of dentinal fluid flow produced by hydrostatic pressure stimulation of exposed dentine in man, *Archives of Oral Biology*; 2007; 52:625-631.

Editor of Archives of Oral Biology comment:.

This is an outstanding manuscript describing elegant and important experiments. To be able to test the tenets of the hydrodynamic hypotheses on living teeth in living people is a substantial achievement. The report is a well written description of novel and significant observations. Rather than being accepted for publication it should be grabbed for publication. The referee points out a few small areas where improvement might be possible.

(**Archives of Oral Biology** impact factor = 1.554, from: Journal Citation Reports, 2008):