รายงานวิจัยฉบับสมบูรณ์
โครงการวิจัยเรื่อง การพัฒนาเทคนิคเพื่อวัดสัญญาณประสาทจากเนื้อฟันในมนุษย์และการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

โดย

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สิงหาคาม 2558

กิตติกรรมประกาศ

ผู้วิจัยใคร่ขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัยเป็นอย่างยิ่งที่ให้ทุนสนับสนุนโครงการวิจัยนี้ ขอกราบขอบพระคุณศาสตราจารย์เกียรติคุณนายแพทย์วิจารณ์ พานิช ท่านผู้อำนวยการกองทุนสนับสนุนการวิจัยท่านแรกที่แนะนำและให้โอกาสผู้วิจัยได้รับทุนจากสำนักงานกองทุนสนับสนุนการวิจัยเป็นครั้งแรกตั้งแต่ปี 2539 ขอกราบขอบพระคุณศาสตราจารย์เกียรติคุณ ดร. วิชัย บุญแสง ที่เมตตาและให้การสนับสนุนผู้วิจัยได้ทำงานวิจัยด้านกลไกความเจ็บปวดจากฟันได้ทำงานวิจัยในเวลาต่อมา ขอกราบขอบพระคุณ ศาสตราจารย์ ดร. สวัสดิ์ ตันตระรัตน์ อดีตผู้อำนวยการกองทุนสนับสนุนการวิจัย และขอกราบขอบพระคุณ ศาสตราจารย์นายแพทย์สุทธิพันธ์ จิตพิมลมาศ ผู้อำนวยการกองทุนสนับสนุนการวิจัยท่านปัจจุบันที่ยังให้การสนับสนุนผู้วิจัยได้ทำงานวิจัยต่อเนื่อง ขอขอบคุณ คุณสุจารี สอนง่ายเจ้าหน้าที่ฝ่ายวิชาการของสกว. ที่ช่วยเหลือเป็นอย่างมาก ขอกราบขอบพระคุณ ศาสตราจารย์เกียรติคุณทันตแพทย์ ดร. สถิตย์ สิริสิงห ที่เมตตาและให้การแนะนำด้านการวิจัยและวิชาการ ขอกราบขอบพระคุณ ศาสตราจารย์เกียรติคุณทันตแพทย์สมศักดิ์ จักรไพวงศ์ อดีตคณบดีคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล ที่เมตตาและให้การสนับสนุนการวิจัยและสนับสนุนอย่างมากด้านเครื่องมือและอุปกรณ์การวิจัย ขอกราบขอบพระคุณรองศาสตราจารย์ทันตแพทย์ ดร. สุขุม ธีรดิลก อดีตหัวหน้าภาควิชาสรีรวิทยาและชีวเคมีและอดีตคณบดีคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล ที่เมตตาและให้การแนะนำด้านการวิจัยและวิชาการตั้งแต่ผู้วิจัยยังเป็นนักศึกษาทันตแพทยศาสตร์ปีที่ 4 เมื่อปีพ.ศ. 2520 และเรื่อยมาจนถึงปัจจุบัน ขอกราบขอบพระคุณ รองศาสตราจารย์ ทันตแพทย์หญิงวรรณะ อภัย รองศาสตราจารย์ทันตแพทย์ชูโชติ ธนะภูมิ ผู้ช่วยศาสตราจารย์ทันตแพทย์หญิงอิงบุญ เทียนศิริ อดีตหัวหน้าภาควิชาสรีรวิทยาและชีวเคมี ที่เมตตาและให้ให้การสนับสนุน ขอกราบขอบพระคุณ รองศาสตราจารย์ทันตแพทย์หญิง ดร. ธีรลักษณ์ สุทธเสถียร อดีตคณบดีคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล และรองศาสตราจารย์ทันตแพทย์สุรินทร์ สูอำพัน อดีตรองคณบดีฝ่ายโครงการพิเศษ และขอขอบพระคุณ รองศาสตราจารย์ รองศาสตราจารย์ ทันตแพทย์พาสน์ศิริ นิสาลักษณ์คณบดีคณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล รองศาสตราจารย์ ดร.ฤดี สุราฤทธ์ หัวหน้าภาควิชาชีววิทยาช่องปากที่ให้การสนับสนุน

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 ที่ให้การสนับสนุนการวิจัยที่ผ่านมา

ขอขอบคุณอดีตนักศึกษาปริญญาเอกสาขาชีววิทยาช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล ผู้ช่วยศาสตราจารย์ ทันตแพทย์หญิง ดร. ปานตา เจริญลาภ ผู้ช่วยศาสตราจารย์ทันตแพทย์หญิง ดร. วรางคณา ชิดช่วงชัย อาจารย์ทันตแพทย์หญิง ดร. กนิษฐา กิจสมานมิตร ขอขอบคุณ ทันตแพทย์หญิง พรนภัส ว่องวานิชวัฒนะ ทันตแพทย์หญิงอะเคื้อ รักษ์ชน ทันตแพทย์หญิง ปิยะนุช ยงรุ่งเรือง ขอขอบคุณคุณศิรินทิพย์ ชูเนตร นักวิทยาศาสตร์ของภาควิชาฯ

BRG5580003

บทคัดย่อ

รหัสโครงการ: BRG5580003

ชื่อโครงการ:การพัฒนาเทคนิคเพื่อวัดสัญญานประสาทจากเนื้อฟันในมนุษย์และการใช้วิธีไอออนโตโฟรีซีสเพื่อให้เนื้อเยื่อในโพรงฟันชา

ชื่อนักวิจัย: รองศาสตราจารย์ ทพ. ดร. นพคุณ วงษ์สวรรค์

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ระยะเวลาโครงการ: วันที่ 31 กรกฎาคม 2555 ถึงวันที่ 30 กรกฎาคม 2558

การศึกษาวิจัยในการศึกษาที่หนึ่งคณะผู้วิจัยประสบผลสำเร็จในการการพัฒนาเทคนิคเพื่อวัดสัญญานประสาทจากเนื้อฟันในมนุษย์ได้เป็นครั้งแรก การศึกษาวิจัยนี้คณะผู้วิจัยได้ศึกษาเปรียบเทียบสัญญานประสาทจากเนื้อฟันในมนุษย์ที่อยู่ไกล้ผิวเคลือบฟัน และสัญญานประสาทจากเนื้อฟันในมนุษย์ที่อยู่ลึกลงไปในเนื้อฟัน 0.5-2 มม แต่ยังคงเหลือเนื้อฟันอีก 1 มม จากการศึกษาพบว่า สัญญานประสาทจากเนื้อฟันในมนุษย์ที่อยู่ไกล้ผิวเคลือบฟัน ไม่แตกต่างจากสัญญานประสาทจากเนื้อฟันในมนุษย์ที่อยู่ลึกลงไปในเนื้อฟัน 0.5-2 มม การศึกษาวิจัยในการศึกษาที่สอง สาม และสี่ คณะผู้วิจัยประสบผลสำเร็จในการการพัฒนาเทคนิควิธีไอออนโตโฟรีซีสเพื่อให้เนื้อเยื่อในโพรงฟันชาในมนุษย์ได้เป็นครั้งแรก กล่าวคือทำให้สามารถบูรณะฟันที่ผุได้โดยไม่ต้องฉีดยาชาและไม่มีอาการเจ็บปวด ในการศึกษาที่สาม ได้ทดลองวิธีไอออนโตโฟรีซีสเพื่อให้เนื้อเยื่อในโพรงฟันชาในฟันกรามน้อยจำนวน 13 ซี่ โดยใช้ยาชาลิกโนเคน 20% ผสมกับอิพินิฟฟรีน 1% ใช้กระแส 120 ไมโครแอมป์ เวลา 90 วินาที พบว่าทำให้เนื้อเยื่อในโพรงฟันชากว่า 40 นาทีในฟันทุกซี่ที่ทดลอง และมีผลทำให้เลือดที่มาเลี้ยงเนื้อเยื่อในโพรงฟันหดตัวเพื่อทำให้อาการชานานขึ้น การวัดปริมาณเลือดที่มาเลี้งฟันใช้วิธีวัดด้วยเครื่องเลเซอร์ ดอปเปลอร์โฟลมีเตอร์ ในการศึกษาที่สี่ ได้ทดลองวิธีไอออนโตโฟรีซีสเพื่อให้เนื้อเยื่อในโพรงฟันชาในฟันกรามที่ผุจำนวน 57 ซี่โดยใช้ยาชาลิกโนเคน 20% ผสมกับอิพินิฟฟรีน 1% ใช้กระแส 200 ไมโครแอมป์ เวลา 2-10 นาที พบว่า 7 ซี่ใช้เวลาไอออนโตโฟรีซีส 2 นาที 17 ซี่ใช้เวลาไอออนโตโฟรีซีส 4 นาที 14 ซี่ใช้เวลาไอออนโตโฟรีซีส 6 นาที 4 ซี่ใช้เวลาไอออนโตโฟรีซีส 8 นาที 7ซี่ใช้เวลาไอออนโตโฟรีซีส 10 นาที อีก 7 ซี่ไม่ชาแม้ใช้เวลาไอออนโตโฟรีซีส 10 นาทีแล้ว โดยสรุปวิธีไอออนโตโฟรีซีสประสบผลสำเร็จโดยสามารถทำให้เนื้อเยื่อในโพรงฟันทีผุชาได้ สารถบูรณะฟันโดยไม่ต้องฉีดยาชา 87.5%

คำสำคัญ: อาการเจ็บปวด ภาวะเสียวฟัน สัญญานประสาท วิธีไอออนโตโฟรีซีส ยาชา ฟันมนุษย์ที่ผุ

Abstract

Project Code: BRG5580003

Project Title: Development of the techniques for recording nerve discharges from human dentine and the iontophoretic delivery of local anesthetic drugs through dentine to obtain pulpal anesthesia in man

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Project Peroid: 31th July 2012- 30th July 2015

The first series of experiments, the technique for recording nerve discharges from human dentine was successfully developed. This recording would be the quantitative pain assessment for pain sensation eg. the number of action potentials or spikes per second. The first objective of these experiments were to determine if dentine at the enamel-dentine junction (EDJ) in man is more sensitive to hydrostatic pressure stimuli then deeper dentine.In all the teeth, the intensity of the pain produced by a stimulus tended to increase as the cavity was deepened, as did the number of action potentials recorded (in 6 of the 8 teeth). The responses were greater from etched than unetched dentine, and negative pressures evoked greater responses than the corresponding positive pressures. It was concluded that there was no evidence that dentine close to the EDJ was more sensitive to hydrostatic pressure stimuli than deeper dentine. The second and third series of experiments aim to use high concentration of lignocaine and to determine the effects of the iontophoretic application of lignocaine and epinephrine to exposed dentine on the sensitivity of the dentine in premolars in human subjects. The lignocaine plus epinephrine solution completely blocked the pain produced by both forms of stimulus immediately, and this continued for 40 min. It also produced an immediate fall in pulpal blood flow that lasted for 40 min. The control solution had the same effect on pulpal blood flow but no effect on dentine sensitivity. The topical application of 20% lignocaine and 0.1% epinephrine, with an iontophoretic current of 120 μA for 90 s, will anaesthetise exposed, normal dentine. The fourth series of experiments aim to determine the effectiveness of the iontophoretic delivery of lidocaine with epinephrine through carious dentine for pain control during cavity preparation. The total duration (mins.) of iontophoresis required to anaesthetize the dentine was: 2 in 7 teeth, 4 in 17 teeth, 6 in 14 teeth, 8 in 4 teeth, and 10 in 7 teeth. The remaining 7 teeth were not anaesthetized even after 14 mins. of iontophoresis. The iontophoretic delivery of lidocaine with epinephrine anaesthetized dentine for cavity preparation in 49 of 56 (87.5%) of carious molars. The restoration was complete without needle anesthesia

Key Words: Pain, Dentine Sensitivity, Nerve discharges, Iontophoresis, Local anaesthetic, Carious human teeth

Research project

Development of the techniques for recording nerve discharges from human dentine and the iontophoretic delivery of local anesthetic drug through dentine to obtain pulpal anesthesia in man

Project no. BRG5580003

This project was composed of four series of experiments. In series I experiments entitled “Effect of cavity depth on dentine sensitivity in man”. These series of experiments obtained from the grant proposal section 1: “Development of the techniques for recording nerve discharges from human dentine in man”. This was the first that the nerve discharges could be recorded from dentine in man. The manuscript was submitted to *Archives of Oral Biology*.

In series II experiments entitled “Effect of the topical application of 50% lignocaine hydrochloride on the sensitivity of dentine in man”. In series III experiments entitled “Effects of the iontophoresis of lignocaine with epinephrine into exposed dentine on the sensitivity of the dentine in man”. In series IV experiments entitled “Effects of the iontophoresis of lignocaine with epinephrine into exposed dentine on the sensitivity of the dentine in man”. These three series of experiments obtained from the grant proposal section 2: “Development of the iontophoretic delivery technique of local anesthetic drugs through dentine to obtain pulpal anesthesia in man”. We demonstrated that pulpal anesthesia in man could be obtained by using iontophoretic delivery of local anesthetic drugs through dentine. This was the first that the pulpal nerve was anesthetized without needle. The technique was proofed that could be used in carious teeth (series IV). Three papers were published in *Archives of Oral Biology* (Impact factor = 1.735, From Journal Citation Reports®, 2014).

Series I experiments

Effect of cavity depth on dentine sensitivity in man

Abstract

***Objective*:** To determine if dentine at the enamel-dentine junction (EDJ) in man is more sensitive to hydrostatic pressure stimuli then deeper dentine.

Design: Cavities (1 mm diam.) were cut at the tips of the buccal and lingual cusps of 8 premolars in 3 subjects (ages: 22 -25 years). Both cavities were initially deepened to expose the EDJ then one (the test cavity) was deepened in steps of 0.5 mm to a maximum of 2.0 mm below the EDJ. The cavities were tested at each stage, before and after etching, with 5 s, hydrostatic pressure stimuli between 400 mm above, and 400 mm below atmospheric. The intensity of any pain produced was recorded on a VAS scale and electrodes were placed in both cavities in an attempt to monitor any action potentials evoked in intradental nerves.

Results: In all the teeth, the intensity of the pain produced by a stimulus tended to increase as the cavity was deepened, as did the number of action potentials recorded (in 6 of the 8 teeth). The responses were greater from etched than unetched dentine, and negative pressures evoked greater responses than the corresponding positive pressures.

Conclusions: There was no evidence that dentine close to the EDJ was more sensitive to hydrostatic pressure stimuli than deeper dentine. It may however may be more sensitive to mechanical stimuli as it is more compliant.

Introduction

It is widely believed that the dentine at the enamel-dentine junction (EDJ) is more sensitive than deeper dentine; but there is no experimental evidence for this, and the statement seems to be based solely on clinical experience during cavity preparation. Even if it is true for cavity preparation, it may not apply to other forms of stimulation. In the present experiments we have investigated the effect of cavity depth on the sensitivity of dentine to hydrostatic pressure stimulation in man. This form of stimulation was used because it can be applied, controlled and measured very easily and it is a very selective method for stimulating the hydrodynamic receptors associated with dentine (Vongsavan and Matthews, 2007).

The hydrodynamic receptors are thought to be situated in the inner ends of the dentinal tubules or adjacent pulp, and to respond to displacement of the contents of the dentinal tubules. Thus, the tubules are thought to form a hydraulic link between the outer dentine and these receptors, so that the tubules and receptors together form the hydrodynamic transduction mechanism whereby stimuli applied to the outer dentine, which appears to contain no nerves or other excitable cells, can excite sensory receptors in the inner dentine or pulp.

Tomes (1856) rejected such a mechanism because he thought it could not explain how dentine in one part of a tooth could be more sensitive than that at another, and particularly referred to the observation that ".. in operating upon the tooth for the removal of carious dentine, it is almost invariably found that the dentine immediately below the enamel is much more sensitive than that situated deeper in the tooth".

But there are several ways in which a hydrodynamic mechanism could account for dentine just below the EDJ being more sensitivity than deeper dentine. It might be due to branching of the dentinal tubules under the enamel, so that stimulation of an area of exposed dentine there would affect more tubules and excite more receptors than stimulation of a similar area of deeper dentine. Another possibility is that the hydraulic conductance of the dentine is higher immediately after it is exposed than a short while later, so that a given pressure change at the opened ends of the tubules would produce a greater displacement of the tubule contents and stimulation of the hydrodynamic receptors during the initial stages of cavity preparation than later. Clotting of tubular fluid (Pashley *et al*., 1984) could cause such a fall in hydraulic conductance. A further possibility is that, after the hydrodynamic receptors have been stimulated during the initial exposure of the dentine, they are damaged during the subsequent deepening of the cavity, with the result that their

sensitivity is decreased. The excitability of the receptors might also be reduced if the cavity preparation resulted in changes in the composition of the extracellular fluid around them. There are several ways in which this might occur: exposing the dentine would allow an outward flow of fluid in the opened tubules, driven by the interstitial fluid pressure of the pulp ((Vongsavan and Matthews, 1992), with the result that the normal dentinal fluid would gradually be replaced with pulpal tissue fluid, which may not have the same composition (Wanachantararak *et al.*, 2011). The composition of the fluid around the receptors might also be affected if the cavity preparation caused damage to the odontoblast cell membranes, with the resultant release of potassium-rich intracellular fluid into the dentinal fluid and tissue fluid of the pulp; or if drilling the outer dentine produced disruption of the tight junctions between the odontoblasts (Turner *et al.* 2011), which would affect the diffusion of ions between the pulp and the dentinal fluid. An increase in the potassium ion concentration of the dentinal fluid is known to produce changes in the sensitivity of the hydrodynamic receptors (Wanachantararak *et al.*, 2011).

A decrease in the intensity of the pain produced by dentine stimulation as a cavity was deepened could also be the result of changes in the central nervous system if, for example, the initial stimulation of the pain pathways when the EDJ was reached resulted in inhibition of the same pathways so that they were less sensitive to afferent input evoked by subsequent stimuli.

The present experiments were carried out to determine if the intensity of the pain evoked by hydrodynamic stimuli does decrease as a cavity is deepened below the EDJ.

An attempt was also made to record the action potentials evoked by the stimuli in intradental nerves, with methods similar to those used to record from dentine in the cat (Horiuchi and Matthews, 1974; Vongsavan and Matthews, 2007). Such data could help to differentiate between a peripheral and a central mechanism for a decrease in the sensitivity of dentine as a cavity was deepened, and also show if the neural discharge evoked by a hydrostatic pressure stimulus in man was likely to be due to a hydrodynamic mechanism.

Materials and methods

The experiments were carried out on 8 healthy, premolar teeth (5 upper and 3 lower) in 3 subjects (ages: 22, 24 & 25 years). All the teeth were scheduled to be extracted for orthodontic reasons. Radiographic and clinical examinations confirmed that the teeth were fully erupted, vital, and free of caries. One tooth had a very small, class II restoration.

The experiments were carried out in the Endodontics Department of the Faculty of Dentistry, Mahidol University in the Maha Chakri Sirithon Dental Hospital at the Salaya Campus of the University. The study was approved by the Ethics Committee on the Use of Human Rights Related to Human Experimentation of the Mahidol University and complied with the principles of the Declaration of Helsinki. The experiment procedures were clearly explained to each subject and their written consent to these procedures being carried out was obtained. The subject could terminate the experiment at any stage. The privacy rights of the subjects were observed at all times.

Experimental design

Cavities were prepared at the tips of the buccal and lingual/palatal cusps of each tooth. Both cavities were 1 mm in diameter and were initially deepened to expose the EDJ. One cavity (the test cavity) was then deepened in steps of 0.5 mm to a maximum of 2.0 mm below the EDJ. The other cavity served as a control and was not deepened beyond the EDJ. Initially, at the level of the EDJ, hydrostatic pressure stimuli between 400 mm Hg above, and 400 mm Hg below atmospheric were applied separately to the both cavities, both before and after etching the exposed dentine. The test cavity was then deepened and re-tested at each step. During each stimulus voltage recordings were made from both cavities in an attempt to detect action potentials, and after each stimulus the subject indicated on a visual analogue scale (VAS) the maximum intensity of any pain that had been produced.

Tooth preparation

A 1.0 mm diameter, 1.0 mm deep cavity was cut into the enamel at the tip of the buccal cusp and the palatal/lingual cusp with a 0.7 mm diam. round, diamond bur in an air-rotor drill under a constant stream of water. The enamel in this area is approximately 1.5 mm thick. The centre of each cavity was then deepened gradually with the same bur until the EDJ was reached, which was indicated when either the subject felt pain or the electrical resistance of the cavity fell by a factor of 10 from several MΩ to several 100 KΩ. The cavity resistance was measured by recording the voltage drop between a silver/ silver chloride electrode inserted into the cavity and a silver/ silver chloride reference electrode on the subject's forehead when an alternating, square-wave current of 40 nA was passed between them.

The floor of the cavity was then flattened to the depth of this central depression with a 1.0 mm diameter, tungsten carbide, flat fissure bur in a very slow hand-piece and with copious water spray. At this stage the centre of the floor of the cavity was assumed to be at the EDJ. The depth of the cavity, from its base to the enamel margin, was recorded using an endodontic probe with a sliding marker, and measured with callipers that incorporated a Vernier scale.

The test and control cavities were selected randomly and hydrostatic pressure stimuli were applied to both, as described below. The test cavity was then deepened in steps of 0.5 mm in the direction of the underlying pulp horn, to a maximum of 1.5 (in the case of a small tooth) or 2.0 mm below the EDJ, This was done with the 1.0 mm diameter, tungsten carbide, flat fissure bur as before. At each level, the exposed dentine was again tested with hydrostatic pressure stimuli, before and after etching.

Stimulation

Hydrostatic pressure stimuli were applied by connecting one of two manometers to a stainless steel tube (needle gauge 21: o.d. 0.8 mm, i.d. 0.5 mm; length 5 mm) that was sealed into each cavity with composite resin. One manometer was set at a pressure of up to 400 mmHg above atmospheric, and the other at an equivalent pressure below atmospheric (which will be referred to as a negative pressure). The stainless steel tubes were filled with Ringer's solution and were connected to the manometers through solenoid valves which were controlled electronically. A standard stimulus sequence was used which consisted of applying a positive pressure for 5 s and an equivalent negative pressure for 5 s, separated by 5 s at atmospheric pressure. The applied pressures were recorded with a pressure transducer (Honeywell, type 24PCCFA6/D. Supplied by RS UK; http://uk.rs-online.com/web/p/pressure-sensors/2355835/).

Each cavity was tested initially at the level of the EDJ with 5 s stimuli of ±400 mm Hg. If either the positive or negative pressure stimulus caused pain, stimuli of ±100, ±200, and ±300 mm Hg were applied in random order, with 2 min. between the stimulus sequences. This procedure was repeated after etching the cavity floor with 35% ortho-phosphoric acid for 30 s. The acid was applied with a fine cannula and removed by rinsing the cavity with Ringer's solution applied similarly. If at any level, stimuli of ±400 mm Hg produced no pain, it was assumed that the thresholds to both positive and negative pressure stimuli were greater than this and that less intense stimuli would have produced no pain.

After each stimulus, the subject indicated the maximum intensity of any pain produced by placing a mark on a simple visual analogue scale (VAS) calibrated from 0 (no sensation) to 100 mm (the most severe pain one can imagine) (Holland et al., 1997). As well as recoding the maximum intensity of any pain felt in the form of a VAS score in this way, the subject provided a continuous record of changes in the intensity of any pain during the stimulus by squeezing appropriately a wrist-exerciser that was equipped with strain-gauges.

Each time a test cavity was deepened, the tube was replaced with a new one and the cavity was tested again, before and after etching. The etched control cavity was re-tested at the end of the experiment.

Dentine Recording

Simultaneous voltage recordings were made from the dentine in both cavities during the application of the stimuli. The recordings were made with electrodes that were incorporated into the stainless steel tubes used to apply the stimuli (Fig. 1). In this way, the recordings were made from the same dentinal tubules that were stimulated. Each stainless steel tube was insulated on its outside with polyimide tubing (PIT) (o.d. 1.0 mm, i.d. 0.9 mm, length 5.5 mm; Cole-Parmer, cat.no. WZ-95820-08) and a piece of Teflon-insulated, silver wire (diameter 0.125 mm; Advent Research Materials, prod. no. AG5494) was trapped between the two tubes. To create space for the wire, a flat area was ground on one side of the stainless steel tube. The PIT extended 0.5 mm, and the silver wire 2 mm, beyond the end of the stainless steel tube; and the assembly was sealed together with epoxy resin. The protruding end of the silver wire was stripped of its insulation and formed into a coil, which was pushed inside the end of the PIT. Just before use, a thin layer of silver chloride was deposited electrolytically onto the silver coil to form a silver/silver chloride electrode. Differential recordings were made between each of these electrodes and an indifferent electrode made of the same wire, which was inserted under one of the contact points of the tooth from which the recordings were made. The reference electrode was a silver/silver chloride ECG electrode that was attached to the subject's forehead.

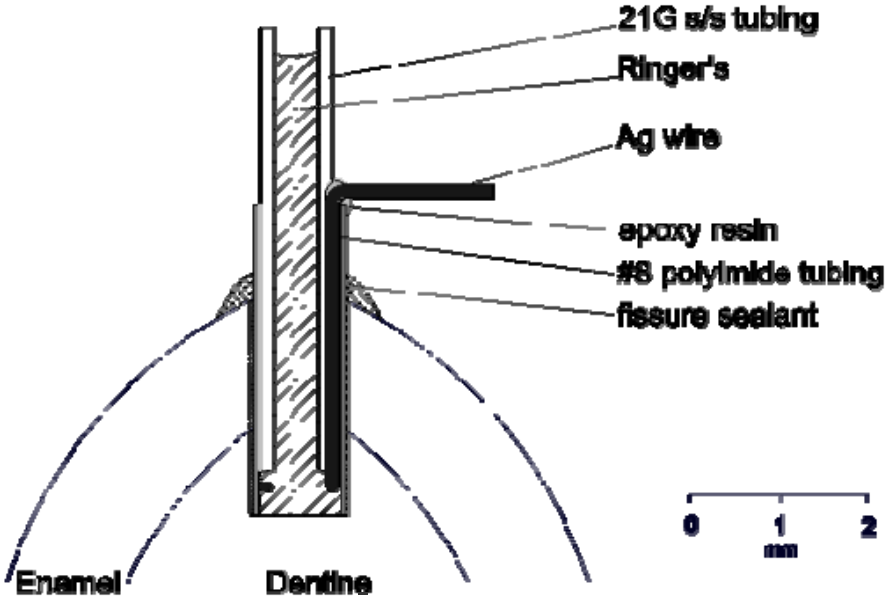

Fig. 1. Diagram of the electrode.

Recordings were made with custom-made, differential amplifiers with variable gain (up to $2x10^6$) and bandpass (10 Hz – 5 kHz and 100 Hz – 1 kHz). Each had a DC-coupled, x20 input stage based on an INA111 instrumentation amplifier (Texas Instruments), which was placed close to the subject's mouth.

Data Collection

The experiments were controlled, and data collected and stored, with a lap-top computer and data acquisition unit (CED Micro1401 Mark II; Cambridge Electronic Design, Cambridge, CB4 0FE, UK). The dentine, VAS and pressure records were digitized at up to 80K samples/sec with 16 bit resolution by the CED unit, which also controlled the solenoids that applied the stimuli.

For safety reasons, all of this equipment was powered from low voltage batteries and was isolated from ground (earth).

Data Analysis

For the VAS data, the median and the 25th and 75th percentile values were calculated. Comparisons between the median values obtained with the same stimulus at different distances from the EDJ were made with Friedman's Repeated Measures Analysis of Variance on Ranks (RMAVR). Where the RMAVR indicated there was a significant difference within the groups of data, multiple paired comparisons were made with the Tukey test. A *P* value of less than 0.05 was considered significant.

For the other, normally-distributed data, means and standard deviations were calculated.

Results

Effect of Cavity Depth on the Pain Produced by Hydrostatic Pressure Stimuli

Unetched Dentine

Stimulation of unetched dentine produced very few responses. Four of the 8 test cavities did not respond to any of the stimuli at any level, and there was no response from the remaining 4 at either the level of the EDJ and at 0.5 mm below the EDJ. Two of these 4 cavities responded at both 1.0 and 1.5 m and the remaining 2 responded only when tested a 2.0 mm. The VAS scores obtained with +400 and -400 mm Hg stimuli at levels down to

1.5 mm below the EDJ from all 8 test cavities are summarized in Fig. 2A. For the unetched dentine, there was no significant effect of cavity depth on the median VAS scores obtained with either the +400 or □400 mm Hg stimuli.

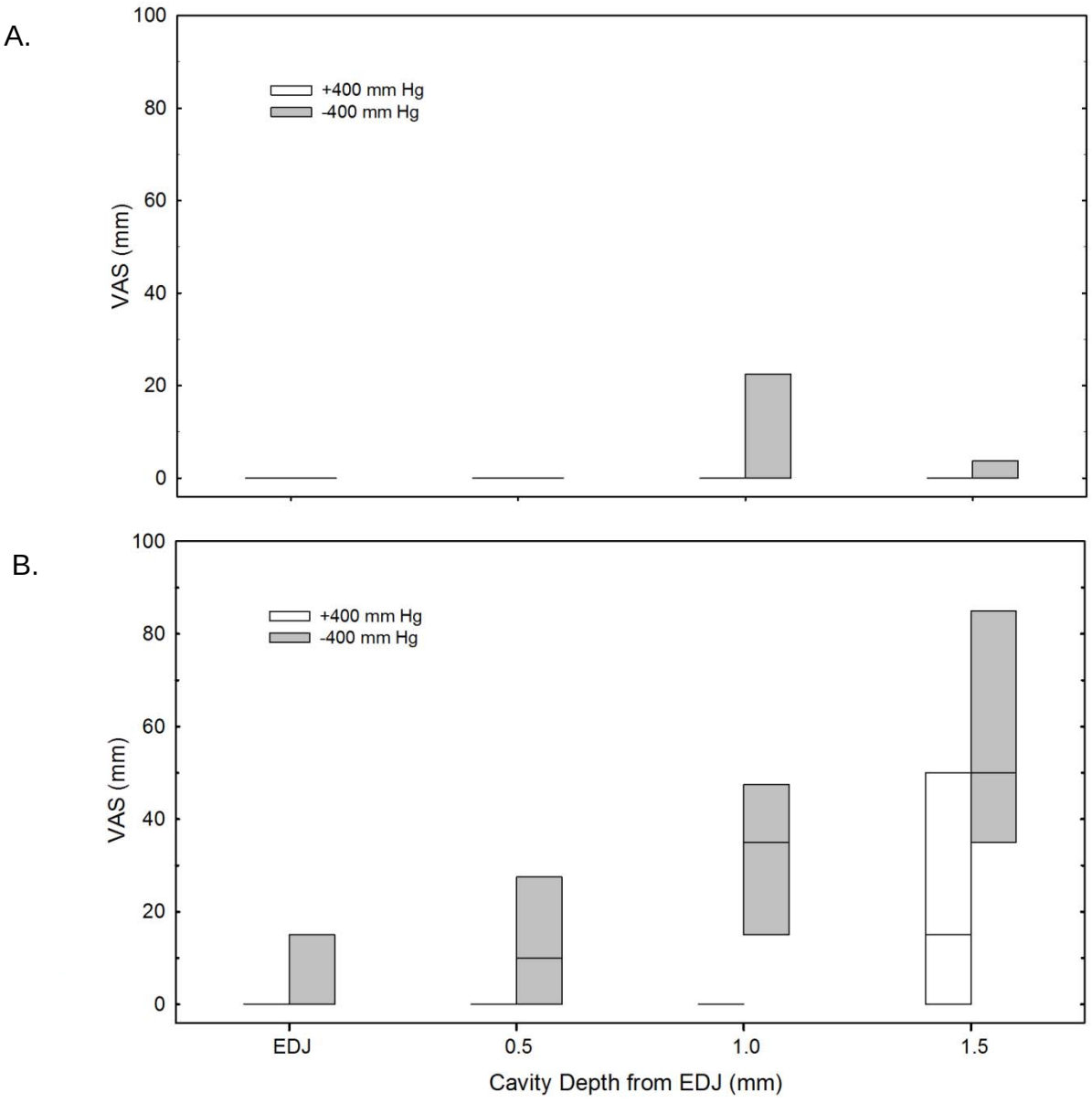

Fig. 2. The effect of cavity depth on the VAS responses produced by +400 and -400 mmHg mm Hg stimuli. The data are represented by box plots in which the lower and upper limits of the box represent the 25th and 75th percentiles respectively of the data. The median VAS score is the horizontal line through the box or is the lower limit of the box...

Etched Dentine

After etching the dentine, more cavities responded to stimulation and the effect of cavity depth on the sensitivity of the dentine became more clear. With the +400mm Hg stimulus, none of the test cavities responded at the level of the EDJ or at 0.5 mm; but 1 of the 8 cavities responded at 1.0 mm, and 5 responded at 1.5 mm. With the □400 mm Hg stimulus, 2 cavities responded at the EDJ, 4 at 0.5 mm, 7 at 1.0 mm and 8 at 1.5 mm. The VAS scores recorded at each level with these stimuli are summarized in Fig. 2B. Overall, with the □400 mm Hg stimulus, the changes in the median VAS were significant ($P<0.001$; RMAVR), and both the increases between 0 and 1.5 mm and between 0.5 and 1.5 mm were significant ($P<0.05$; Tukey Test). The responses to the +400 mm Hg stimulus also tended to increase as the cavity was deepened, but overall the effect of cavity depth on the median responses was not significant. The median VAS scores obtained by stimulating etched dentine with a range of pressures at each level are shown in Fig. 3.

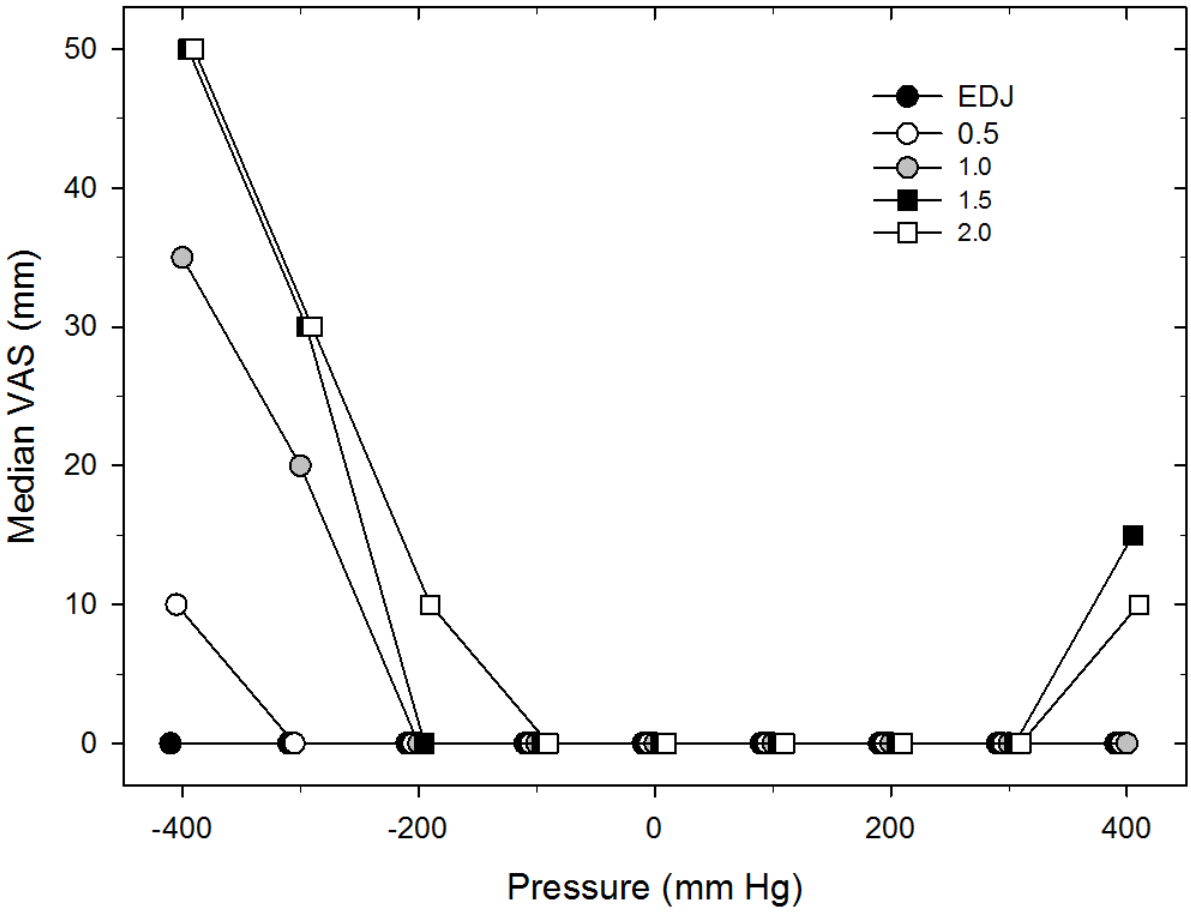

Fig. 3. The effect of stimulus intensity on the mean VAS responses produced from the test cavities at different levels through the dentine. Eight cavities were tested at all depths except 2.0 mm, for which data were available from only four.

The responses to stimulation of the dentine in the control cavity increased after etching, as in the test cavity, but they did not increase further as the test cavity was deepened.

Recordings from Dentine

Action potentials were recorded from 6 of the 8 teeth; no action potentials were recorded from the other 2 although stimulation of the dentine caused pain. Action potentials were recorded from only the test cavity in 3 of the 6 teeth and from both cavities in the other 3. Successful recordings were not obtained from all levels of the dentine in each cavity; although when recordings were obtained at one level, they were also obtained at deeper levels. Action potentials were recorded from the EDJ in some cases. Deepening the test cavity never resulted in recordings being lost from either the test or control cavities. In some teeth, stimulation of one cavity resulted in action potentials being recorded from both. Examples of recordings from one tooth, in which action potentials were recorded from both the test and control cavities, are shown in Fig. 4.

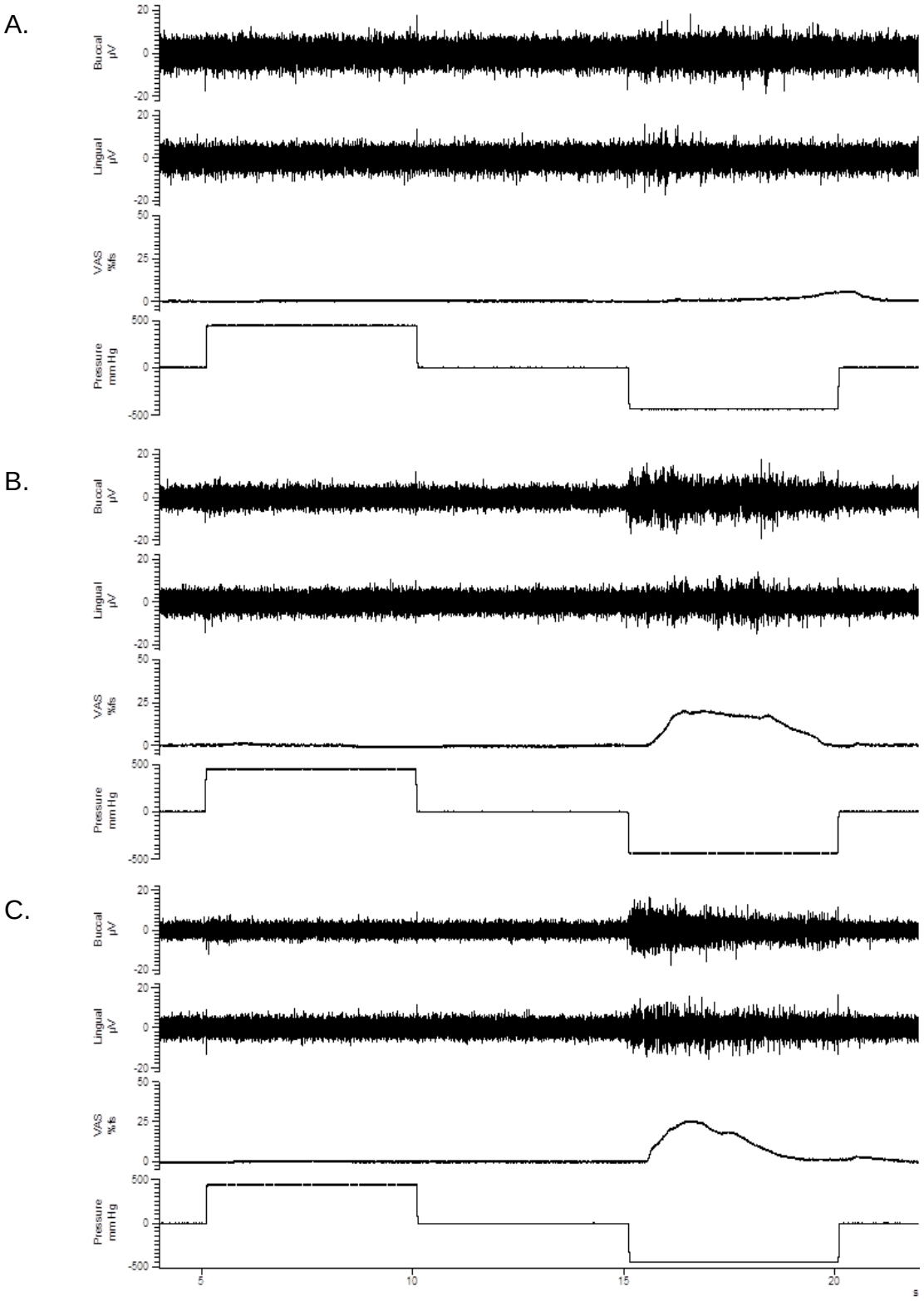

Fig. 4. Recordings of responses to 5 s, +400 and -400 mm Hg stimuli. In each panel, the top record is from the electrode in the test (buccal) cavity, the second record is that in the control (distal) cavity, the third trace is the output of a device by which the subject indicated the intensity of any pain felt (VAS), and the fourth trace is a record of the stimulus. In the upper 2 records, the bandpass of the amplifier was 100 Hz – 1 kHz in both cases.

The records in ***A*** were obtained with both control and test cavities deepened to the EDJ, those in ***B*** were obtained after deepening the test cavity by 0.5 mm, and those in ***C*** after deepening it by a further 0.5 mm, to 1.0 mm below the EDJ. Both cavities had been etched in all cases.

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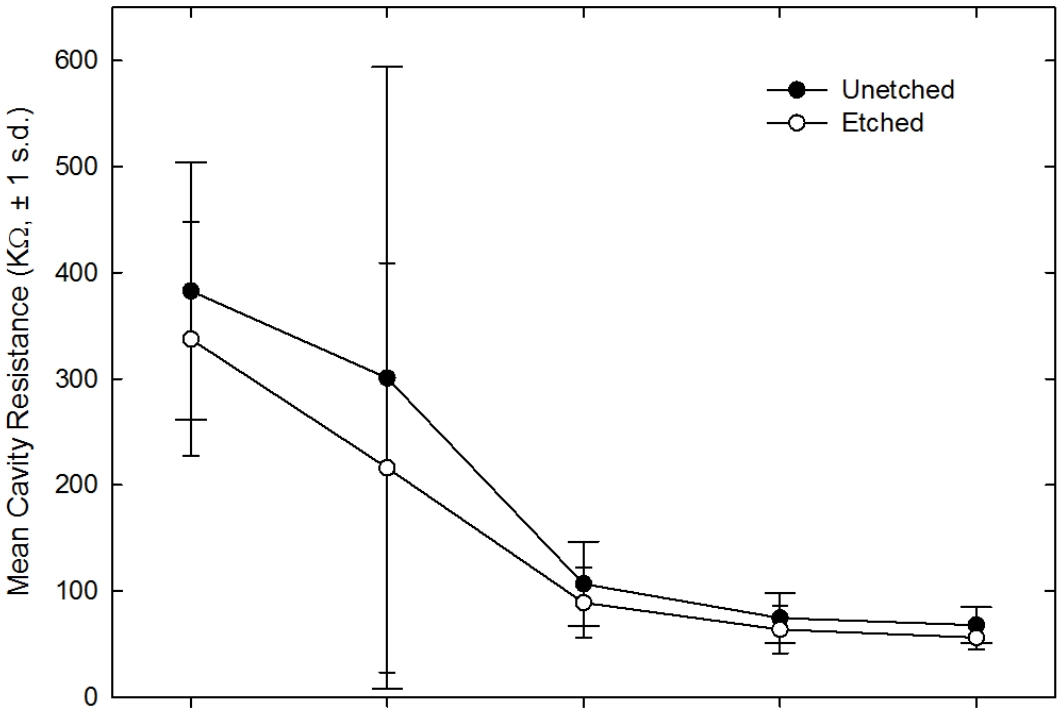
As in the example shown in Fig. 4, the peak-to-peak amplitude of the action potentials changed little as the cavity was deepened (the average of a sample of the largest spikes at all levels in 6 test cavities was 25 μV); but as the cavity was deepened, the amplitude of the noise in the recordings decreased and, as a result, the signal : noise (S:N) ratio increased. The decrease in noise was associated with a decrease in the cavity resistance (Fig. 5A & 5B). None of the recordings was of sufficiently good quality to enable the action potentials of individual sensory units to be identified on the basis of their waveform and amplitude. In the six teeth in which action potentials could be detected, the responses were measured by counting the total number of spikes evoked by a stimulus. Spikes were identified as transient voltage changes that exceeded a threshold that was set at 25% above the maximum voltage recorded during the 5 s before stimulation.

The effects of cavity depth and of stimulus intensity on the spike counts obtained with stimulation of the test cavity (Fig. 6) were similar in all 6 teeth: the responses tended to increase as the stimulus intensity was increased, with a negative pressure eliciting a greater response than the corresponding positive pressure; and responses increased as the cavity was deepened. The responses recorded from the control cavities during stimulation of the test cavity followed a similar pattern although the spike counts were smaller. No spontaneous action potentials were recorded in the absence of stimulation.

In individual teeth, there tended to be a positive correlation between the number of spikes evoked by a stimulus and the VAS score reported by the subject following the stimulus; but in the pooled data from 6 teeth, the correlation was not significant.

A.



B.

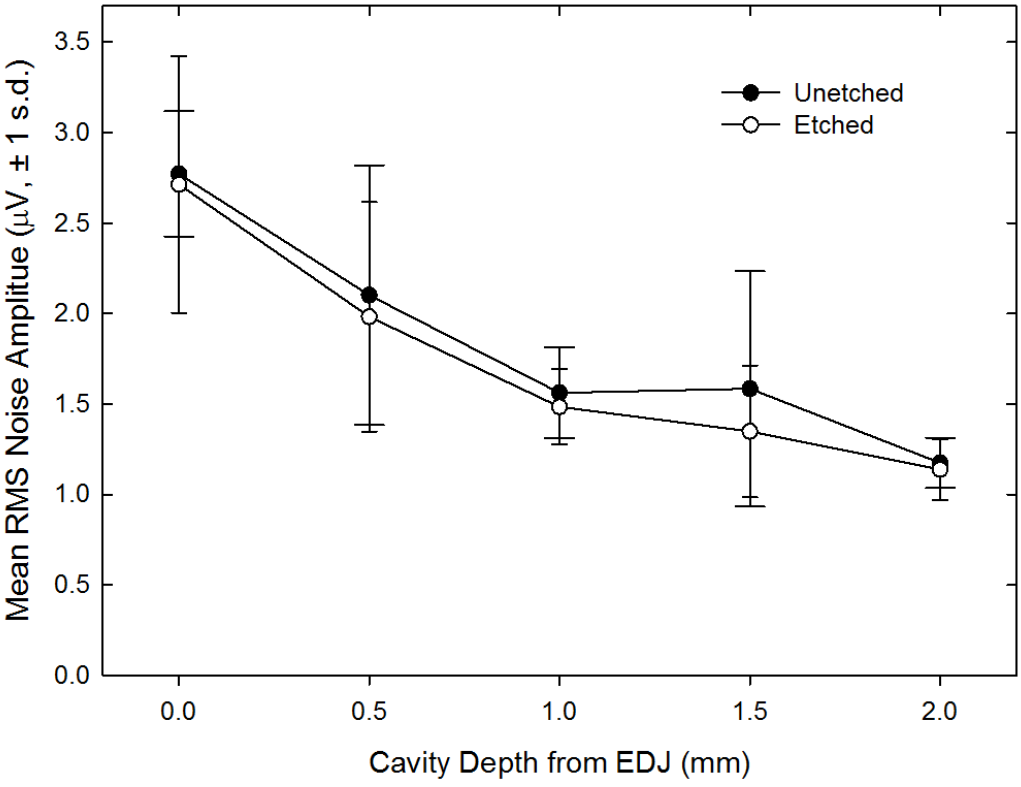


Fig. 5. The effects of cavity depth on the electrical resistance of the test cavities (***A***) and on the amplitude (RMS value) of the noise in the recordings (***B***) from these cavities when no stimulus was applied. The bandpass of the amplifier was 100 Hz – 1 kHz. Data are included for all 8 cavities for depths down to 2.0 mm, and for 4 at 2.0 mm.

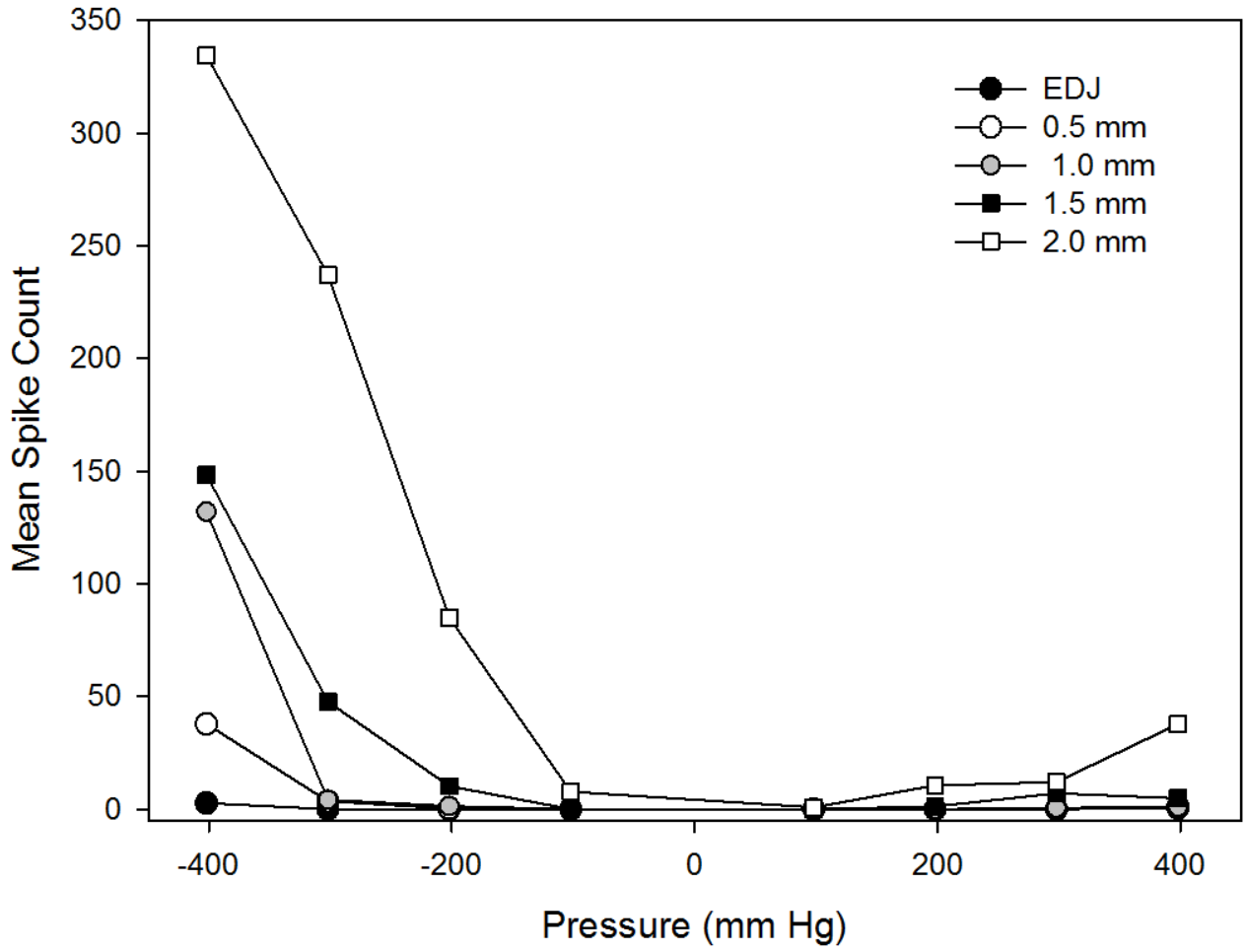


Fig. 6. The effect of stimulus intensity on mean number of spikes recorded from the etched test cavity at different cavity depths. Data are included for all 8 cavities for depths down to 2.0 mm, and for 4 at 2.0 mm.

Discussion

We found no evidence that dentine just below the EDJ is more sensitive than deeper dentine. On the contrary, the sensitivity increased progressively as the cavity was deepened. Also, as was found in another study (Charoenlarp *et al.*, 2007) the dentine was more sensitive to negative than to positive pressure stimuli, and etching the dentine increased its sensitivity to both forms of stimulus. These trends were present at all levels of the dentine. The recordings from dentine indicate that the properties of the sensory receptors that respond to hydrostatic pressure stimuli in human teeth are similar to those in cat teeth (Vongsavan and Matthews, 2007).

Our results are consistent with the hydrodynamic mechanism of dentine sensitivity, in which action potentials are generated in nerve terminals in the inner dentine or superficial pulp as a result of movement of the contents of the dentinal tubules. The effects of cavity depth and of etching, on the sensitivity of the dentine to hydrostatic pressure stimulation can be explained by the effects that these procedures would have had on the hydraulic conductance of the dentine.

This study was prompted by the impression gained by clinicians that drilling or probing dentine near the EDJ, particularly in carious teeth, is often more painful than drilling or probing deeper dentine. A possible explanation for such a difference is that the dentine close to the EDJ is more compliant than the underlying dentine (Wang and Weiner, 1998), so that mechanical stimulation just below the EDJ tends to produce more tubular flow than similar stimulation of deeper dentine. Partially decalcified dentine under caries is also likely to be more compliant than normal dentine, and hence hypersensitive to mechanical stimuli; although an additional factor under these conditions would be inflammation of the underlying pulp.

It would be possible to investigate the effect of cavity depth on the sensitivity of dentine to mechanical stimuli in man by repeating the present experiments but with probing or drilling as the test stimulus. Drilling has been used to investigate topical anaesthesia of dentine under caries (Smitayothin *et al*., 2015).

It is not known why impulses were not recorded from the test cavity in two of the teeth in the present study: it may have been that the cavity did not communicate with tubules that either contained nerve terminals or terminated close to nerve terminals in the pulp horn. When we have deliberately placed a recording cavity in the cervical region of a

tooth, where there are likely to be fewer nerve terminals (Byers and Dong, 1983; Holland *et al*., 1987), we have never recorded action potentials.

In three teeth, action potentials were recorded from the control cavity during stimulation of the test cavity. This pattern of response can be explained by axons branching and having terminals in the pulp horns of both cusp, so that some action potentials generated under the test cavity would produce secondary action potentials in other branches that would be propagated antidromically into the pulp horn under the control cavity. In other experiments (Ajcharanukul *et al*., 2006), we did not record from the area of exposed dentine to which stimuli were applied, but used simple recording electrodes that did not incorporate a method of applying stimuli, and we relied upon branching of the nerve fibres to enable action potentials to be recorded from one area of dentine that had been generated in another. A disadvantage of this method compared with that used in the present experiments is that a smaller proportion of the action potentials generated are likely to be recorded.

The electrodes we used were selected after preliminary experiments in which we investigated many different designs. The best recordings were obtained from cavities that were as small as possible in diameter, extended deep into the dentine, and exposed tubules that terminated over the pulp horn, where a high proportion contain nerve terminals (Byers and Dong, 1983; Holland *et al*., 1987). Also, as noted above, the probability of recording action potentials was greatest with electrodes that incorporated a method of applying stimuli to the tubules from which the recordings were made. These findings, together with evidence obtained in cats by recording orthodromic and antidromic action potentials from single pulpal nerve fibres (Horiuchi and Matthews, 1974) and the effects on the antidromic responses of sectioning either the pulp horn (Horiuchi and Matthews, 1974) or the pulpal nerves outside the tooth (Holland *et al*., 1987), indicate that the action potentials that can be recorded from dentine originate from nerve terminals that are located in the exposed tubules and the superficial pulp close to the ends of those tubules.

In other trials to find a better way of recording from human dentine, we recorded from an array of 5, 0.2 mm diameter electrodes that were formed in the floor of a 2 mm diameter cavity. The aim was to improve the identification of single units by making multiple, closely-spaced recordings for analysis with spike-sorting software (Buzsaki, 2004). We also thought the array would increase the chances of at least one electrode making contact with tubules that terminated over the pulp horn. But the method was

unsuccessful: no impulses were recorded. It seems that exposing the larger area of dentine resulted in impulses being blocked for some reason; although the subjects still felt pain when the dentine was stimulated.

It is not possible to record nerve action potentials from the surface of any other tissue with relatively large electrodes such as were employed in the present experiments. They can be recorded from the surface of the cornea (Carr, *et al*., 2009) but this requires micro-electrodes. In dentine, the tubules exposed in the floor of a cavity appear to function as a bundle of parallel, biological microelectrodes in series with the much larger electrode in the cavity. The potentials recorded from the cavity floor, 2 mm or more from the nerve terminals, must be produced by extracellular currents flowing through the tubules during the propagation of impulses in the nerves.

The action potentials we recorded from human premolars were much smaller than those recorded with similar techniques from cat canines (Horiuchi and Matthews, 1974; Vongsavan and Matthews, 2007). The reason for this is probably that the pulp horn is much more slender in a cat canine than in a human premolar. As a result, the resistance of the tissues around the nerve terminals is higher in the cat teeth and the extracellular current densities associated with the propagation of action potentials in the terminals, correspondingly larger. Even larger action potentials, up to 400 μV, have been recorded from the cusps of rat molars (Matthews et al., 2008), and in these teeth the pulp horns are even more slender than that in a cat canine.

To detect the action potentials in the present study, the noise in the recordings had to be kept to a minimum. Surprisingly, despite working in a clinical environment with no Faraday cage, mains electrical interference was not a problem and was avoided by using batteries to power all the equipment, turning off all mains-powered equipment in the immediate vicinity of the subject, and by using a simple 50 Hz notch filter in the amplifiers. The mains supply in the building in which the experiments were carried out is channelled through earthed, metal ducting.

The first recordings of action potentials from human dentine were made by Ewall and Olgart, (1977) using methods similar to those developed by (Scott and Tempel, 1965) in the cat. They recorded from cavities that were cut on the buccal side of the teeth. A similar method was used later by Ahlquist and others (Ahlquist et al., 1984; Ahlquist et al., 1994).

The only alternative to dentine recording for monitoring the discharge evoked in peripheral sensory nerve fibres by pain-producing stimuli in man, is micro-neurography (Hagbarth, 2002). This technique involves inserting a microelectrode through the skin or mucous membrane into a peripheral nerve trunk. It has been used to record from pulpal nerve fibres in the inferior alveolar nerve (Iwata et al., 1991; Ikeda and Suda, 1998).

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Series II experiments

Effect of the topical application of 50% lignocaine hydrochloride on the sensitivity of dentine in man

Abstract

***Objective*:** To determine the effect of topical applications of 50% w/v lignocaine HCl on the sensitivity of human dentine .

Design: The experiments were carried out on 12 premolars scheduled for extraction as part of orthodontic treatment in nine subjects (ages: 16 – 29 years). Dentine was exposed by cutting a cavity at the tip of the buccal cusp of each tooth, and etched with 35% phosphoric acid. The sensitivity of the exposed dentine to probing and air-blast stimuli was assessed before and after applying either 50% w/v lignocaine HCl solution or distilled water to the exposed dentine for 10 minutes. Changes in the sensitivity of the dentine were monitored for up to 160 minutes. The subject indicated the intensity of any pain produced by marking a 100 mm visual analogue scale (VAS).

Results: Before treatment, both forms of stimulus evoked pain in all the teeth. The median VAS score with probing was 40 mm and, with air-blast stimulation, 30 mm. 50% lignocaine HCl produced a progressive fall in these scores and after 30 minutes there was no response to either probing or air-blast stimulation. The responses started to return 30 – 160 minutes after the lignocaine had been washed off. Water had no effect.

Conclusions: Lignocaine will diffuse into exposed dentine and block the pain evoked by probing and air-blast stimuli provided that a sufficiently steep diffusion gradient is created. A topical application of a 50% w/v solution of lignocaine HCl for 10 minutes will anaesthetise dentine within 30 minutes.

Introduction

Soon after the introduction of cocaine as a local anaesthetic for surgery in 1884 (Koller, 1884; Calatayud and González, 2003) it was found that solutions of cocaine that could block all sensations when applied topically to skin, mucous membranes and the cornea, were ineffective when applied to exposed dentine (Gysi, 1900). Gysi (1900), on the basis of this and other evidence (to be discussed below), concluded in 1900 that the nerve endings responsible for the sensitivity of dentine were not present in the dentinal tubules but were in the underlying pulp. He also concluded that, when dentine was stimulated to cause pain, these nerve endings were activated by movement of fluid in the dentinal tubules (now referred to as the hydrodynamic hypothesis). It was subsequently shown by Anderson *et al.* (1958) that the topical application of 2% lignocaine failed to block the pain produced by osmotic stimulation of the dentine, and they reached similar conclusions. But neither Gysi nor Anderson *et al* had evidence on how far into the tubules the local anaesthetic diffused in a concentration sufficient to block the propagation of nerve impulses.

Although there may be no structural barrier to the diffusion of these anaesthetics into dentine, other factors will affect the rate of diffusion. Both cat (Vongsavan and Matthews, 1991). and human (Anderson and Ronning, 1962; Vongsavan *et al*., 2000). dentine have been shown to be permeable to Evans' blue dye when tested in recently extracted teeth, but the dye did not penetrate dentine as readily when it was tested *in viv*o. This difference was attributed to the presence of an outward flow of fluid in the dentinal tubules *in vivo* (Vongsavan and Matthews, 1992) which opposed the inward diffusion of the dye and which was not present in the extracted teeth. The rate of diffusion of a solute through dentine will depend on its concentration gradient, the pore size of the diffusion channels, and the length of the channels. The main diffusion channels are the dentinal tubules and the pore size of these channels will be affected by the presence or absence of a smear layer. The effective length of the channels will be increased if there is any outward flow of fluid in the tubules, and decreased if there is inward flow. As a result, the rate of inward diffusion will be reduced by outward flow (Matthews and Vongsavan, 1994; Pashley *et al*., 1993).

Thus, outward flow of dentinal fluid could account for the observations of both Gysi (1900) and Anderson *et al.* (1958). The rate of inward diffusion of the cocaine and the lignocaine would have been increased if they had been applied at a higher concentration, or

at a raised hydrostatic pressure to slow or reverse the tubular flow, or if a current of appropriate polarity had been passed through the tubules to add an electromotive force to speed up the movement of the anaesthetic ions (iontophoresis).

Evidence that 2% lignocaine HCl is able to block impulse conduction in some nerve terminals when it is applied to exposed dentine in the cat was obtained by Horiuchi and Matthews (1974). In those experiments the solution was applied at atmospheric pressure to unetched dentine in which the rate of outward flow of dentinal fluid would have been restricted by the smear layer formed by the dental drill use to expose the dentine. Higher concentrations (10 and 28% w/v lignocaine HCl) have also been shown to block the neural discharge evoked by mechanical stimulation of the dentine when applied to exposed, etched dentine at atmospheric pressure in the cat (Amess and Matthews, 1996; Matthews *et al*., 1996). The onset of this effect was more rapid when the lignocaine was applied at a pressure of 25 mm Hg, which would have slowed or reversed the outward flow of dentinal fluid and facilitated diffusion into the tubules. Topical applications of 50% lignocaine HCl at atmospheric pressure to exposed, etched dentine in man have also been shown to reduce the sensitivity of the treated dentine (Matthews *et al*., 1996; Amess et al., 1996). Iontophoresis has been shown to facilitate the penetration of lignocaine through human dentine *in vitro* (Pashley *et al*., 1978) and Gysi (1900) was able to anaesthetise dentine with cocaine if he facilitated its inward diffusion with an iontophoretic current. He also showed (although unfortunately no details are given in the report of his communication) that the inward diffusion of an aniline dye mixed with the cocaine was similarly facilitated, but the extent of the penetration of a visible concentration of the dye would not have given an accurate indication of the penetration of an effective concentration of the cocaine. More recently, evidence has also been presented that an AC current in some way facilitates the inward diffusion of lignocaine through human dentine and enamel *in vitro* (Ikeda and Suda, 2013) but this procedure has not been tested *in vivo*. Gangarosa (1974) iontophoresed lignocaine through the oral mucosa to anaesthetise deciduous teeth for extraction.

The objective of the present study was to investigate the effect of topical applications of a high concentration (50% w/v) of lignocaine HCl at atmospheric pressure on the sensitivity of human dentine.

Materials and methods

Brief Outline of Experiments

Dentine was exposed by cutting a cavity at the tip of the buccal cusp of a human premolar and etched. The sensitivity of the dentine to probing and air-blast stimuli was assessed before and after applying either 50% w/v lignocaine (synonym: lidocaine) HCl solution or sterile distilled water to the exposed dentine for 10 minutes. After the application of lignocaine, changes in the sensitivity of the dentine were monitored for up to 160 minutes after the drug had been washed off.

Subjects and Teeth

The experiments were carried out on 12 healthy premolar teeth in nine subjects (ages: 16 – 29 years). The teeth were to be extracted as part of orthodontic treatment. All the teeth were vital and healthy, as determined by clinical and radiographic examination. The study was approved by the Ethics Committee on Human Rights Related to Human Experimentation of Mahidol University, and complied with the principles of the Declaration of Helsinki. Informed consent was obtained from each subject, or for those under 18 years, a parent or guardian.

Cap and Cavity Preparation

An acrylic cap was prepared that covered most of the crown of the test tooth. The cap was formed *in situ* by covering the buccal surface, the buccal cusp and the occlusal one third of lingual cusp with a 2 mm thick layer of self-curing, acrylic resin (Unifast G C Dental Industrial Corp. Japan). Once the resin had cured, the cap was removed.

The tooth was then isolated with a rubber dam and a 3 mm diameter, 3 mm deep cavity was cut at the tip of the buccal cusp (Fig. 1). The cavity was prepared with a round, diamond bur (#201) and finished with a cylindrical diamond bur (#204, Intensive, Switzerland); both in an air-rotor hand-piece under water spray. A wet cotton pellet was placed in the cavity to keep it moist. The surrounding enamel was etched with 37% phosphoric acid gel (3M Dental Products, USA) for 30 seconds, washed with distilled water, and dried with air from a triple syringe. A 3 mm diameter hole was drilled through the cap at a location corresponding to the position of the cavity, and the cap was sealed to the enamel with light-cured, composite resin (Z-100, 3M Dental Product, USA). The hole in the cap and the cavity together formed a reservoir with a volume of approx. 25 µl to hold the test solution.

The exposed dentine in the cavity was etched with 37% phosphoric acid gel for 30 seconds to remove the smear layer, and then rinsed with distilled water.

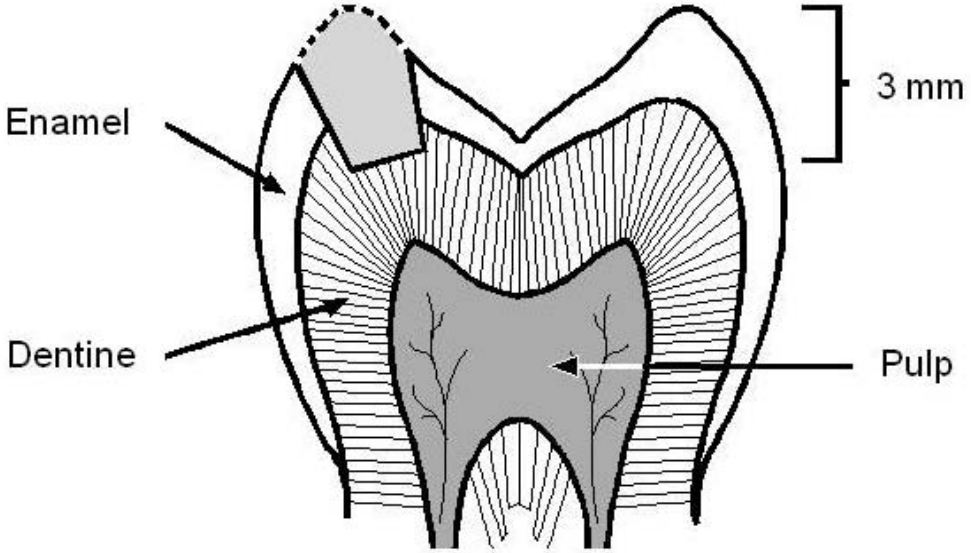

Fig. 1. Diagram of the preparation of the tooth.

Dentine Sensitivity Tests

After it had been blotted dry with cotton pellets, the sensitivity of the exposed, etched dentine was tested with two forms of stimuli: gently stroking the floor of the cavity with an explorer (tip diameter, 0.2 mm; force, approx. 20 g) and a 3 sec air-blast in which air at room temperature was directed onto the exposed dentine from a triple syringe (reservoir pressure = 41 Pa; distance from syringe tip to mouth of cavity: 1-2 mm). The stimuli were always applied in this order. After each stimulus, the subject was asked to rate the intensity of any pain experienced by placing a mark on a 100 mm visual analogue scale (VAS) which was divided into 10 mm steps, in which 0 indicated no pain and 100, the most severe pain that could be imagined (Holland *et al*., 1997). Thus, in scoring the severity of the pain, the subject was asked to select from eleven possible values.

Experimental design

In six teeth (Group 1), selected randomly, a baseline assessment of dentine sensitivity was made, then the cavity and the reservoir in the cap were filled with a 50% w/v (1.85 mol/l, 3.55 mol/Kg) solution of lignocaine HCl (Sigma-Aldrich, Dorset, England) in sterile, distilled water for 10 minutes. After this, the cavity was blotted dry with cotton pellets, washed thoroughly with 3 ml of sterile, distilled water, blotted dry, and the sensitivity of the dentine tested again. The sensitivity testing was repeated twice at 10 minute intervals, with a cotton pellet moistened with water in the cavity between tests to prevent drying. At this stage the responses to stimulation of the dentine were abolished in all of the teeth. The sensitivity tests continued at 10 minute intervals to determine the time taken for the responses to return. . Thirty minutes after the lignocaine had been washed off, the cavity was filled with sterile distilled water for 10 minutes, blotted dry and retested (see Fig. 2). Testing continued for at least a further 10 minutes after the water rinse, or until responses to simulation returned.

In the other six teeth (Group 2), essentially the same procedures were carried out but, before the application of lignocaine, a control 10 minute application of water was made. The cavity was then blotted dry and two assessments of dentine sensitivity were made at 10 minute intervals, with moist cotton pellet in the cavity between tests. A 10 minute application of 50% lignocaine followed, and this was washed out with water as before. Testing continued at 10 minute intervals until the sensitivity of the dentine had been abolished and returned.

After these procedures had been completed, the tooth was extracted with forceps under local anaesthesia at the same visit. Each tooth was sectioned longitudinally through

the cavity with a rotating diamond disc and the minimum remaining dentine thickness overlying the pulp horn along the course of the cut dentinal tubules was measured.

Statistical Analysis

The median and the 25^{th} and 75^{th} percentile values were calculated for each set of data. In addition, the 10^{th} and 90^{th} percentiles were calculated for pooled data with 12 values. Comparisons between the median values of two groups of paired data were made with the Wilcoxon Signed Rank Test. Comparisons between the median values of several groups were made with Friedman's Repeated Measures Analysis of Variance on Ranks (RMAVR) and where this indicated there was a significant difference within the groups of data, multiple paired comparisons were made with the Tukey test. A P value of less than 0.05 was considered significant.

Results

None of the patients reported any pain while the exposed dentine was being etched, but both the probing and air-blast stimuli produced pain from all the teeth prior to application of the test or control solutions. Combining the results from both Groups of experiments, the median baseline VAS score with probing was 40 mm (range 30 - 100, N=12) and the corresponding value with air-blast stimulation was 30 mm (range 10 - 100, N=12). The difference between these medians is significant ($P < 0.01$, Wilcoxon Signed Rank Test).

Group 1 *Experiments*

The effects of a 10 minute application of 50% lignocaine in the 6 teeth of Group 1 are summarised in Fig. 2. The responses to both probing and air-blast stimuli had decreased in some teeth by the time the lignocaine was washed off, were completely abolished in all the teeth after a further 20 minutes, and had started to recover in some teeth 10 minutes later. The recovery continued following the 10 minute application of water. These effects of both lignocaine and water are considered further below.

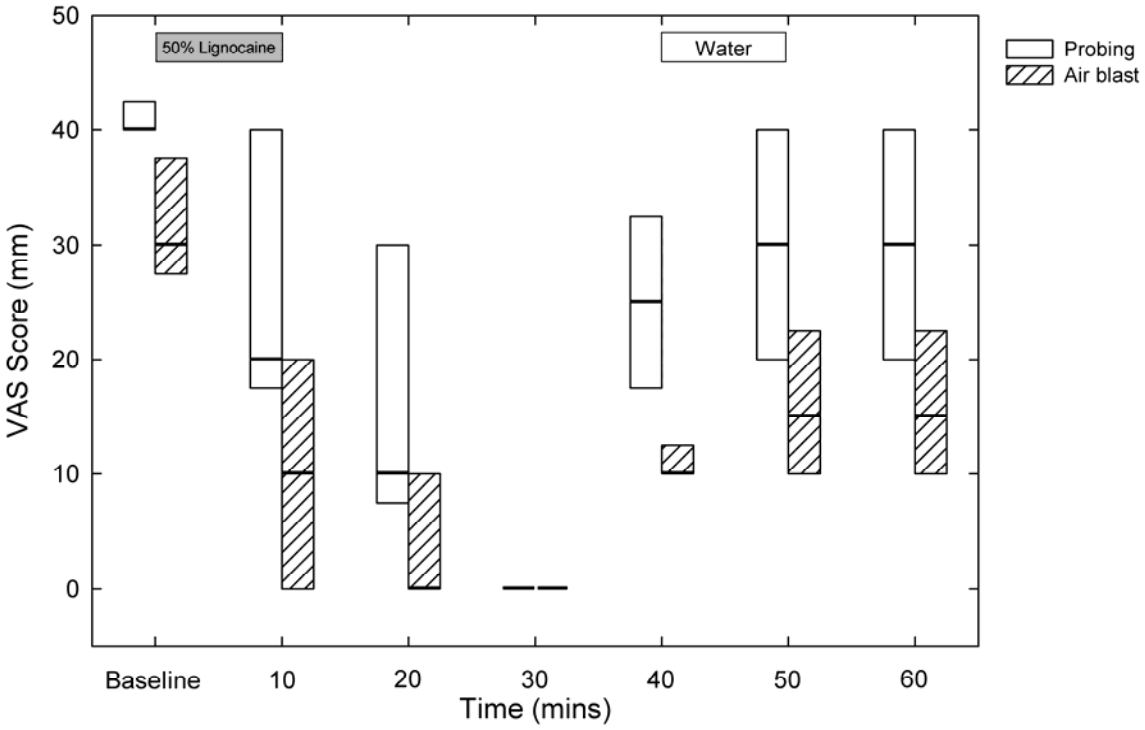

Fig. 2. The effect of a 10 minute application of 50% w/v lignocaine HCl and of water to exposed, etched dentine on the intensity of pain (VAS score) evoked in 6 teeth by probing (open boxes) and by air-blast (hatched boxes) stimulation of the dentine. The data shown were collected at 10 minute intervals for 60 minutes from immediately before the application of the lignocaine solution (Baseline), The water was applied 30 minutes after the lignocaine was washed off, The thick line through each box indicates the median VAS score and the lower and upper limits of the box, the 25th and 75th percentiles respectively. The grey bar indicates the period during which the lignocaine solution was applied to the dentine; and the open bar, the period of water application.

Group 2 *Experiments*

In the Group 2 experiments, in which water was applied before lignocaine to the cavity for 10 minutes, neither the median VAS scores with probing nor those with air-blast were changed significantly by the water treatment ($P>0.05$, RMAVR). Before the application of the water, the median VAS score with probing was 45 mm (range 30 – 100, N=6) and after the treatment, it was 55 mm (range 10 – 80, N=6). Ten minutes later it was 55 mm (range 30 – 80, N=6). The corresponding figures for air-blast were: baseline, 15 mm (range 10 – 100, N=6); immediately after treatment, 30 mm (range 10 – 50, N=6); and 10 minutes later, 30 mm (range 10 – 50, N=6). There was no evidence that repeated stimulation produced a consistent decrease or increase in the sensitivity of the dentine under these conditions. It was concluded that any change in the VAS scores seen after lignocaine treatment could be attributed to effects of the local anaesthetic and not to other aspects of the experiment or the passage of time. The results obtained after the lignocaine application in both groups of experiments were therefore combined for further analysis.

Effects of 50% lignocaine HCl on responses to probing

In the combined data from both Groups, the 10 minute application of 50% lignocaine HCl solution reduced the median VAS response to probing from 40 mm (range 30 – 80, N=12) to 20 mm (range 0 – 70, N=12) by the time the solution had been washed off. After a further 10 minutes, the median VAS score had decreased to 10 mm (range 0 – 30, N=12); and 10 minutes later, no pain was evoked from any of the teeth. The overall effect of the lignocaine in reducing the median VAS score was significant ($P<0.001$, RMAVR). When compared with the median baseline VAS score, the reduction observed immediately after treatment was not significant but those recorded 10, 20 and 30 minutes later were ($P<0.05$, Tukey test). These data are summarised in Fig. 3.

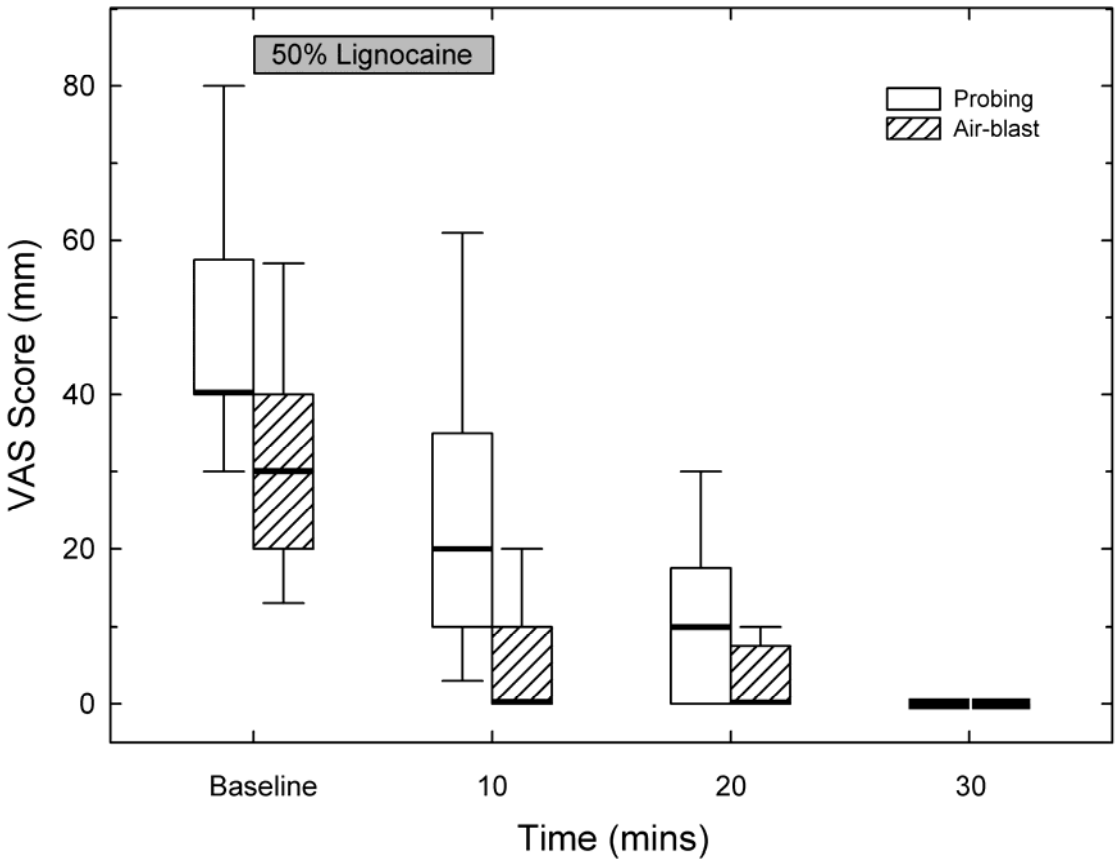

Fig. 3. The effect of a 10 minute application of 50% w/v lignocaine HCl to exposed, etched dentine on the intensity of pain (VAS score) evoked in 12 teeth by probing (open boxes) and by air-blast (hatched boxes) stimulation of the dentine. The data were collected before the application of the lignocaine solution (Baseline), immediately after the solution had been washed off, and after a further 10 and 20 minutes. The thick line through each box indicates the median VAS score and the lower and upper limits of the box, the 25th and 75th percentiles respectively. The bars below and above each box indicate the 10th and 90th percentiles. The grey bar indicates the period during which the lignocaine solution was applied to the dentine.

The application of the lignocaine solution (ideal osmotic pressure: 170 atmospheres) did not cause pain.

The sensitivity of the dentine to probing started to return 30 – 140 minutes (median 50 min, N=12) after the lignocaine had been washed off.

Effects of 50% Lignocaine HCl on Responses to Air-blast Stimulation

The 10 minute application of lignocaine also reduced the median VAS response to air-blast stimulation from 30 mm (range 10 – 60, N=12) to 0 mm (range 0 – 20, N=12). After a further 10 minutes, the median VAS score was 0 mm (range 0 – 10, N=12); and 10 minutes later, no pain was evoked from any of the teeth. The overall effect of the lignocaine in reducing the median VAS score was significant ($P<0.001$, RMAVR). When compared with the median baseline VAS score, the reductions observed immediately after treatment and 10, 20 and 30 minutes later were all significant ($P<0.05$, Tukey test). These data are summarised in Fig. 3.

The sensitivity of the dentine to air-blast started to return 40 – 160 minutes (median 70 min, N=12) after the lignocaine had been washed off. The time taken for the response to air-blast stimuli to return was significantly longer than that of the response to probing (Wilcoxon Signed Rank Test, $P<0.005$).

Effects of the Application of Water on Responses to Probing and to Air-blast Stimulation after Lignocaine.

In the Group 1 experiments, filling the cavity with water 30 minutes after washing off the lignocaine had no significant effect on the median VAS scores to either probing or air-blast stimulation ($P>0.05$, RMAVR). With probing the median score was 25 mm (range 10 - 40, N=6) before the application of water and 30 mm (range 20 - 40, N=6) after the 10 minute application. There was also no significant change after a further 10 minutes (median, 30 mm; range, 20 - 40; N=6).

With air-blast stimulation, the medial score was 10 mm (range 10 - 20, N=6) before the application of water and 15 mm (range 10 - 30, N=6) after the 10 minute application. There was also no significant change after a further 10 minutes (median, 15 mm; range, 10 - 30; N=6).

Remaining Dentine Thickness.

The shortest distance along the course of the dentinal tubules between the floor of the cavity and the underlying pulp horn (RDT) was measured in a ground section of each tooth. It ranged from 1.9 – 3.0 mm (mean 2.16, S.D. 0.39, N=12). There was no apparent

correlation between the RDT and either the VAS score to probing or to air-blast stimulation before applying the lignocaine, the rate of decrease in the VAS score with either form of stimulus after applying the lignocaine, or the time taken for recovery from the anaesthetic..

Discussion

The results of the present experiments have shown that the topical application of a 50% lignocaine HCl solution for 10 minutes to etched dentine in a human premolar tooth can block the response of intradental nerves to mechanical and air-blast stimulation of the treated dentine. This finding supports the results of the earlier investigation (Matthews *et al.*, 1996; Amess *et al*., 1996).

It seems that, by applying a very high concentration of lignocaine to the dentine surface, it was possible to cause the drug to diffuse into the dentinal tubules, against the direction of flow of the dentinal fluid, at a rate which was sufficient to raise the concentration in the fluid in the pulpal ends of the tubules to the level required to block impulse generation and conduction in the nerve terminals located there and in the adjacent pulp.

The sensitivity of the dentine was not fully blocked in all the teeth until 20 minutes after the drug was washed off. When the lignocaine was washed off, a high concentration would have been present in the outer ends of the tubules but there was not sufficient in the inner ends of the tubules to block impulse conduction in all the nerve terminals there in all of the teeth. During the subsequent 20 minutes, the concentration in the inner dentine would have increased as a result of inward diffusion from the reservoir in the outer dentine. Some of the lignocaine in the outer dentine would also have been carried out of the tubules by the continuing outward flow of the tubular fluid; and some would have been lost from the inner ends of the tubules by diffusion into the pulpal tissue fluid and ultimately carried away in the pulpal circulation. The duration of the anaesthesia varied widely between teeth and, when tested with probing, ranged from 30 to 140 minutes after the lignocaine had been washed off. This variation can be accounted for by differences in rate at which the lignocaine was cleared from the inner dentine and pulp due to differences in pulpal blood flow and in the rate of fluid flow in the dentinal tubules. Differences between the teeth in the diffusion distance appear not to have been an important factor in determining the rate at which the lignocaine diffused either into or out of the inner dentine since there was no

correlation between the RDT and either the speed of onset of the anaesthesia or the time taken for recovery.

The precise site of action of the lignocaine is not known. Nerve terminals branch extensively under the odontoblast cell layer in the superficial pulp and some of the terminals pass through this layer and penetrate up to 100 μm into the dentinal tubules, particularly over the pulp horns (Lilja, 1979; Holland *et al*., 1987) Both the probing and air-blast stimuli are thought to generate impulses in the nerve terminals as a result of the movement they produce in the dentinal fluid (the hydrodynamic hypothesis), (Vongsavan and Matthews, 2007; Charoenlarp *et al*., 2007) but neither the ionic mechanisms of this transduction process nor the site at which the impulses are generated is known. Ionic channels in the odontoblasts may be involved (Magloire *et al*., 2009).

The fact that the pain evoked by the air-blast stimulus was blocked more readily than that evoked by probing may have been because the former was the weaker stimulus in terms of the movement produced in the tubular fluid, because the sites of impulse generation were different for the two stimuli, or because different types of ionic receptor channel were involved. The air-blast stimulus will have caused an increase in the normal rate of outward flow in the tubules due to the capillary forces generated as a result of the loss of water by evaporation from the dentine surface. Probing would have produced a more complex effect: the initial contact of the probe with the etched dentine surface would have blocked the tubules and arrested the normal outward flow, then the force applied at the probe tip (approx. 64 Kg/cm^2, despite the sensation of only "gently" stroking the dentine) would have compressed the compliant, etched dentine matrix and caused inward flow. When the probe was removed from the dentine, not only would the normal outward flow have been restored but there would probably have been a brief acceleration in this outward flow generated by negative pressures created by the recoil of the compressed dentine and by the probe being pulled away from the etched dentine to which it appears to become adherent during stimulation. The latter is suggested by recordings made with a probe fitted with strain-gauges during mechanical stimulation of etched dentine in the cat *in vivo*, and by recordings of dentinal fluid flow made from extracted human teeth (Wanachantararak, Vongsavan and Matthews; unpublished observations).

While the responses to air-blast stimuli were blocked more readily than those to probing, they recovered more slowly. This supports the suggestion that the sites of impulse generation were different for the two stimuli; the air-blast stimuli evoking responses at the more superficial location where with the lignocaine concentration would have built up

more rapidly initially and have decreased more slowly after the lignocaine had been washed off.

Although a very high concentration solution of lignocaine was used, there was no danger of this producing toxic effects, either systemically or locally in the pulp. The 25 μl of solution inserted into the cavity contained only 12.5 μg of lignocaine HCl which was washed off after 10 minutes, with the result that the amount that diffused into the dentine was sufficient to produce only transitory local anaesthesia of the dentine.

The use of a topical application of 50% lignocaine HCl as a method for anaesthetising exposed dentine is unlikely to have wide clinical application, although it could be useful where cavity preparation had been started without anaesthetic but was becoming very painful and the patient preferred not to have an injection. It might also be useful in cases in which it proved difficult to achieve adequate anaesthesia with either a regional nerve block or infiltration of anaesthetic around the tooth. A disadvantage of the method is that up to 30 minutes would be required for the anaesthetic to become effective. This delay might be reduced by passing a small DC current through the dentine to both drive lignocaine ions into the tubules by iontophoresis, and slow or even reverse the outward flow of tubular fluid by electro-osmosis.

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Series III experiments

Effects of the iontophoresis of lignocaine with epinephrine into exposed dentine on the sensitivity of the dentine in man

Abstract

***Objective*:** To determine the effects of the iontophoretic application of lignocaine and epinephrine to exposed dentine on the sensitivity of the dentine in human subjects.

***Design*:** *:* The experiments were carried out on 13 healthy premolars (13 subjects) that were scheduled for extraction. Dentine was exposed at the tip of the buccal cusp by cutting a cavity which was etched with 35% phosphoric acid. The sensitivity of the dentine was tested with probing and air blast stimuli. The subject indicated the intensity of any pain produced by marking a 100 mm visual analogue scale. In 7 teeth, the cavity was filled with a solution containing 20% w/v lignocaine HCl and 0.1% w/v epinephrine HCl, and an iontophoretic current of 120 μA was passed for 90s. The sensitivity of the dentine was tested before and immediately after the treatment and then at 10 min. intervals for 40 min. Pulpal blood flow was recorded at each stage. Control experiments were carried out on 6 teeth using a solution containing only the epinephrine.

***Results*:** The lignocaine plus epinephrine solution completely blocked the pain produced by both forms of stimulus immediately, and this continued for 40 min. It also produced an immediate fall in pulpal blood flow that lasted for 40 min. The control solution had the same effect on pulpal blood flow but no effect on dentine sensitivity.

Conclusion: The topical application of 20% lignocaine and 0.1% epinephrine, with an iontophoretic current of 120 μA for 90 s, will anaesthetise exposed, normal dentine.

Introduction

The possibility of delivering local anaesthetic to the dental pulp through the dentinal tubules has been investigated, but generally this has met with little success. Anderson *et al*. (1958) applied 2% lignocaine to dentine that had been exposed by drilling in human premolars, and found no decrease in the intensity of pain produced when glucose syrup was applied to the same dentine. Brännström (1966) obtained a similar result with the application of a solution of Citanest (8% prilocaine hydrochloride) to fractured human dentine. These results indicated that the local anaesthetic agents could not diffuse through the dentinal tubules in sufficient amounts to block conduction in nerve terminals in the dentine or underlying pulp. It was later shown that this was likely to be due to an outward flow of dentinal fluid opposing the diffusion of the anaesthetic towards the pulp rather than to the size of the pores in the dentine acting as a barrier to diffusion (Vongsavan and Matthews, 1991; Vongsavan and Matthews, 1992). If this were the case, one possible method of achieving successful topical anaesthesia would be to increase the concentration gradient of the anaesthetic. This was confirmed by Amess and Matthews (1995) who found that the topical application of 10% lignocaine to exposed dentine in cats for 3 minutes at atmospheric pressure blocked reversibly the response of intradental nerves to mechanical stimulation of the dentine , whereas a 2% solution applied ion the same way had no effect. Amess and Matthews (1996) also showed that the application of 50% lignocaine HCl to human dentine for 10 minutes blocked the pain produced by air blast stimuli. More recently, Rirattanapong *et al*. (2013). demonstrated that 50% lignocaine HCl could anaesthetize dentine to air-blast and probing stimuli but this effect required an application of up to 30 minutes, which is too long for routine clinical use.

An alternative way of increasing the rate of transfer of an anaesthetic into the dentinal tubules would be to apply the solution at a raised hydrostatic pressure to slow or even reverse the outward flow of dentinal fluid (Vongsavan and Matthews, 1991; Ozawa *et al*., 2002). This would have the effect of increasing the rate of inward diffusion and, if the outward flow of dentinal fluid was reversed, of transferring the anaesthetic solution by bulk flow. But there are practical problems in doing this in a decayed or fractured human tooth because of difficulties in making a seal with the tooth to enable the solution to be applied at a raised pressure.

A further method of facilitating the diffusion of a local anaesthetic into dentine is to use iontophoresis. Gysi (1900) showed that this method could be used to anaesthetize dentine

with cocaine, and several other studies have shown that the rate of diffusion of local anaesthetics into tissues can be influenced by the passage of an electric current (Gangarosa, 1974; Sloan and Soltani, 1986; Kostouros *et al*., 1996; Puapichartdamrong *et al*., 2003; Ikeda and Suda, 2013).

In the present study, the combined effects of the iontophoresis of lignocaine and epinephrine into dentine were investigated. The reason for including the epinephrine was to produce pulpal vasoconstriction and thereby a fall in pulpal interstitial fluid pressure and a reduction in the rate of outward flow of dentinal fluid, to further facilitate the inward diffusion of the lignocaine (Matthews and Vongsavan, 1994). Since lignocaine and epinephrine ions are both positively charged, they can be iontophoresed together into dentine by an anodal current (Kostouros *et al*, 1996 a&b). Thus, in the present experiments we attempted to anaesthetize dentine by produced optimal conditions for the inward diffusion of lignocaine by creating a steep concentration gradient with a solution of 20% lignocaine, including epinephrine to produce pulpal vasoconstriction and reduce the rate of outward flow of dentinal fluid, and by passing an anodal current between the solution and the dentine to enhance the inward movement of both the lignocaine and epinephrine ions towards the pulp.

Materials & methods

Brief Outline of Experiments

A cavity was cut at the tip of the buccal cusp of a premolar and the sensitivity of the exposed dentine was tested with mechanical stimulation and with an air-blast stimulus. A solution of 20% w/v lignocaine (synonym: lidocaine) with 1:1000 epinephrine was then applied to the cavity and an iontophoretic current of 120 uA passed between the solution and the underlying dentine for 90 s. Immediately after this, the sensitivity of the dentine was tested again. The testing was repeated at 10 min. intervals for a further 40 min. Pulpal blood flow was recorded after each of these tests. In control experiments, the same procedure was followed except the solution contained only the epinephrine and data were recorded for only 20 min. after the treatment.

Subjects and Teeth

The experiments were carried out on 13, non-carious premolar teeth in 13 healthy subjects (age: 16-24 years, mean 19.4) that were scheduled for extraction as part of orthodontic

treatment. All teeth were healthy and had completely formed roots, as determined by clinical and radiographic examination. The study was approved by the Ethics Committee on Human Rights Related to Human Experimentation of Mahidol University, and complied with the principles of the Declaration of Helsinki. The experiment procedures were clearly explained to each subject and informed consent was obtained from the subject, or for those under 18 years, a parent or guardian. The privacy rights of the subjects were observed at all times.

Tooth preparation

An acrylic, clip-on splint was made on a plaster model to cover the crowns of the test tooth and the two adjacent teeth. A socket was incorporated into the splint to support a laser Doppler blood flow probe over the cervical area of the test tooth (see below). A hole was cut in the splint to expose the occlusal surface of the test tooth. The teeth were isolated with an opaque black rubber dam (Four D Rubber Co., Ltd., Heanor, DE75 7SJ, England; S type, thickness: 0.33 mm) to minimise the contamination of the signal from the pulp by blood flow in adjacent tissues (Soo-ampon *et al*., 2003). The dam was held in place by the splint. After the splint had been clipped in place, the joint between it and the occlusal enamel of the test tooth was sealed with light-curing composite resin.

A cylindrical cavity (diameter, 3 mm; depth, 3 mm) was cut without anaesthetic at the tip of the buccal cusp using diamond burs in an air-rotor with water coolant. The cavity was etched with 35% phosphoric acid for 30 seconds to remove the dentine smear layer. The volume of the cavity plus the overlying hole in the splint was approx. 25 µl.

Dentine Sensitivity Tests

After it had been blotted dry with cotton pellets, the sensitivity of the exposed, etched dentine was tested with two forms of stimulus: gently stroking the middle of the floor of the cavity with an explorer (tip diameter 0.15 mm., force approximately 20 g.), and by directing a 3 second blast of air at room temperature onto the exposed dentine from a triple syringe (reservoir pressure = 41 Pa; distance from syringe tip to mouth of cavity: 1-2 mm). The stimuli were always applied in this order. After each stimulus, the subject was asked to rate the intensity of any pain experienced by placing a mark on a 100 mm visual analogue scale (VAS) which was divided into 10 mm steps, in which 0 indicated no pain and 100, the most severe pain that could be imagined (Holland *et al*, 1997)Thus, in scoring the severity of the pain, the subject was asked to select from eleven possible values.

Test Solution and Iontophoresis

The test solution contained 20% w/v (0.69 mol/l) lignocaine HCl monohydrate (Sigma-Aldrich, Dorset, England) in sterile, distilled water to which was added 0.1% w/v (1:1000) epinephrine hydrochloride (GPO, The Government Pharmaceutical Organization, Thailand). The control solution contained only the epinephrine.

In each tooth, 25 µl of either the test (7 teeth) or the control (6 teeth) solution was placed in the cavity and a direct current of 120 µA was passed for 90 s between an electrode (anode) that was inserted into the solution and another electrode (cathode) that was held in the subject's hand (Fig. 1). The current was applied from a battery-operated device (Dentaphore-II, model 611 D; Life-tech, Inc., Houston, Texas, USA). In some cases, the current was increased gradually over a period of approx. 5 s and in others, 120 µA was applied from the start. The accuracy of the calibration of this device was confirmed by recording the current passed in a model system.

Pulpal Blood flow Recording

Pulpal blood flow was recorded with a laser Doppler blood flow monitor (Periflux 4001; Perimed AB, Järfälla, Sweden) which was equipped with an infrared laser (wavelength 780-820 nm). The probe (Perimed type 407: ext. diam. 1.0 mm; optical fibre diam. 0.125 mm, fibre separation 0.25 mm) was supported in a mini probe holder (type PH 07-5) that was incorporated into the removable acrylic splint (see above). The probe holder was positioned so that the probe was perpendicular to the labial enamel surface on the labial surface of the tooth, with its tip over the central long axis of the crown, and with its centre 2 mm from the gingival margin.

Pulpal blood flow was recorded after each of the sensitivity tests, before and after the iontophoretic treatment with the test and control solutions. Data were recorded from the digital output of the flow meter with a computer running the PeriSoft (version 1.13) software program. The probe was calibrated according the manufacturer's instructions and the results are expressed in arbitrary perfusion units (PU).

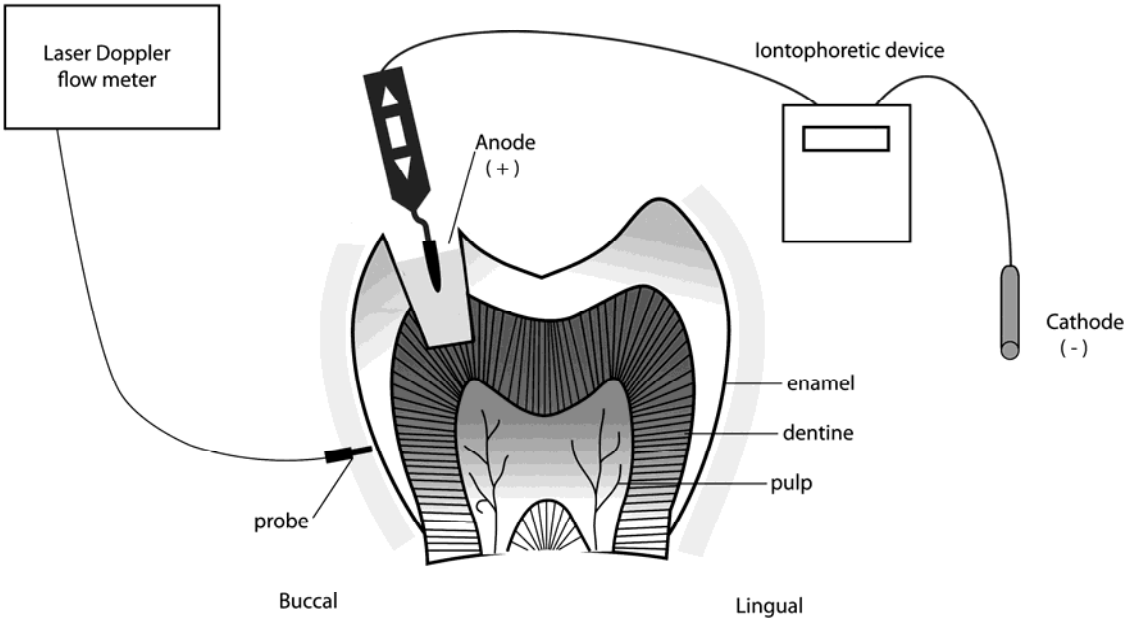

Fig. 1. Diagram of the experimental set up for treatment with the test solution (not to scale.

Remaining Dentine Thickness

The thickness of the dentine remaining beneath the cavity was measured with calipers after extraction of the tooth.

Statistical Analysis

Non-parametric statistical methods were used to compare VAS and blood-flow data. The median and the 25th and 75th percentile values were calculated for each set of data. Comparisons between the median values of several groups were made with Friedman's Repeated Measures Analysis of Variance on Ranks (RMAVR) and where this indicated there was a significant difference within the groups of data, multiple paired comparisons were made using Dunnett's Method. A *P* value of less than 0.05 was considered significant. The values of remaining dentine thickness were normally distributed and group mean values were compared with Student's t-test.

Results

Treatment with the test solution produced a very clear effect: it reduced the median VAS response to probing from 60 mm (range 20 – 80, N=7) to 0 mm (range 0 – 0), and to air-blast from 80 mm (range 50 – 100, N=7) to 0 mm (range 0 – 0). These effects were recorded when the sensitivity of the dentine was first tested immediate after the 90 s iontophoretic treatment, and in every tooth they persisted throughout the subsequent 40 min. period of observation (Fig. 2A). In contrast, treatment with the control solution produced no significant change in the median VAS responses to either probing or air-blast stimuli ($P > 0.05$, RMAVR) (Fig. 2B).

Applying the 120 μA iontophoretic current caused no pain if the current was increased from zero over 5 s, but if it the full intensity was applied from the start, some patients felt pain for a few seconds.

The iontophoresis of lignocaine with epinephrine produced a decrease in pulpal blood flow from a median baseline value of 0.92 PU (range 0.25 to 2.36) to 0.61 PU (0.13 to 1.85) immediately after the treatment and to 0.47 (0.12 to 2.10), 0.27 (0.07 to 2.11), 0.28 (0.10 to 2.16), and 0.50 (0.50 to 1.98) at successive 10 min. intervals after this (Fig. 3). All the changes in median blood flow from the median baseline value were significant ($P < 0.05$, RMAVR and Dunnett's Method). Treatment with epinephrine alone produced a similar effect (Fig. 3).

The mean remaining dentine thickness of the teeth used for the test solution was 0.74 (s.d. 0.30, n = 7) and for the control solution, 0.95 (s.d. 0.35, n = 6). These mean values are not statistically different ($P > 0.05$, Student's t-test).

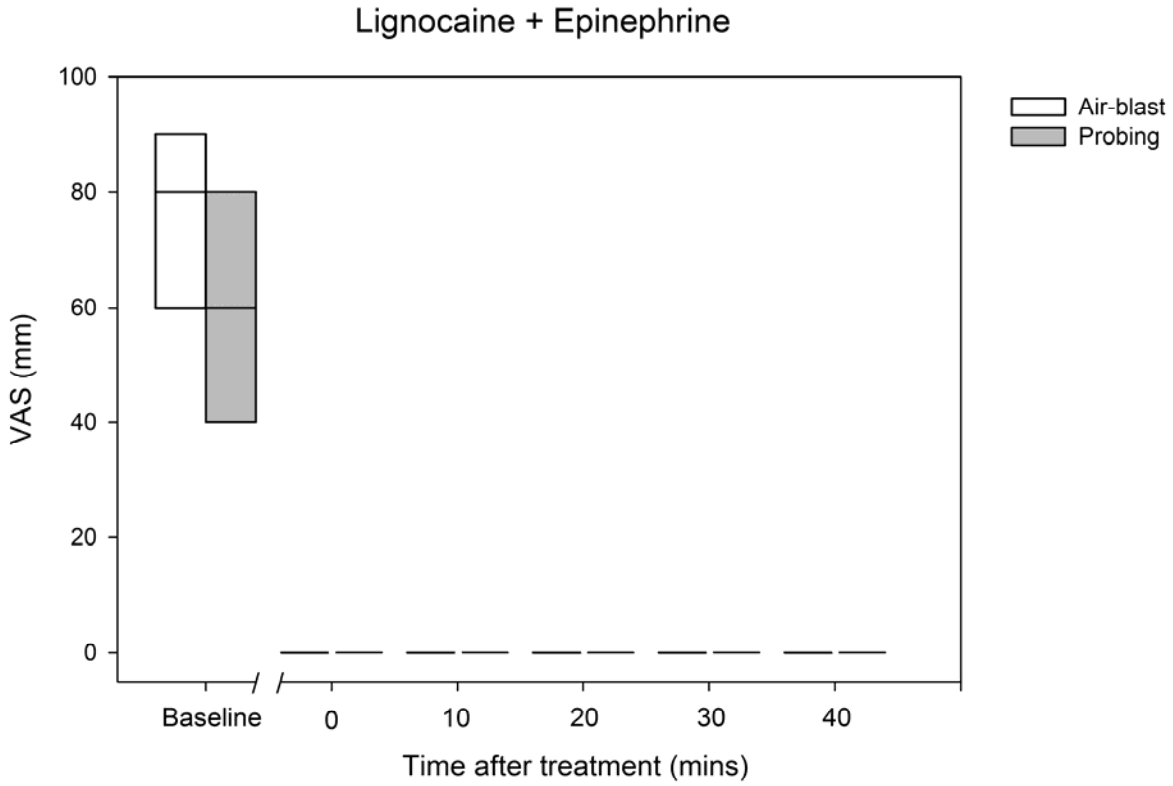

Fig. 2A. The effect of the iontophoresis of 20% w/v lignocaine HCl with 0.1% w/v epinephrine HCl to exposed, etched dentine on the intensity of pain (VAS score) evoked in 7 teeth by air-blast (open boxes) and by probing (grey boxes) stimulation of the dentine. The data were collected before (Baseline) and immediately after (0 mins.) treatment, then at 10 minute intervals for the next 40 minute, The line through each of the Baseline boxes indicates the median VAS score and the lower and upper limits of the box, the 25th and 75th percentiles respectively. After treatment, the VAS scores were all 0.

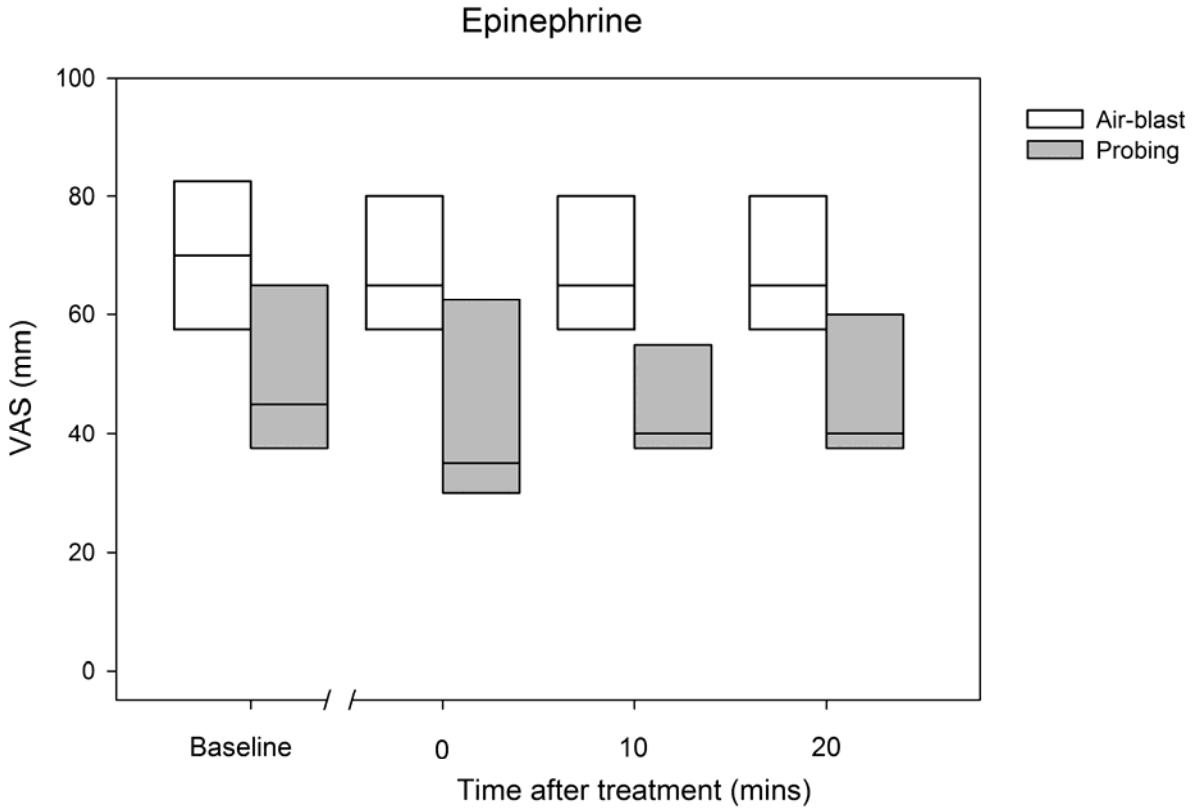

Fig. 2B. The effect of the iontophoresis of 0.1% w/v epinephrine HCl to exposed, etched dentine on the intensity of pain (VAS score) evoked in 6 teeth by air-blast (open boxes) and by probing (grey boxes) stimulation of the dentine. The data were collected before (Baseline) and immediately after (0 mins.) treatment, then at 10 minute intervals for the next 20 minute, The line through each box indicates the median VAS score and the lower and upper limits of the box, the 25th and 75th percentiles respectively.

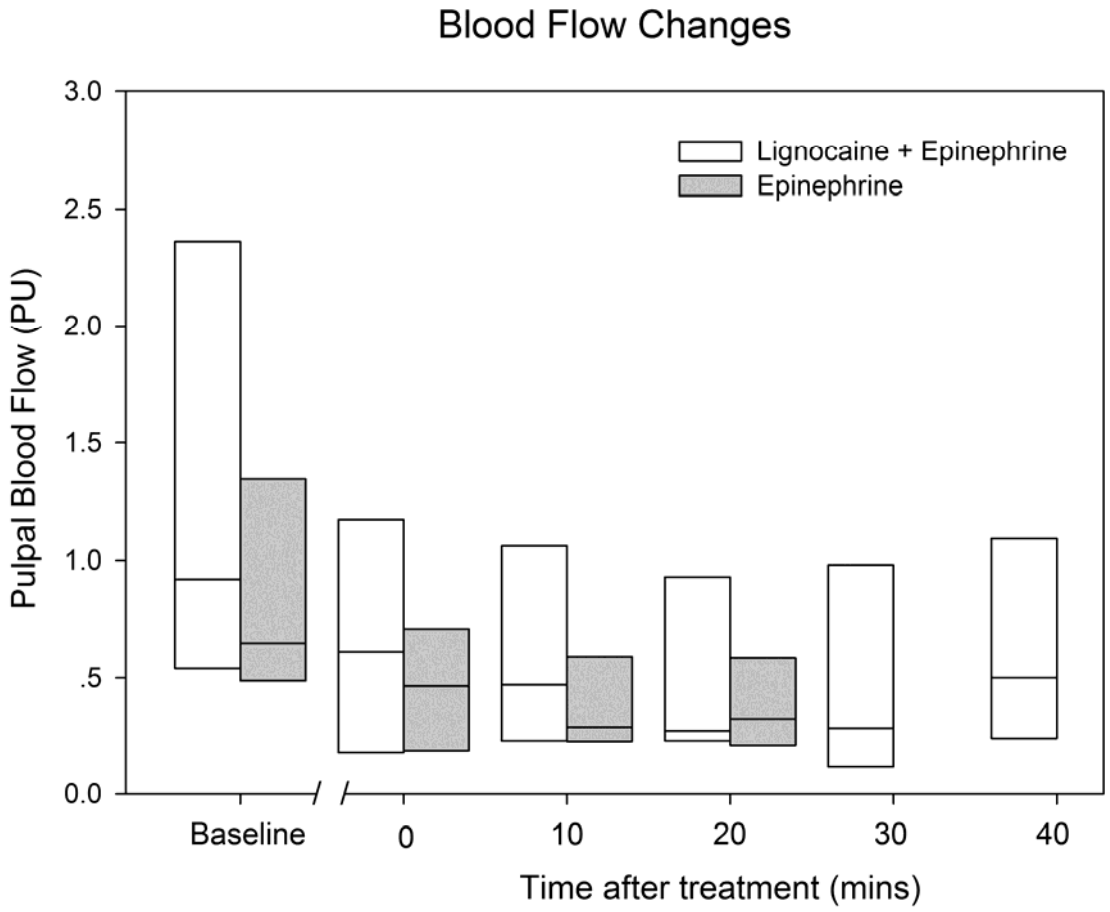

Fig. 3. Pulpal blood flow values recorded before (Baseline) and immediately after (0 mins.) treatment, then at 10 minute intervals for up to 40 minutes subsequently, Data recorded from 7 teeth treated with 20% w/v lignocaine HCl and 0.1% w/v epinephrine HCl are represented by the open boxes, and those from 6 teeth treated with 0.1% w/v epinephrine only are represented by the grey boxes. The line through each box indicates the median value and the lower and upper limits of the box, the 25th and 75th percentiles respectively.

Discussion

The present experiments demonstrate that dentine can be anaesthetized by the topical application of a solution containing 20% w/v lignocaine HCl monohydrate and 0.1% w/v epinephrine HCl when diffusion of these substances into the dentine is facilitated by the application of an iontophoretic current of 120 μA for 90 s. This is a simple procedure, which provides an alternative to the administration of the anaesthetic by injection, that could find application clinically.

In the control experiments, the epinephrine alone produced the same fall in pulpal blood flow but no anaesthesia. In preliminary experiments (Vongsavan, unpublished observations) it was found that the iontophoresis of 20% lignocaine without epinephrine produced anaesthesia lasting less than 10 mins. The inclusion of epinephrine in the test solution had the effect of increasing the duration of the anaesthesia to a value that would be adequate for most clinical procedures. Without iontophoresis, even a 50% solution of lignocaine applied topically produced anaesthesia for a maximum of 10 min., and after a latent period of up to 30 min (Rirattanapong *et al*., 2013).

These conclusions apply to normal dentine; further experiments are require to determine if they also apply to carious dentine and to dentine overlying inflamed pulp.

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Series IV experiments

The iontophoresis of lignocaine with epinephrine into carious dentine for pain control during cavity preparation in human molars

Abstract

***Objective*:** To determine the effectiveness of the iontophoretic delivery of lidocaine with epinephrine through carious dentine for pain control during cavity preparation.

***Design*:** The experiments were carried out on 56 carious molars that required class I restorations in 42 subjects (aged 15-20 years). The overhanging enamel and soft caries were removed then the sensitivity of the exposed dentine was tested with drilling, probing and air blast stimuli. The subject indicated the intensity of any pain produced by marking a visual analogue scale (VAS). The cavity was then filled with 20% w/v lidocaine with 0.1% w/v epinephrine and a 200 μA iontophoretic current applied for 2 min. after which the sensitivity of the dentine was re-tested. If the dentine was not anaesthetized, the treatment and testing were repeated up to 6 times.

***Results*:** The total duration (mins.) of iontophoresis required to anaesthetize the dentine was: 2 in 7 teeth, 4 in 17 teeth, 6 in 14 teeth, 8 in 4 teeth, and 10 in 7 teeth. The remaining 7 teeth were not anaesthetized even after 14 mins. of iontophoresis.

***Conclusion*:** The iontophoretic delivery of lidocaine with epinephrine anaesthetized dentine for cavity preparation in 49 of 56 (87.5%) of carious molars.

Introduction

It has been shown recently that dentine can be anaesthetised rapidly by the topical application of a solution containing 20% w/v lignocaine HCl and 0.1% w/v epinephrine HCl if an iontophoretic, anodal current of 120 μA is passed for 90 s between the solution and the dentine (Thongkukiatkun *et al.*, 2015). These experiments were carried out on freshly exposed, healthy dentine; if the technique could be used for cavity preparation in carious teeth, it would avoid the need for the administration of local anaesthetics by injection, which causes patients pain and anxiety. Injections are one of the most anxiety-provoking procedures in dental treatment for both children and adults (Corah, 1985; Giangrego, 1986; Milgram et al, 1986; Weine, 1982).

In the present experiments, the possibility of anaesthetising carious molars for cavity preparation using a technique similar to that described by Thongkukiatkun *et al*. (2015) was investigated. Preliminary experiments indicated that the original technique did not provide adequate anaesthesia for the treatment of carious teeth and for this reason the iontophoretic current was increased from120 to 200 μA and it was applied for 2 min. rather than 90 s. In every other respect, the method of treatment was as described by Thongkukiatkun *et al*. (2015).

Materials & methods

Brief Outline of Experiments

Molar teeth were selected that required a class I cavity to remove caries. The cavity was cut by first removing the overhanging enamel with an air-rotor, and then removing the soft caries with a spoon excavator. The sensitivity of the dentine in the floor of the cavity was then tested with a 3 s period of drilling with a diamond bur, with a 3 s blast of air from a triple syringe, and by gently stroking the centre of the cavity floor with an explorer. After this, a solution of 20% w/v lignocaine (synonym: lidocaine) with 0.1% w/v epinephrine was applied to the cavity and an anodic iontophoretic current of 200 uA passed for 2min. Immediately after this, the sensitivity of the dentine was tested again. If the patient felt no pain during these tests, the cavity preparation was completed and the cavity was filled with composite resin.

If, after the first treatment, the patient felt pain during any of the three dentine sensitivity tests, the treatment was repeated and the sensitivity of the dentine tested again. This

procedure of treatment and testing was repeated up to 7 times until the patient felt no pain. If the dentine was anaesthetised, cavity preparation was completed as described above. If the dentine was not anaesthetised by seven treatments, the tooth was anaesthetised by injection of local anaesthetic and the cavity preparation completed.

Subjects and Teeth

The experiments were carried out on 56 carious molar teeth that required class I restorations, in 42 healthy subjects (age: 15-20 years, mean 16.7). The experiments were carried out either in the Department of Paediatric Dentistry of the Faculty of Dentistry of Mahidol University or in the Dental Surgery of a local school. In all the teeth, the caries extended into the dentine, and in some the caries was of moderate depth. Teeth with deep caries, under which the pulp might have been exposed, were excluded. The teeth were not x-rayed.

The study was approved by the Ethics Committee on Human Rights Related to Human Experimentation of Mahidol University, and complied with the principles of the Declaration of Helsinki. The experiment procedures were clearly explained to each subject and informed consent was obtained from the subject, or for those under 18 years, a parent or guardian. The privacy rights of the subjects were observed at all times.

Tooth preparation

The enamel over-hanging the caries was drilled away with a diamond bur in an air-rotor hand-piece with water-spray and the soft caries was removed with a spoon excavator. To prevent the spread of the local anaesthetic solution a temporary wall of composite resin was built up on the enamel around the cavity (height approx.. 2 mm) and rubber dam was applied to the tooth. The cavity was not etched.

After the tooth had been anaesthetised, the final stage of cavity preparation was completed with diamond and steel burs in an air-rotor hand-piece with water-spray.The cavity was filled with composite resin (Filtex ™, 3M Dental Products, USA).

Dentine Sensitivity Tests

After it had been blotted dry with cotton pellets, the sensitivity of the dentine in the floor of the cavity was tested in three ways: by gently drilling for 3 s with a diamond bur (no. 204) in an air-rotor hand-piece with water-spray, by gently stroking the middle of the floor of the cavity with an explorer (tip diameter 0.15 mm., force approximately 20 g.), and by

directing a 3 second blast of air at room temperature onto the exposed dentine from a triple syringe (reservoir pressure = 41 Pa; distance from syringe tip to mouth of cavity: 1-2 mm). After each stimulus, the subject was asked to rate the intensity of any pain experienced by placing a mark on a 100 mm visual analogue scale (VAS), in which 0 indicated no pain and 100, the most severe pain that could be imagined (Hollandet al., 1997).

Anaesthetic Solution and Iontophoresis

The anaesthetic solution contained 20% w/v (0.69 mol/l) lignocaine HCl monohydrate (Sigma-Aldrich, Dorset, England) in sterile, distilled water 0.1% w/v (1:1000) of epinephrine hydrochloride (GPO, The Government Pharmaceutical Organization, Thailand). In each tooth, 50 µl of the this solution was placed in the cavity and a direct current of 200 µA was passed for 2 min. between an electrode (anode) that was inserted into the solution and another electrode (cathode) that was held in the subject's hand (Fig. 1). The current was applied from a battery-operated device (Dentaphore-II, model 611 D; Life-tech, Inc., Houston, Texas, USA). To prevent the current causing pain by stimulating intradental nerves, its intensity was increased gradually from 0 over a period of approx. 10s. here

Statistical Analysis

The median and the 25th and 75th percentile values of the VAS scores were calculated for each set of data. In addition, the 10^{th} and 90^{th} percentiles were calculated for each set with more than 9 values. Comparisons between the median values of several groups were made with Friedman's Repeated Measures Analysis of Variance on Ranks (RMAVR) and where this indicated there was a significant difference within the groups of data, multiple paired comparisons were made with the Tukey test. A *P* value of less than 0.05 was considered significant.

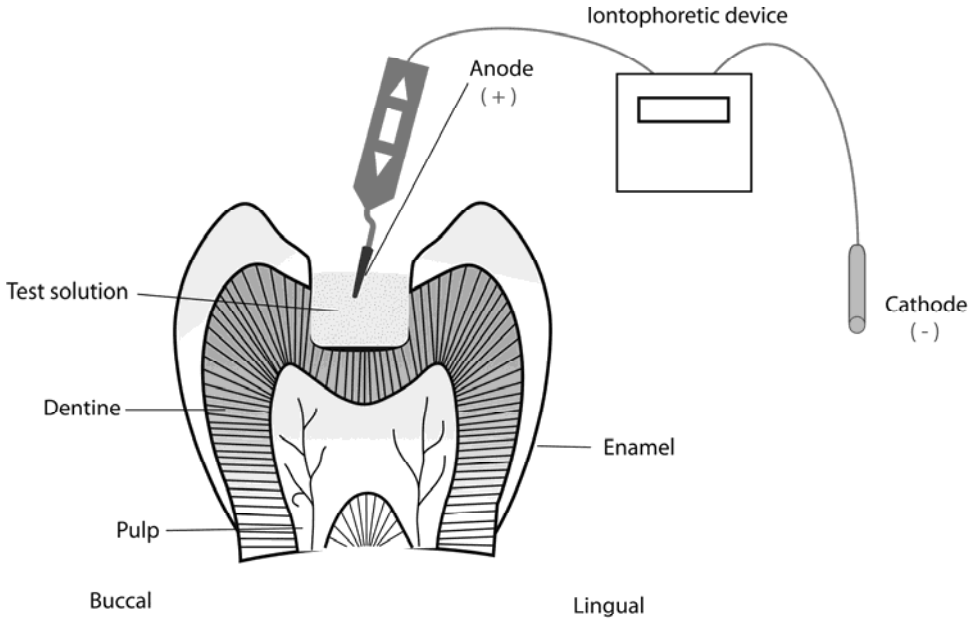

Fig. 1. Diagram of the experimental set up (not to scale).

Results

The median VAS scores when the dentine was first tested after removal of the soft caries were 40 mm (range 5 to 75, n = 56) with the drilling stimulus, 10 mm (range 0 to 80, n = 56) with the air-blast stimulus, and 0 mm (range 0 to 30, n = 56) with probing. The value for drilling was significantly greater than those for air-blast and probing ($P < 0.05$; RMAP and Tukey test), but the values for air-blast and probing were not significantly different. All the teeth responded to at least one form of stimulus.

After the first treatment, the corresponding values for the 56 teeth were 20 (0 to 60); 0 (0 to 60); and 0 (0 to 30). The reduction in the median VAS score with drilling was significant, but the changes in the responses to the other stimuli were not. None of the three stimuli produced pain in 7 of the 56 teeth and the cavity preparation was completed in these without further iontophoresis.

A second anaesthetic treatment was carried out on the remaining 49 teeth and after this the median responses to the test stimuli were 20 (0 to 50) with drilling; 0 (0 to 50) with air-blast and 0 (0 to 30) with probing. Again, only the change in the response to drilling was significant. There was no response to any of the test stimuli in 17 teeth and the cavity preparation was completed in these without further iontophoresis.

The results obtained with up to 7 treatments (14 mins. total) are summarised in Fig. 2. The drilling stimulus consistently caused more pain than either air-blast or probing.

The numbers of teeth that required more than 4 mins. of iontophoresis were as follows: 14 required 6 mins., 4 required 8 mins., and 7 required 10 mins. The remaining 7 teeth were not fully anaesthetised even after a further 2 treatments, and an injection of local anaesthetic was required for the cavity preparation to be completed in these teeth.

The cumulative proportions of the teeth that were successively anaesthetised with different periods of iontophoresis were 12.5% after 2 mins., 42.9% after up to 4 mins., 67.9% after up to 6 mins., 75.0% after up to 8 mins., and 87.5% after up to 10 mins. The remaining 12.5% were not anaesthetised even after a total of 14 mins. iontophoresis.

Once the stage had been reached in a tooth at which none of the three forms of test stimulus caused pain, the cavity preparation could be completed without causing further pain.

The teeth were examined after 3 months and 1 year. On each occasion, all were symptomless and vital.

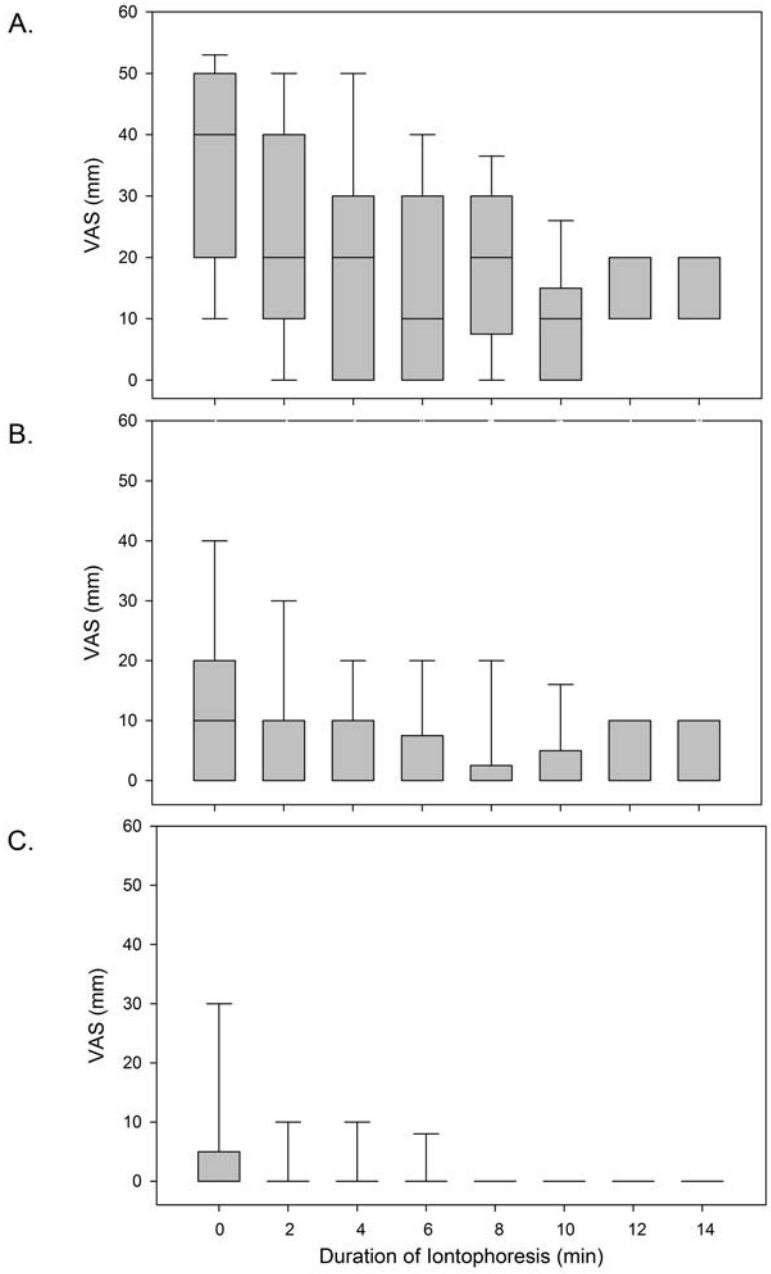

Fig. 1. The effect of the iontophoresis of 20% w/v lignocaine HCl with 0.1% w/v epinephrine HCl to dentine in a carious cavity on the intensity of pain (VAS score) evoked by: A: drilling, B: air-blast, and C: probing stimulation of the dentine.
The data were collected before treatment (Duration 0) and after a variable number of 2 min. periods of iontophoresis up to a maximum of 14min. The data are represented by box plots in which the lower and upper limits of the box represent the 25th and 75th percentiles respectively of the data. The median VAS score is the horizontal line through the box or is the lower limit of the box. The bars below and above each box indicate the 10th and 90th percentiles.
The number above each box in A shows the number of teeth treated with that duration of iontophoresis.

Discussion

These experiments have shown that it is possible to anaesthetise a high proportion of carious teeth, to permit cavity preparation to be carried out without pain, by the iontophoresis of a mixture of lignocaine and epinephrine into the dentine. The carious dentine in these teeth was however much more resistant to anaesthesia than the freshly exposed, normal dentine that was investigated using a similar procedure in a previous study (Thongkukiatkun *et al*, 2015). The same solution was applied to the dentine in both studies (20% w/v lignocaine HCl with 0.1% w/v epinephrine HCl) but whereas the normal dentine was immediately anaesthetised when an anodal current of 120 μA was passed from the solution into the dentine for 90 s, the carious dentine required a current of 200 μA, and this had to be applied for much longer. Most teeth were anaesthetised after between 2 and 10 minutes, but even after a total of 14 minutes, 7 out of 56 teeth (12.5%) were not anaesthetised to the level that would permit dentine to be drilled without pain. Despite this relatively long period of induction of anaesthesia, most of the subjects preferred it to an injection.

The most likely explanation for the greater resistance to anaesthesia of carious dentine compared with normal dentine is that, as a result of inflammatory changes in the pulp associated with the caries, Na+ channels of a type that are not sensitive to lignocaine were expressed in the nerve terminals and that these continued to support the propagation of action potentials despite the presence of the anaesthetic (Renton *et al*., 2005; Wells *et al*., 2007; Warren *et al*., 2008; Kistner *et al*., 2010; Suwanchai *et al*., 2012). There are several other possible explanations: for example, the dentine under the caries is likely to have been less permeable to the lignocaine than the normal dentine due to the presence of secondary and tertiary dentine. The hydraulic conductance of dentine under a carious lesion has been shown to be much lower than that of normal dentine (Pashley *et al*., 1991; Elgalaid *et al*., 2007). Also, if the pulp under the caries was inflamed and the pulpal interstitial fluid pressure was raised above normal, the rate of outward flow of dentinal fluid in the opened tubules may have been higher than in normal teeth and this would have reduced the rate of inward diffusion of the lignocaine (Pashley, 1994; Heyeraas and Berggreen, 1999; Ajcharanukul *et al*., 2011).

The VAS scores recorded from the carious teeth when tested with air-blast and probing stimuli before treatment, were substantially lower than those obtained with the same

stimuli under similar conditions from normal teeth (Thongkukiatkun *et al*, 2015). This was probably because the dentine in the normal teeth had been etched, which would have both removed the smear layer left by drilling, unblocking the mouths of the tubules, and left the dentine surface more compliant than normal. These changes may have allowed the air-blast to cause more fluid to be lost from the tubules and the probing to cause a greater displacement of the tubule contents; both resulting in a greater excitation of the hydrodynamic receptors in the normal than in the carious teeth. There are no data to compare the sensitivity to drilling of normal dentine with that of dentine under caries, although, from our experience of cutting cavities in normal teeth, we believe that the dentine in these teeth is much less sensitive than dentine under caries.

It may be possible to improve the technique we have employed to anaesthetise dentine by for example using an alternative anaesthetic that blocks a wider range of ion channels. Even in its present form, the method could be useful for anaesthetising teeth for conservatory procedures in patients who should avoid injections, such as those suffering from haemophilia, and in those who very much dislike injections.

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Outputs จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานที่ตีพิมพ์ในวารสารระดับนานาชาติ

1.1 ผลงานที่ได้รับการตีพิมพ์ในวารสารระดับนานาชาติแล้ว

1. Rirattanapong P, Vongsavan K, Kraivaphan P, Vongsavan N, Matthews B. Effect of the topical application of 50% lignocaine hydrochloride on the sensitivity of dentine in man. *Archives of Oral Biology* 2013;58(10), pp. 1549-1555.
(impact factor = 1.735, From Journal Citation Reports®, 2014)

2. Thongkukiatkun W, Vongsavan K, Kraivaphan P, Rirattanapong P, VongsavanN, Matthews B. Effects of the iontophoresis of lignocaine with epinephrine into exposed dentine on the sensitivity of the dentine in man. *Archives of Oral Biology* 2015;60(8), pp. 1098-1103.
(impact factor = 1.735, From Journal Citation Reports®, 2014)

3. Smitayothin K, Vongsavan K, Rirattanapong P, Kraivaphan P, Vongsavan N, Matthews B. The iontophoresis of lignocaine with epinephrine into carious dentine for pain control during cavity preparation in human molars. *Archives of Oral Biology* 2015;60(8), pp. 1104-1108.
(impact factor = 1.735, From Journal Citation Reports®, 2014)

1.2 ผลงานที่ส่งเพื่อตีพิมพ์ในวารสารระดับนานาชาติ

1. Wanachantararak S, Ajcharanukul A, **Vongsavan N**, Matthews B. Effect of cavity depth on dentine sensitivity in man. Submitted to *Archives of Oral Biology*.
(impact factor = 1.735, From Journal Citation Reports®, 2014)

2. การนำผลงานวิจัยไปใช้ประโยชน์

ได้รับการอ้างอิงระดับนานาชาติ (cited)

Rirattanapong P, Vongsavan K, Kraivaphan P, Vongsavan N, Matthews B. Effect of the topical application of 50% lignocaine hydrochloride on the sensitivity of dentine in man. *Archives of Oral Biology* 2013;58(10), pp. 1549-1555.
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2. Thongkukiatkun W, Vongsavan K, Kraivaphan P, Rirattanapong P, VongsavanN, Matthews B. Effects of the iontophoresis of lignocaine with epinephrine into exposed dentine on the sensitivity of the dentine in man. *Archives of Oral Biology* 2015;60(8), pp. 1098-1103.
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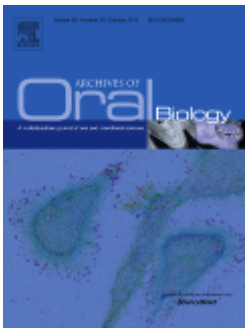

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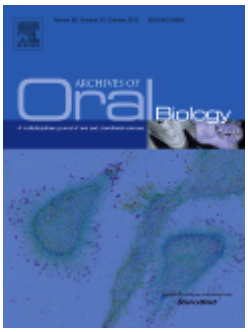

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