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The nociceptive and anti-nociceptive effects of white mineral trioxide aggregate

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Abstract

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Aim To assess the nociceptive and antinociceptive effects of white mineral trioxide aggregate (WMTA) using the orofacial formalin test in rats.

Methodology Rats ($n = 10$ in each group) were separately injected into the ipsilateral upper lip with either 40 μL of a 2.5% formalin solution and eugenol (50 mg kg^{-1}) or WMTA (5, 10 and 20 mg dissolved in 0.2 mL saline) alone. In a second experiment to evaluate antinociception effects, 15 min prior to formalin injection, rats were pre-treated with either white ProRoot MTA (20 mg dissolved in 0.2 mL saline) or eugenol. The time each rat spent rubbing the injected site with its paw, as an index of nociception, was recorded for a period of 45 min.

Results Administration of 40 μL white ProRoot MTA (5, 10 and 20 mg per 0.2 mL) alone did not produce any significant nociceptive response. Moreover, prior treatment with WMTA caused significant ($P < 0.001$) inhibition of formalin-induced nociception. Injection of eugenol (50 mg kg^{-1}) provoked the first phase of a nociceptive response, although its intensity was reduced compared with that produced by formalin. Pretreatment with eugenol significantly ($P < 0.0001$) inhibited the induction of nociception by formalin. Comparison of the behavioural responses observed in WMTA and eugenol-treated rats alone or in combination with formalin revealed that WMTA did not only induce pain behaviour but also prevented formalin-induced nociception.

Conclusion White mineral trioxide aggregate, when compared with eugenol, was more effective in treating nociceptive pain in the orofacial formalin test.

Keywords: antinociception, eugenol, nociception, orofacial formalin test, white mineral trioxide aggregate.

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Introduction

Pain and discomfort are often associated with endodontics therapy and can be caused by inflammation of the dental pulp and/or periapical tissues. Inflammation may lead to tissue acidification (Rukwied *et al.* 2007), which in turn, can make an important contribution to postoperative pain (Woo *et al.* 2004). It has been shown that protons can evoke pain through acid-sensing ion channel activation (Chen *et al.* 1998) which is present in primary sensory neurons of the trigeminal nerve (Lingueglia 2007). Dental filling materials which may come into direct contact with the oral tissue can also cause inflammatory reactions and it is therefore crucial to use materials without or with only limited toxic or irritant effects.

Mineral trioxide aggregate (MTA) is a relatively novel dental material with numerous clinical applications in endodontics, including root-end filling, pulp capping and perforation repair (Lee *et al.* 1993, Schwartz *et al.* 1999, Torabinejad & Chivian 1999, Holland *et al.* 2001, Aeinehchi *et al.* 2003, Main *et al.*

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2004, Accorinte *et al.* 2009). It has been reported that MTA possesses adequate physical (Bidar *et al.* 2007, Roberts *et al.* 2008), chemical (Torabinejad *et al.* 1995a, Camilleri *et al.* 2005) and biological (Economides *et al.* 2003) properties. The biocompatible nature of MTA has been well described previously, based on its biological and physicochemical properties (Torabinejad *et al.* 1995b, Koh *et al.* 1997, Camilleri *et al.* 2004, Ribeiro *et al.* 2006). Evidence also exist that MTA biomaterials possess antibacterial (Eldeniz *et al.* 2006, Tanomaru-Filho *et al.* 2007) and antifungal (Al-Nazhan & Al-Judai 2003, Al-Hezaimi *et al.* 2005, 2006) properties. Several reports have also described no cytotoxic (Torabinejad *et al.* 1995b, 1997, Keiser *et al.* 2000, Camilleri *et al.* 2005,) and genotoxic (Huang *et al.* 2003, Braz *et al.* 2006, Ribeiro *et al.* 2006) effects of the material. Therefore, based on the existing data, it is unlikely that MTA is a carcinogenic and/or cytotoxic substance. Additionally, white ProRoot MTA was reported to cause an increase in DNA synthesis, indicating positive effects on cellular proliferation (Moghadam-Jafari *et al.* 2005) and no affect on cell viability or the prostaglandin E2 synthesis of murine macrophages and fibroblasts (Melegari *et al.* 2006). Moreover, clinical and *in vivo* histological studies have demonstrated that white ProRoot MTA is highly effective in healing of pulp tissue in humans (Maroto *et al.* 2005, 2006). It has been suggested that possibly the release of Ca^{2+} from MTA could yield a desirable healing effect (Estrela & Pesce 1996). According to the available evidence, high extracellular Ca^{2+} increases the Ca^{2+} influx through voltage-gated calcium channels, which in turn can activate several types of Ca^{2+}-dependent K^+ channels (K_{Ca2+}). These channels are found in all excitable cells where they play a pivotal role in regulating the cell excitability and in regulating nocicepive input to the central nervous system (Li *et al.* 2007).

Based on this, it could be hypothesized that white ProRoot MTA treatment produces an antinociceptive effect by reducing pain-related behaviour at least in part due to the activation of K_{Ca2+}channels. Therefore, the present study was designed to investigate the nociceptive and/or antinociceptive effects of white ProRoot MTA on an experimental orofacial pain model in rats.

The possible antinociceptive effect of white MTA (WMTA) on both the early (neurogenic pain) and late (inflammatory pain) phases might be potentially useful in endodontics by reducing the postoperative pain so that following therapy with WMTA no prescription of anti-inflammatory pain killer may be required.

Method and materials

Animals

Experiments were carried out on 78 male Sprague–Dawley rats weighing 180–200 g, housed at 23 ± 1 °C on a 12/12 h light/dark cycle (lights on at 08 : 00 hours), acclimatized to the laboratory conditions for at least 72 h before use, with free access to food and water. Testing sessions took place during the light phase (between 10 : 00 hours and 17 : 00 hours) in a quiet room. As a standard procedure, animals were subjected to the test substances once only and sacrificed at the end of the experiments. All experimental procedures were carried out according to the protocols approved by the local ethics committee of the University of Shahid Beheshti (Medical Campus).

This study was divided into two parts. In part I, the nociceptive effect of white ProRoot MTA (Dentsply, Tulsa Dental, OK, USA) was examined in comparison with formalin and eugenol treatments and in part II the possible antinociceptive effect of white ProRoot MTA on formalin-induced pain behaviour, using a rat model of orofacial test was investigated.

Part I: the nociceptive assessment

In the first stage, rats were assigned randomly to three experimental groups (n = 10 in each group) and the rats received a 40 μL subcutaneous injection of either 2.5% formalin, WProRoot MTA at different concentrations of 5, 10 or 20 mg dissolved in 0.2 mL saline or eugenol (50 mg kg^{-1}) onto the ipsilateral upper lip, just lateral to the nose, respectively. An additional group of rats was assigned with saline as a vehicle (n = 8, control). The 2.5% formalin was prepared by diluting the stock aqueous 37% formaldehyde solution (Sigma, UK) in 0.9% isotonic saline. MTA was prepared according to the manufacture's direction as a mixture of powder (5, 10 or 20 mg) and saline (0.2 mL) in a slurry form.

Part II: the antinociceptive assessment

In the second stage, two groups of rats (n = 10 per group) were given a local injection (40 μL) of white ProRoot MTA (20 mg dissolved in 0.2 mL saline) or eugenol (50 mg kg^{-1}) 15 min before the injection of 2.5% formalin into the upper lip.

Formalin test

The orofacial formalin test was conducted as described by Clavelou *et al.* (1989). Briefly, each rat was placed before injection for 30 min, in a transparent Plexiglas observation chamber ($30 \times 30 \times 30$ cm^3 with a mirror placed at an angle of 45°) to minimize any stress-related behavioural changes. The animals were gently held and then received a subcutaneous 40 μL injection of the substances using a thin sterile needle (30 gauge). Immediately, the rats were returned to the transparent box for a 45-min observation period. The parameter of nociceptive response measured in this study was rubbing of the injected area. The number of seconds that each animal spent rubbing the injected area with its fore- or hind paws was recorded cumulatively (using a stopwatch) in consecutive 5-min blocks over a period of 45 min, and was considered as an index of nociception. The nociceptive response was clearly biphasic, with an initial component that normally peaked about 5 min after formalin injection (first – neurogenic phase) and subsided transiently over the next 5 min, followed by a second rise in rubbing incidence (second – inflammatory phase) over the remainder of the 45-min observation period (which peaked between 20 and 25). In view of these characteristics, the responses were considered over the first 5–10 min period following formalin injection as the first phase of nociception, and those occurring between 20 and 35 min as the second phase. Responses were scored according to the procedure described by Clavelou *et al.* (1995). This score was based on following four scales: 0 for normal behaviour, including grooming; 1, abnormal head movements including continuous placement of the jaw on the floor or the wall of the cage; 2, abnormal continuous shaking of the lower jaw and 3, excessive rubbing of the mouth. Nociceptive scores were calculated at the end of observation period by blocks, 5 min each, according to the following formula, where T represents time: Nociceptive score = $[(1 \times T$ in scale 1) + $(2 \times T$ in scale 2) + $(3 \times T$ in scale 3)] per 300 s (Dubuisson & Dennis 1997, Chidiac *et al.* 2002).

Data analysis

The nociceptive behavioural responses were averaged into 5-min blocks to decrease minute by minute variability. Parametric tests [two-tailed Student's t-test and ANOVA (analysis of variance with *post hoc* comparisons via Tukey's Honesty Significant Difference test)] were used as appropriate using Statistica software (Version 6, StatSoft, Tulsa, OK, USA). All results were presented as the mean ± SEM of 10 observations. Values were considered statistically significant at $P < 0.05$.

Results

Part I

Injection of formalin into the upper lip produced a biphasic nociceptive response (Fig. 1) consisting of an immediate and short period of activity lasting approximately 5–10 min, followed by a tonic period of activity starting at minute 15, peaking between 20 and 30 min and subsiding by 45 min after the injection. Little nociceptive behaviour was observed during 5-min period from minute 12 to minute 15. To assess the possible nociceptive effect of WMTA, animals were treated subcutaneously with different concentrations of WMTA. The effectiveness of white ProRoot MTA on both phases 1 and 2 of the pain response is shown in Figs 1 and 2. No pain-related behaviours were observed after injection of all doses of WMTA when compared with the formalin-treated rats (Fig. 3). The duration of face rubbing in MTA-treated rats was significantly less than that observed in the formalin group ($P < 0.0001$, Figs 1 and 2). There was also no significant difference in the behavioural responses between rats receiving different concentrations of WMTA.

Part II

Further experiment was undertaken to address the antinociceptive potential of WMTA in orofacial nociception induced by formalin. As shown in Fig. 1, the duration of the nociceptive response to 2.5% formalin reduced significantly by prior treatment with WMTA ($P < 0.01$). In addition, WMTA pre-treatment reduced significantly in the first phase (Fig. 2a; $P < 0.0001$) and the second phase (Fig. 2b; $P < 0.0001$ and $P < 0.01$) of the nociception induced by 2.5% formalin, respectively, compared with formalin and MTA injections alone. The nociceptive behavioural score was also suppressed when animals received MTA, either alone or in combination with formalin (Fig. 3).

Eugenol which has been previously reported to cause analgesia in the rat hind-paw formalin model (Ohkubo & Shibata 1997, Kurian *et al.* 2006) was tested to see if it would also prevent the orofacial nociception induced by formalin injection. Pre-treatment with eugenol, 15 min before formalin administration, inhibited significantly both phases of the nociceptive response to

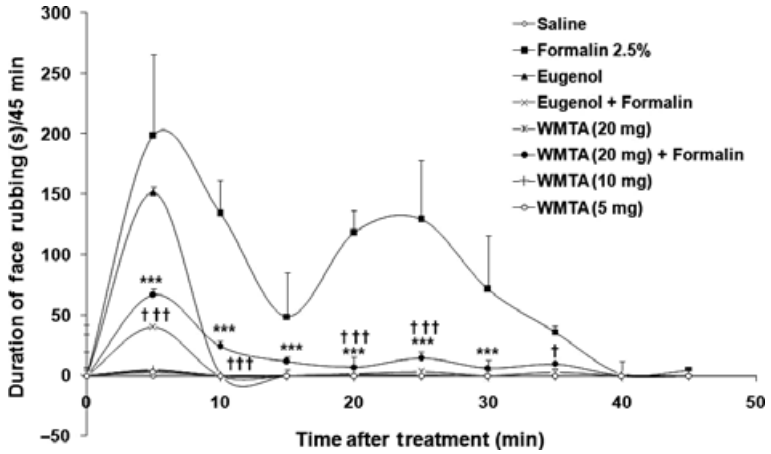

Figure 1 Time course of the face rubbing activity observed after subcutaneous injection of 2.5% formalin, white mineral trioxide aggregate (WMTA; 5, 10 and 20 mg per 0.2 mL) and WMTA (20 mg per 0.2 mL) + Formalin and (b) 2.5% formalin, eugenol, eugenol + Formalin. The mean number of seconds that rats (n = 10 per group) spent rubbing is plotted for each 5-min blocks over 45 min post-injection period. *Significant difference between WMTA (20 mg per 0.2 mL) and WMTA (20 mg per 0.2 mL) + Formalin versus 2.5% formalin-treated alone rats. †Denotes significant difference between eugenol and eugenol + Formalin-treated rats versus 2.5% formalin injection alone. There was a significant difference ($P < 0.001$) between WMTA injection alone (at all concentrations) and 2.5% formalin-induced nociceptive responses but the statistical symbols have been omitted from the figure 1 for sake of clarity. †$P < 0.05$; ***†††$P < 0.001$; ****$P < 0.0001$ (one-way ANOVA test).

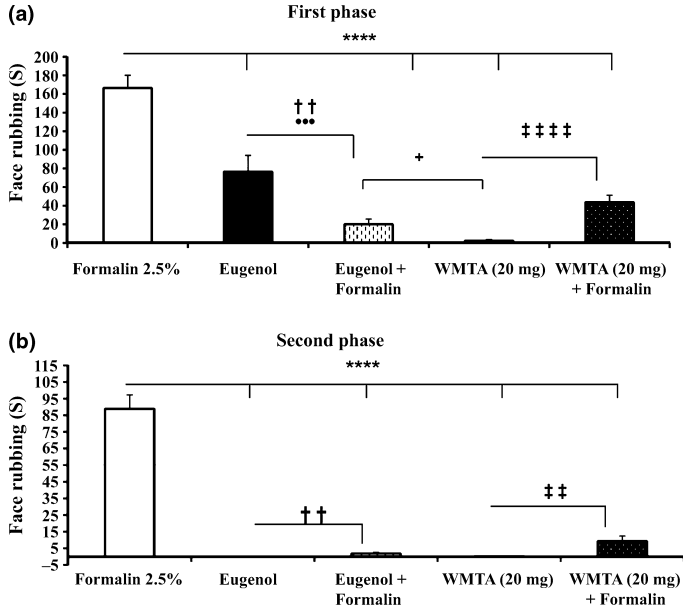

Figure 2 Effect of different treatment on (a) the first phase (5–10 min post-injection) and (b) the second phase (20–35 min post-injection) of face rubbing. Asterisks represent a significant difference when compared with formalin response. †Denotes significant difference between eugenol versus eugenol + formalin treated rats. ‡Represents significant difference between white mineral trioxide aggregate (WMTA; 20 mg per 0.2 mL) versus WMTA + Formalin. +Denotes significant difference between eugenol + Formalin against WMTA (20 mg per 0.2 mL). •Shows significant difference between eugenol versus WMTA. Data are mean ± SEM (n = 10 in each group). ****, ‡‡‡‡$P < 0.0001$; •••$P < 0.001$; ‡‡, ††$P < 0.01$; +$P < 0.05$ (two-tailed Student's t-test).

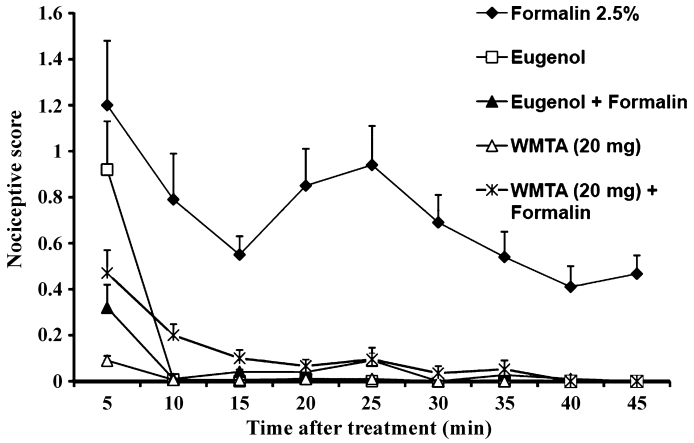

Figure 3 Nociceptive scores recorded in 2.5% formalin-treated rats and after injection of eugenol, eugenol + Formalin, mineral trioxide aggregate (MTA) at concentrations of 5, 10 or 20 mg per 0.2 mL and MTA (20 mg per 0.2 mL) + Formalin. Data are mean ± SEM (n = 10 in each group).

formalin ($P < 0.0001$) and decreased the total time spent in face rubbing nociceptive behaviour compared with formalin injection alone (Figs 1 and 2). In contrast, local administration of eugenol elicited the first phase of nociceptive behavioural response, although its intensity was significantly less than rats that received formalin alone (Figs 1 and 2a) and the pain score was lower in the eugenol group than in the formalin-treated rats (Fig. 2).

Although, eugenol alone, like white ProRoot MTA, significantly reduced the face rubbing in phase 2 (Fig. 2b, $P < 0.0001$ and $P < 0.01$, respectively, compared with formalin and eugenol + formalin), it did not reduce completely the nociceptive response in phase 1 as much as it did in phase 2 (Fig. 2a; $P < 0.01$ and $P < 0.001$, respectively, compared with eugenol + formalin and WMTA alone). Thus, WMTA was effective in attenuating both the first and second phases of formalin-induced pain-related behaviour, whilst eugenol was particularly potent in reducing the inflammatory pain. Treatment with normal saline (control group) as a vehicle *per se* did not significantly induce nociceptive or antinociceptive responses (Fig. 1).

Discussion

Physiological and biological aspects of root-end filling materials are important in relation to their clinical use. On the other hand, endodontic treatments may give rise to persistent pain whose origin is sometimes difficult to determine. Pain is an essential sensation that usually signals tissue injury inflicted by external or internal damaging events. The purpose of this study was to document the nociceptive and antinociceptive effects of white ProRoot MTA in comparison with formalin and eugenol in the orofacial formalin test. The orofacial region including teeth and oral cavity is one of the most densely innervated areas of the body, which is considered to be involved in nociceptive signalling (Jahnsen 1986, Raboisson & Dallel 2004).

Injection of formalin in the upper lip of the rat generates a characteristic nociceptive behavioural response, which appears to be a valuable tool for the study of clinical pain (Clavelou *et al.* 1995). Most reported studies used face rubbing as the measure of assessing nociception (Clavelou *et al.* 1995, Cadet *et al.* 1998, Raboisson & Dallel 2004). This nociception response consisted of two distinct phases: phase 1 – a short but immediate response lasting the first 5 min after the upper lip was injected; phase 2 – a prolonged response starting at approximately minute 15 and ending at approximately 45 min. Between phase 1 and 2, there was an intermittent period where little nociceptive behaviourwas observed.

It has been shown that the nociception seen in phase 1 is a result of direct nerve stimulation by the formalin (Hunskaar & Hole 1987) whilst the nociception produced in phase 2 was a result of chemical insult resulting in tissue damage (Rosland *et al.* 1990). Results of the present study with white ProRoot MTA and eugenol showed that white ProRoot MTA administration alone eliminated both phases of nociception, whilst eugenol injection caused a significant reduction in phase 1 but complete elimination of phase 2. On the

other hand, pre-treatment with white ProRoot MTA or eugenol significantly suppressed the induction of nociception by formalin.

Mineral trioxide aggregate has become a popular material to seal communication between the root canal system and external environment (Torabinejad *et al.* 1995c, Wu *et al.* 1998). It has also been recommended for root perforations, root-end filling and apexification (Göhring *et al.* 2004, Parirokh *et al.* 2005).

Mineral trioxide aggregate is commercially available in two versions: grey MTA (GMTA) and WMTA. In recent years, the use of WMTA has become more popular due to tooth discolouration caused by GMTA (Glickman & Koch 2000, Mohammadi *et al.* 2006). Both MTA products have been reported to consist of fine hydrophilic particles, but with slightly different composition, as the WMTA is iron-free and primarily composed of tricalcium silicate and bismuth oxide, whilst GMTA is composed primarily of tricalcium silicate, dicalcium silicate and bismuth oxide (Camilleri *et al.* 2005). Both GMTA and WMTA have been reported to produce effective antifungal (Al-Nazhan & Al-Judai 2003 Al-Hezaimi *et al.* 2005 &, 2006) and antimicrobial (Tanomaru-Filho *et al.* 2007) activities, which may be attributed to releasing calcium hydroxide or presumably due to its high pH.

Here, the cellular mechanism(s) responsible for the antinociceptive action of MTA has not been determined, although it can be speculated that the release of calcium ions from MTA may be involved. The calcium elevation outside the nerve would be associated with enhanced transmembrane Ca^{2+} influx which in turn, activates Ca^{2+}-dependent K^+ channel currents leading to K^+ efflux that mediates membrane hyperpolarization and decreases neuronal excitability. However, further research using electrophysiological technique needs to confirm this hypothesis. The high pH and calcium ion release has been reported to be responsible for the biocompatibility of MTA (Holland *et al.* 2001, Sarkar *et al.* 2005) and it has been shown that Ca^{2+} release from MTA could be the main factor contributes to the pulp repair (Bortoluzzi *et al.* 2008).

On the other hand, eugenol, a phenolic dental medicament, has been widely used as a topical treatment for pain and inflammation in pulpitis and dentine hyperalgesia (Kurian *et al.* 2006). The agent has sedative and anodyne effects, but concomitantly shows an irritant action (Sneddon & Glew 1973).

The analgesic action of eugenol had long been attributed to its action as a nonspecific counterirritant (Ohkubo & Shibata 1997). It has been previously reported that the agent inhibits sensory nerve activity (Ozeki 1975, Trowbridge *et al.* 1982), or has a potent inhibitory action on prostaglandin (PG)12 production (Hirafuji 1984). It has also been indicated that eugenol inhibits sodium and calcium channel currents in dental afferent neurons (Lee *et al.* 2005, Park *et al.* 2006).

In the second set of experiments, eugenol, which is widely used in the dentistry as a pain relieving agent, was considered as a gold standard of antinociception. As shown in Figs 1b and 2b, application of eugenol alone induced the first phase of the nociceptive response with a low intensity but not the second phase. However, when it was applied prior to the application of formalin, there was a complete suppression of the formalin-induced nocieceptive behaviour. In agreement with this, Kurian *et al.* (2006) found that eugenol exhibited more pronounced antinociceptive effect in the inflammatory phase which was attributed to the peripheral mechanism. It has been demonstrated that the nociception produced in phase 2 of the formalin test is a result of chemical insult resulting in tissue damage (Rosland *et al.* 1990). The results also showed that eugenol itself can induce nociceptive (neurogenic) response, whilst WMTA injection did not elicit any pain behaviour such as face rubbing.

Taken together these results suggest that WMTA does not appear to have any irritant effect on the nerve tissue, but is also more effective than eugenol, a palliative agent against dental pain, in treating an experimental animal model of orofacial pain. The antinociceptive effect of WMTA on both the early (neurogenic pain) and late (inflammatory pain) phases could have important clinical application by reducing postoperative pain.

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