Vasorelaxant Effect of a Self-etch Adhesive System through Calcium Antagonistic Action

Feridun Basak, DDS, PhD, Ismail Mert Vural, MD, PhD,† Ertugrul Kaya, MD, PhD,† Cunay Ulku, MD, PhD,† Gunseli Guven, DDS, PhD,* S. Burcak Cebreli, DDS, PhD,‡ Zafer C. Cebreli, DDS, PhD,§ Melik Seyrek, MD, PhD,† and Oguzhan Yildiz, MD, PhD†*

Abstract

Etch-and-rinse adhesives can cause vasorelaxation via mechanisms occurring in the endothelium and smooth muscle, including the release of nitric oxide (NO). This effect might promote or aggravate bleeding if such adhesives are placed inadvertently on iatrogenic pulp microexposures. The present study assessed the vasoactive potential of a newer generation self-etch adhesive, Clearfil Protect Bond (CPB), on isolated rat aorta. Cumulative concentrations of the primer, bond, and mixture of CPB elicited concentration-dependent relaxation of phenylephrine-induced active tonus in the rat aorta, demonstrating that the tested self-etch adhesive can lead to vasorelaxation of pulp vessels that is mediated by Ca^{2+} antagonistic effect. The vasorelaxant effect of CPB or its components was mediated neither via endothelium-dependent NO and prostanoid-dependent mechanisms nor by K^+ efflux through K^+ channels. Mechanical removal of the endothelium did not significantly alter the relaxation induced by CPB. Assuming these data can be extrapolated to the clinical situation, CPB, either in mixed form or by its components, can lead to vasorelaxation of pulp vessels that is mediated by a Ca^{2+} antagonistic effect. If CPB is placed inadvertently on pulp microexposures during direct pulp capping, this effect might promote bleeding that might impair healing and, via plasma exatravasation, might compromise resin infiltration and polymerization. *(J Endod 2008;34:1202–1206)*

Key Words

Calcium antagonistic action, rat aorta, self-etch adhesive system, vasodilation

From the *Department of Pediatric Dentistry, Center of Dental Sciences, Gulhane School of Medicine, Ankara, Turkey; †Department of Medical Pharmacology, Gulhane School of Medicine, Ankara, Turkey; ‡Department of Pediatric Dentistry, Faculty of Dentistry, Baskent University, Ankara, Turkey; and §Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, Ankara, Turkey.

Address requests for reprints to Oguzhan Yildiz, MD, PhD, Department of Medical Pharmacology, Gulhane School of Medicine, Ankara, Turkey. E-mail address: oyildiz@gata.edu.tr.

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Resin-containing adhesives are widely used for bonding dental restorative biomaterials to replace lost tooth structure caused by dental caries, fracture, or abrasion. Adhesive resins have been shown to release unreacted monomers into the adjacent aqueous phase and diffuse through dentin to the pulp (1). These residual unpolymerized monomers might be responsible for pulp inflammation and necrosis, especially when adhesive resins are placed directly over a thin remaining layer of dentin (ie, <500 μm) or when placed inadvertently on iatrogenic pulp microexposures (2). In the latter scenario, tissue response to bonding resins might be even more severe (3, 4) because complete polymerization of adhesive is hampered as a result of the high oxygen tension (5) and humidity (ie, presence of blood, clot, and exudates) of the exposure site (6, 7).

Despite a plethora of structure-cytotoxicity evaluations published to date, very few studies have addressed the vasoactive potential of dental resin monomers, which might promote or aggravate pulpal hemorrhage (8–11). Recent studies have indicated that direct exposure of single-bottle etch-and-rinse dental bonding systems to vascular tissue can induce vasorelaxation via mechanisms occurring in the smooth muscle and endothelium in vitro (11). Tested separately, methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), and dimethylaminoethyl methacrylate (DMAEMA) have been shown to relax the isolated rat aorta (10–12).

Self-etch adhesives are a relatively newer generation of dentin bonding systems that do not require a separate etch and rinsing step and were developed to reduce technique sensitivity associated with etch-and-rinse adhesive systems (13). Clearfil Protect Bond (CPB) (Kuraray, Osaka, Japan) is a self-etch adhesive system that contains antibacterial monomers within its primer and adhesive components (14). With newer adhesives continuously being introduced for clinical use, their possible adverse effects on vasculature need to be evaluated. In this study, we examined the effects of the primer, bond, and mixture of CPB on rat thoracic aorta. We also evaluated the possible mechanism of its vascular action.

Materials and Methods

Twenty locally bred Sprague-Dawley male adult rats (weighing 250–300 g) were used in this study. The animals were cared for under controlled temperature (23.2°C) and humidity (55.5%), with a 12-hour light/12-hour dark cycle. They were fed standard laboratory food and given tap water ad libitum. The study protocol was approved by the institutional animal care and use ethics committee, which followed the Animal Welfare Act and National Institutes of Health guidelines for the care and use of laboratory animals (publication no. 86-23).

Thoracic aortae were obtained as described previously (3, 6). Briefly, thoracic aortae were dissected out, and the isolated aortae were carefully cleaned of adhering fat and connective tissue. Aortic ring segments (3 mm in length) were cut, after which the endothelium was denuded from 12 rings, and 12 rings were left with the endothelium intact to test whether mediators such as nitric oxide (NO) and prostacycline released from the endothelium are involved in vasoactive effect. The aortic rings were suspended in isolated tissue baths containing oxygenated 10 ml Krebs solution (composition in mmol/L: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4.7H_2O$, 1.2; K_2HPO_4, 1.2; 2.04; $NaHCO_3$, 25; and D-glucose, 10) at 37°C for isometric tension measurements. Tissues were tested for endothelium integrity with acetylcholine (10^{-6} mol/L) after contracting them

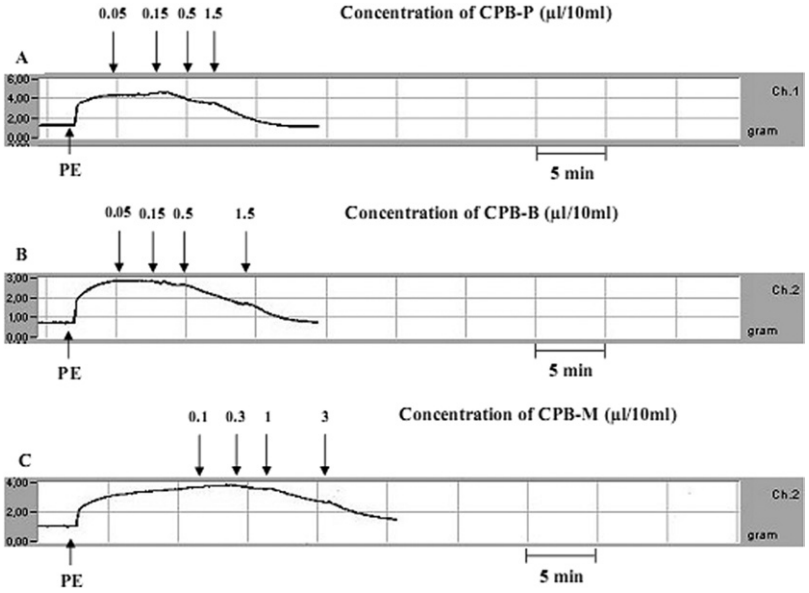

Figure 1. Original tracings of the relaxation effect of CPB components and its mixture. The cumulative relaxation to the primer (*A*), the bond (*B*), and the mixture (*C*) of CPB induced relaxation responses.

with phenylephrine (PE) (10^{-6} mol/L). Vasorelaxant effects of the primer, the bond, and the mixture of CPB were determined in rings precontracted with PE (10^{-6} mol/L). Possible contractile effects of these products were also tested on tissues maintained only under basal tonus.

In another set of experiments, arterial rings were challenged with 68 mmol/L KCl after the equilibration period to test the viability. Because a higher contraction indicated functional integrity of vascular smooth muscle, the rings that were contracted more than 1.5 g were included in the experimental protocol (n = 62). The tissues were washed every 10 minutes during an additional 30-minute waiting period. Tissues were tested for endothelium integrity with acetylcholine (10^{-6} mol/L) after contracting them with PE (10^{-6} mol/L). In endothelium-intact tissues, control relaxations to 4 different concentrations of the primer (0.05–1.5 µL/10 mL), the bond (0.05–1.5 µL/10 mL), and the mixture (0.1–3 µL/10 mL) of CPB were recorded. The mixture contained equal volumes of the primer and the bond. Then the rings were washed every 15 minutes for 1 hour. After the washing period, the rings were incubated with the cyclooxygenase (COX) inhibitor indomethacin (10 µmol/L), nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) (100 µmol/L), nonselective large conductance calcium activated K^+ channel (BK_{Ca}) and voltage-dependent K^+ channel (K_V) inhibitor tetraethylammonium (TEA) (1 mmol/L), adenosine triphosphate (ATP)–sensitive K^+ channel (K_{ATP}) inhibitor glibenclamide (GLI) (10 µmol/L), or K_V inhibitor 4-aminopyridine (4-AP) for 30 minutes; and after contracting the rings with PE (10^{-6} mol/L), relaxations to the test materials were repeated.

In different rings to investigate the Ca^{2+}-channel antagonistic effect of compounds, concentration-response curves to $CaCl_2$ (10 µmol/L–30 mmol/L) were obtained in the absence and in the presence of the primer (0.5 µL/10 mL), the bond (0.5 µL/10 mL), and the mixture (1 µL/10 mL) of CPB, and the L-type Ca^{2+}-channel blocker nifedipine (1 µmol/L), as described previously (15). After thoracic aorta rings were allowed to equilibrate for 30 minutes, the rings were washed 3 times at 10-minute intervals with Ca^{2+}-free Krebs solution containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA) (disodium salt). The rings were then bathed with Ca^{2+}-free (containing 1 mmol/L EDTA), high KCl (68 mmol/L) Krebs solution with or without the test solutions for 30 minutes. In vehicle-control experiments, absolute ethanol was added in the same volume as compounds. For comparison, nifedipine (1 µmol/L), instead of compounds, was assayed in adjacent segments in parallel.

Chemicals

KCl and TEA were purchased from Merck Co (Darmstadt, Germany); PE, EDTA, GLI, indomethacin, nifedipine, and L-NAME were purchased from Sigma Chemical Co (St Louis, MO). 4-AP was purchased from Acros Organics (Morris Plains, NJ).

TABLE 1. Relaxation Effect of CPB Components (0.05–1.50 µL/10 mL) and its Mixture (0.10–3.00 µL/10 mL) in Endothelium Intact and Denuded (−) Rings

Primer of CPB	0.05 µL/10 mL	0.15 µL/10 mL	0.50 µL/10 mL	1.50 µL/10 mL
Control	3.50 ± 1.40	5.67 ± 2.12	36.62 ± 4.04	87.05 ± 4.20
Endothelium (−)	1.25 ± 1.25	2.00 ± 2.00	28.57 ± 3.62	76.07 ± 8.96
Time control	3.07 ± 1.91	6.09 ± 3.95	37.68 ± 6.95	75.49 ± 10.95
Bond of CPB	0.05 µL/10 mL	0.15 µL/10 mL	0.50 µL/10 mL	1.50 µL/10 mL
Control	4.73 ± 1.84	9.26 ± 2.04	31.37 ± 5.20	93.02 ± 1.94
Endothelium (−)	3.16 ± 2.18	7.82 ± 4.18	38.49 ± 13.68	96.37 ± 2.33
Time control	2.28 ± 1.53	12.91 ± 5.99	26.89 ± 9.21	89.65 ± 4.02
Mixture of CPB	0.10 µL/10 mL	0.30 µL/10 mL	1.00 µL/10 mL	3.00 µL/10 mL
Control	2.60 ± 1.54	8.88 ± 2.52	53.66 ± 5.97	92.29 ± 1.41
Endothelium (−)	0.00 ± 0.00	5.72 ± 2.06	42.66 ± 9.62	98.61 ± 1.20
Time control	0.00 ± 0.00	4.95 ± 2.17	46.15 ± 8.55	90.88 ± 3.02

CPB, Clearfil Protect Bond.

NOTE. Data are expressed as % relaxation of tone. Each value is expressed as mean ± SEM.