ORIGINAL ARTICLE

Effects of applying glutaraldehyde-containing desensitizer formulations on reducing dentin permeability

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Abstract  Background/purpose: The efficacy of dentin-desensitizing agents is commonly evaluated in clinical studies by measuring patients’ pain response upon stimulation. Although indispensable, such trials are time-consuming, and the results depend on an individual’s subjective pain rating. Therefore, in vitro efficacy screening prior to clinical testing is highly desirable. The objective of this study was to investigate in vitro dentin permeability of two glutaraldehyde-containing desensitizer formulations after different modes and times of application.

Materials and methods: Coronal tooth slices, 1.3 mm thick, were dissected from 60 freshly extracted third molars. Specimens were treated with EDTA to remove the cutting smear. The dentin disks were clamped in a split chamber device to determine the baseline permeability under a liquid pressure of 2.5 kPa for 2 minutes and 13 kPa for 1 minute, to record liquid flow through the dentin using a photochemical method. Slices were soaked in a 2% albumin solution and reevaluated under the same pressure cycles prior to active or passive application for 15, 30, or 60 seconds of either Gluma Desensitizer or Gluma PowerGel (GDP) (Heraeus Kulzer, Hanau, Germany) and then reevaluated. Dentin-disk permeability was determined as the area under the photo signal output voltage line during the pressurizing period (mV s). The statistical data analysis used a Kruskal-Wallis ANOVA and Mann-Whitney post-hoc test with the significance level set to 5%.

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Introduction

Dentin hypersensitivity (DH) is by definition a common complaint, mainly in adult populations in their third and fourth decades. DH is characterized by a sharp transient pain in response to thermal, evaporative, tactile, osmotic, or chemical stimulation of exposed dentin in teeth, without evidence of other defects or pathology.\(^1\)\(^-\)\(^4\) DH is a result of fluid movement within the dentin complex.\(^5\) This phenomenon was described as the "hydrodynamic theory".\(^6\)\(^,\)\(^7\) Based on this widely accepted theory, the clinical treatment goal is to provide a permanent seal of the patent tubules or at least a reduction in their functional diameter to eliminate or minimize outward fluid flow from the tubules.\(^8\)\(^,\)\(^9\)

Numerous topical agents were suggested for professional relief of DH. Examples of frequently used topically applied agents include oxalates, creating tubule obstruction by precipitating fine-grained calcium oxalate crystals,\(^1\)\(^2\)\(^-\)\(^5\) dentin adhesives,\(^6\)\(^-\)\(^9\) protein-precipitating fixative agents,\(^1\)\(^0\)\^-\(^1\)\(^2\) and restorative materials.\(^1\)\(^3\) Gluma Desensitizer (GDL; Heraeus Kulzer, Hanau, Germany) is a spin-off of the Gluma Bonding System (Heraeus Kulzer). According to the manufacturer, GDL is an aqueous solution of 5% glutaraldehyde (GA) and 35% 2-hydroxyethyl methacrylate (HEMA).\(^1\)\(^4\) Bergenholz et al\(^1\)\(^5\) reported that application of Gluma Primer (which is identical to GDL) effectively inhibited the discharge of serum albumin from freshly cut dentin cavities in monkey teeth. They hypothesized that GA, a biological fixative and one of the major components of Gluma Primer, was responsible for the coagulation of plasma proteins and thus tubular blockage. This explanation was corroborated by results of morphological and clinical studies with GDL demonstrating peripheral tubular blockage\(^1\)\(^6\) and significant pain relief following topical application to hypersensitive dentin.\(^1\)\(^7\)\^-\(^1\)\(^9\)\(^,\)\(^2\)\(^0\)\^-\(^2\)\(^2\)

In order to improve handling procedures for GDL, the manufacturer developed an analogous gel formulation, Gluma Desensitizer PowerGel (GDP), for consistent application of the compound to sensitive tooth target sites, and to avoid or minimize the risk of inadvertent contact with adjacent gingival tissues.

Although clinical trials, i.e., pain studies, are the ultimate proof of the effectiveness of desensitizing agents,\(^2\)\(^8\) carefully designed in vitro trials are considered useful tools to predict clinical effects of DH treatments, although such trials cannot fully mimic the complexity of vital teeth. The dentin-disc model, designed to assess dentin permeability and hydraulic conductance,\(^1\)\(^2\)\(^,\)\(^3\)\(^1\) was suggested as a valuable tool for in vitro evaluation of dentin-desensitizing compounds.\(^1\)\(^5\)\^-\(^1\)\(^9\)\(^,\)\(^2\)\(^0\)\^-\(^2\)\(^2\)

The aim of this in vitro investigation was to evaluate and compare the effects of the duration and mode of application of GDL and GDP on the permeability of human dentin, using a modified dentin-disc method.\(^3\)\(^2\) The null hypothesis was that there would be no difference in permeability reduction among the liquid and gel formulations.

**Materials and methods**

**Materials investigated**

In this in vitro study, the effects of the two desensitizing agents, GDL (lot 010092, expiration date December, 2012) and GDP (lot VP1808098Q1, expiration date February, 2010), on reducing fluid flow through human dentin discs were investigated.

GDL is an aqueous solution of 5% GA and 35% HEMA, whereas GDP is an analogous aqueous gel formulation including 5% GA and 35% HEMA, which is thickened with pyrogenic silica and rendered opaque by the addition of pigments.

**Testing device**

The testing device described by Ishihata and colleagues\(^3\)\(^2\) was used to determine dentin permeability. The apparatus (Fig. 1) consists of two cylindrical acrylic chambers (5 mm in diameter and 5 mm high), mounted and fitted with O-rings on each side of a dentin disc, and clamped in a metal frame. Each chamber has a liquid inlet and a drainage outlet. The chamber mounted on the occlusal side of the disc is sealed with a clear glass coverslip and filled with a chemical illuminant reagent (aqueous solution of 0.02% luminol (5-amino-2,3-dihydro-1,4-phtalazinesodium) and 1% sodium hydroxide). The opposite chamber, fitted to the pulpal side of the dentin disc, is filled with an activator liquid (1% potassium ferricyanide and 0.3% hydrogen peroxide). Upon pressurizing the activator liquid, the solution passes through the dentin disc’s tubules to the illuminant-containing chamber and produces a luminescence reaction. This photo signal is recorded with a highly sensitive photo diode (S 9295, Hamamatsu Photonics, Hamamatsu, Japan), mounted 5 mm from the coverslip of the occlusal chamber. In order to prevent outer

**Results:** Permeability at the baseline and after albumin soaking did not significantly differ. For both desensitizing compounds, 30 and 60 seconds of active and passive applications resulted in significantly reduced dentin permeability. After the 15 second application, only the actively treated samples with GDP showed a significant reduction in permeability.

**Conclusions:** The liquid and the gel desensitizing agents both significantly reduced dentin permeability. The obvious advantage of a gel formulation is the controlled application, limited to the hypersensitive tooth area, thus avoiding inadvertent contact with adjacent gingival tissues.

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light interference with the weak luminescence signal, the entire equipment is enclosed inside a lightproof box. The output voltage of the photodiode is recorded with an AD converter at 1 kHz, stored in a central processing unit controlling the system, and transferred to a personal computer for data processing and analysis. The entire procedure is automated in a programmable sequencer.

**Specimen preparation and measuring procedures**

This trial was approved (Number 21–15) by the Ethical Committee of the Graduate School of Dentistry, Tohoku University, Sendai, Japan. Sixty non-identifiable, freshly extracted human third molars, free of decay and restorations, were used. Immediately after extraction, the teeth were frozen until further processing, for 2 weeks at the longest. Coronal 1.3 mm thick tooth slices were cut under copious water-cooling with a diamond wafer saw microtome (model SP 1600, Leica Microsystems Nussloch, Nussloch, Germany) perpendicular to the vertical tooth axis between the occlusal enamel portion and pulp horns. The cut sides of the specimens were cleaned with a 0.5 M EDTA solution (pH 7.4), applied using a soaked microbrush with a slight dabbing action during 60 seconds to remove the cutting smear and open the dentinal tubules prior to thorough rinsing with deionized water and slight air-drying. Dentin specimens were mounted between the chambers of a measuring device. Then the illuminant reagent liquid was injected into the occlusal chamber, and the activator solution was injected into the chamber fitted to the pulpal side. The activator solution was automatically pressurized for 2 minutes at 2.5 kPa, followed by a 2 minute pressure-free interval. The resulting photochemical signal was continuously registered. At the end of this first measuring cycle, the illuminant liquid was discharged, and new liquid was injected for the second pressurizing cycle under the same conditions. Then, a wash cycle with deionized water was automatically initiated before both chambers were filled again with the respective reagent solutions, pressurized in two consecutive runs with 13 kPa for 1 minute followed by a 1 minute pressure-free interval. This entire procedure for determining the baseline permeability was executed in duplicate for each dentin specimen. The areas under the output voltage lines during the pressurizing periods of the two cycles at each pressure were integrated (mV·s). Means of the four pressure runs at each of the two pressures applied served as measures for the dentin specimens’ baseline permeability.

Figure 1  Schematic illustration of the split-chamber device. The activator solution is pressurized from the apical side of the dentin disc. Upon penetration to the occlusal side and contact with the lumino-containing solution a photochemical signal is generated and recorded with a photodetector in mV.

Following the baseline permeability evaluation, specimens were removed from the split-chamber column; the pulpal sides were covered with a few droplets of 2% bovine albumin solution (albumin, from bovine serum, Cohn Fraction V, pH 5.2; Wako Pure Chemical Industries, Osaka, Japan). A vacuum-connected chamber was sealed with O-rings on the opposite specimen side for aspiration of the albumin solution into the dentinal tubules. The dentin samples were then rinsed with deionized water for 3 seconds prior to re-mounting in the split-chamber device at exactly the same position for the same duplicate pressurizing cycles as described above for the baseline characterization.

In the third test run with the same specimens, one of the desensitizing agents, GDL or GDP, was applied on the gently air-dried occlusal dentin side. GDL was applied with a soaked microbrush, while GDP was dispensed to the target area from a syringe fitted with a blunt needle tipped with a small brush. With both desensitizing agents, the effects of 15, 30 and 60 seconds of dwell times on dentin permeability were evaluated. During the dwell time, the agents were either left undisturbed (passive application) or slightly agitated with a microbrush (active application). Samples treated with GDL were air-dried with compressed air for 3–5 seconds, whereas dentin samples treated with GDP were rinsed with deionized water for 5 seconds and gently air-dried for 3 seconds. Subsequently, the dentin slices were re-mounted in the split-chamber device, and the permeability was measured using the same pressurizing cycles as described above for determining the baseline permeability and the permeability of the albumin-soaked specimens.

Specimen preparation and permeability measurement took place in an ambient laboratory atmosphere. Five samples were used for each of the six variable conditions tested and each desensitizing agent. The results were statistically analyzed by a non-parametric Kruskal-Wallis analysis of variance (ANOVA) and post-hoc Mann-Whitney test with statistical significance set to $P = 0.05$ (PASW Statistics 18.0 for Macintosh, Chicago, IL, USA).

**Results**

Figs. 2 and 3 show the mean values and standard deviations of the permeability of dentin specimens, challenged with 2.5 kPa hydrostatic pressure at the baseline, after albumin soaking, and after respective treatment with GDL and GDP. The Kruskal-Wallis ANOVA revealed that there were no significant differences among the six baseline groups or among the related albumin-soaked specimen groups, for specimens allocated to treatments with either GDL or with GDP. The ANOVA calculated for all baseline and albumin-treated samples showed no significant differences ($P = 0.899$ for GDL; $P = 0.749$ for GDP). After GDL application, 30 and 60 seconds of active and passive dwell times...
resulted in significantly reduced permeability compared to the baseline and albumin soaking, whereas a 15 second application showed no significant reduction in permeability. For GDP-treated samples, all groups except that with 15 seconds of passive application showed significant reductions in permeability compared to data at the baseline and after albumin soaking.

Figs. 4 and 5, respectively, show the permeability results for the same specimens as in Figs. 2 and 3, allocated to the GDL and GDP groups, after 13 kPa of pressurization for 1 minute. As with 2 minutes of 2.5 kPa of pressure, no significant differences were found among the baseline and albumin groups, or among the pooled baseline and albumin groups (P = 0.998 for GDL, P = 0.721 for GDP). Thirty and 60 seconds of active and passive dwell times produced significantly reduced permeability compared to the baseline and albumin soaking, whereas 15 seconds of application only showed a significant reduction in permeability for the active application mode of GDP.

Discussion

Although clinical studies are the ultimate proof of desensitizing agents’ efficiency, in vitro testing is a suitable alternative to acquire relevant screening results, provided the test setup simulates the conditions necessary for and are suitable for individual desensitizers’ modes of action. Desensitization of hypersensitive tooth areas implies inhibiting or hampering outward fluid flow from patent dentin tubules. Therefore, in vitro testing of human dentin samples should be used to evaluate their hydraulic conductance or permeability before and after application of a desensitizing agent. Use of freshly extracted third molars without decay or restorations, is presumably the best choice to ensure a reasonably homogeneous substrate, because such teeth are commonly removed from younger patients and their tubules are not yet obstructed.
In this study, we prepared coronal sections from extracted teeth, although hypersensitivity is commonly related to cervical tooth areas. However, several published reports confirmed that there was no significant difference between the permeability of occlusal and buccal dentin, or between tubule densities of occlusal and cervical dentin.

To determine the baseline permeability, the smear produced during diamond-blade cutting was removed from both sides of the specimens in order to simulate the condition of hypersensitive teeth, where tubules are patent at both ends. The physiologic pulp pressure is reportedly 15 cmH₂O (= 1.5 kPa). In the present study, we pressurized the dentin discs from the pulpal side with 2.5 kPa, which is close to the physiologic pressure, and additionally with 13 kPa to investigate the tubule-occluding effects of the desensitizing agents under extreme non-physiologic conditions. The results showed that 2 minutes of pressure at 2.5 kPa corresponded to the chemiluminescence output after 1 minute of pressure at 13 kPa. All pressurizing cycles were repeated in order to remove any possibly remaining reagent from the preceding run and to verify the first reading.

GA is supposedly the main component of GDL and GDP which is responsible for the tubular plugging effect seen after topical application. Among the many available protein cross-linking agents, GA, a fairly small molecule with two aldehyde groups separated by a flexible chain of three methylene bridges, rapidly reacts with several functional groups of proteins and is more efficient than other aldehydes in generating stable crosslinks. Knutsson et al quantitatively determined the release of plasma proteins in dentinal fluid from cavities prepared in healthy young human teeth. Albumin and immunoglobulin G (IgG) were found in all dentin samples, whereas fibrinogen was seen in only four of 16 dentin samples. The amount of serum albumin always exceeded IgG by a ratio of 10.5:1. Based on this analysis, it was reasonable to soak the dentin discs in albumin to prepare them to evaluate the effects of the desensitizing agents. Bovine albumin, selected for our experiments as the protein in the perfusion fluid, has a molecular mass of 66 kDa, low enough not to significantly reduce the permeability of dentin, in contrast to globulins and lipoproteins, that can cause marked reductions in permeability. The permeability of the albumin-soaked discs did not significantly differ from the same dentin discs at the baseline evaluation. This indicates that the 2% aqueous albumin solution used had no appreciable effect on the perfusion fluid’s viscosity.

Both GDL and GDP significantly reduced the permeability of the dentin discs to similar extents. Zero permeability was only registered in a few cases. Fluid flow through the dentinal tubules can appropriately be described by the Hagen-Poiseuille equation, where fluid flow through a capillary is proportional to the radius raised to the fourth power. Therefore, reducing the tubular diameter by one-half would result in a 16-fold lower hydraulic conductance. Hence, the striking reduction in dentin permeability found after GDL and GDP application may indicate that such fluid flow reduction in vivo might be adequate to eliminate or at least greatly reduce pain sensations perceived by patients.

The present data show that the reduction in permeability of albumin-soaked dentin was almost identical after GDL and GDP application, although it could be hypothesized that diffusion of the active desensitizer components from a gel might be lower compared to a liquid compound. Apparently, there is sufficient GA or GA and HEMA available at the dentin interface and to some depth inside the albumin-soaked tubules for coagulation and plugging of the tubules with proteins. In agreement with the manufacturer’s instruction for use, the dwell time of the desensitizing agents on dentin should be 30 or 60 seconds rather than 15 seconds, in order to obtain the most pronounced reduction in permeability. Although the mode of application had no practically significant effect on the in vitro permeability, we suggest using the active mode, i.e., moving the applied desensitizing agent gently throughout the dwell time with an application microbrush for GDL or the brush-tip of the application cannula for GDP, as slight agitation always enhances diffusion at interfaces.

The null hypothesis that there would be no difference in permeability reduction between applications of GDL or the GDP gel formulation is therefore accepted.

In summary, this in vitro investigation proved that GDL and GDP have similar efficiencies of reducing the permeability of human dentin. The obvious advantage of the gel formulation is well-controllable application to treatment sites, limiting the risk of inadvertent spread of the desensitizing agent to adjacent gingival tissues.

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References


