Vasorelaxant Effect of Resin-Based, Single-Bottle Dentin Bonding Systems

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Abstract

Single-bottle dentin bonding systems are currently in wide use. Because these materials are sometimes inadvertently placed on microscopic pulp exposures while at other times deliberately on frank exposures, their effects on pulpal soft tissues need to be evaluated. The present study assessed the vascular effects of 3M Single Bond (3MSB) and Prime & Bond NT (PBNT), using rat aortic ring preparations. It is hypothesized that these bonding agents induce relaxation of these preparations. Both 3MSB and PBNT caused endothelium-dependent and -independent relaxations in a concentration-dependent manner. The endothelium-dependent relaxation was associated with the release of nitric oxide. However, the responses to both agents did not involve the generation of prostanoids or K_{ATP} channel activation. At relatively low concentrations, the responses of endothelium-denuded tissues to 3MSB were greater than those to PBNT, indicating certain differences in the vascular action between these products. The data suggest that 3MSB and PBNT interfere with vascular function by causing vasorelaxation via mechanisms occurring in the smooth muscle and endothelium, including the release of nitric oxide. Among others, this effect may promote bleeding if these adhesives are placed on pulp exposures.

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The increasing use of resin-based adhesive materials in dentistry has raised the question of their compatibility with oral tissues. Some resin components have been shown to be bioactive in the vasculature. Recently, we demonstrated that methyl methacrylate (MMA), hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), and dimethylaminoethyl methacrylate (DMAEMA) relaxed the isolated rat aorta (1, 2).

Single-bottle dentin bonding systems containing mixtures of primer and adhesive resins together with organic solvents are currently in wide use. These products may sometimes inadvertently be placed on microscopic pulp exposures while at other times deliberately on frank exposures (3) during restorative treatment. Previous investigators examined the effects of the dentin bonding systems, Prime & Bond NT (PBNT), Prime & Bond 2.1, Syntac Single Component and Single Bond, on the reactivity of the isolated rat carotid artery (4, 5). All the products, except Single Bond, induced contraction of the tissue. Single Bond did not elicit any response. However, these results do not provide information on the mechanisms of action of the products, and are contrary to the results of previous studies testing individual resin components on the rat aorta (1, 2).

In the present investigation, we examined the effects of the single-bottle dentin bonding systems, 3M Single Bond (3MSB) and PBNT, on the reactivity of the rat aorta and the possible mechanisms involved in their actions. Based on our previous data (1, 2), it is hypothesized that these bonding agents produce relaxation of this tissue.

Materials and Methods

Dentin bonding products used in the present study are shown in Table 1. Drugs and nitrite assay chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Thoracic aortas were obtained from male Wistar-Kyoto rats (350–400 g, Harlan Laboratories, Indianapolis, IN), as described previously (1, 6). Aortic rings 3 to 5 mm long were prepared, and one half of the rings had the endothelium denuded while the remaining rings were left with endothelium intact (1, 6). The aortic rings were suspended in isolated tissue baths containing oxygenated Krebs solution at 37°C for isometric tension measurements as previously detailed (1, 6). Tissues were tested for endothelium integrity with acetylcholine (Ach, 10^{-6}M) after contracting them with norepinephrine (NE, 10^{-7}M) (1). Vasorelaxant effects of the single-bottle dentin bonding products, 3MSB and PBNT, were determined is rings contracted with NE (10^{-7}M). Possible contractile effects of these products were also tested on tissues maintained only under basal tension. Possible responses of aortas to the nonmethacrylate adhesive system components, ethanol, water, acetone, camphorquinine, and 4-methoxyphenol, were also evaluated. After dentin bonding agent treatment, tissue viability was assessed by contracting them with NE (10^{-7}M), followed by relaxation with Ach or sodium nitroprusside (SNP).

To investigate possible mechanisms of vasorelaxation induced by the dentin bonding products, aortic ring responses were obtained after treatment with the nitric oxide synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME, 10^{-5}M), the prostanoid synthesis inhibitor, indomethacin (10^{-5}M) and the ATP-sensitive K^+ (K_{ATP}) channels inhibitor, glybenclamide (10^{-6} M) (1). Tissues were incubated with these inhibitors for 20 min before adding NE and then dentin bonding products (1, 2).

Dentin bonding product-induced generation of nitrite was assayed in endothelium-intact aortic rings following the methods previously published (7). Tissues equilibrated in oxygenated Krebs solution were incubated with 10^{-7}M NE alone or in com-

TABLE 1. Compositions of single-bottle dentin bonding agents used in the study.

Bonding agent	Composition
3M Single Bond (3MSB), 3M Dental Products, St. Paul, MN	Bis-GMA, HEMA, glycerol dimethacrylate, UDMA, polyalkenolic acid, copolymer (MW 20,000) ethanol, water
Prime & Bond NT (PBNT), Dentsply Caulk, Dentsply Int. Inc., Milford, DE	Di- and trimethacrylate resins, PENTA, nanofillers (amorphous silicone dioxide), photoinitiators, stabilizers, cetylamine hydrofluoride, acetone

Abbreviations: Bis-GMA = 2,2-bis(4-2-hydroxy-3-methacryloyloxyproproxyphenyl) propane; HEMA = 2-hydroxylethyl methacrylate; UDMA = urethane dimethacrylate; PENTA = dipentaerythritol penta-acrylate monophosphate.

bination with either 3MSB or PBNT (0.04 μl/ml) for 20 min at 37°C. The incubation medium was removed and assayed for nitrite concentration using the Griess reaction (7, 8).

Relaxant responses to 3MSB and PBNT were calculated as the percentage of reduction in tension produced by NE. Maximum responses and the amounts of dentin bonding products eliciting 50% of the maximum responses (EC_{50} values as μl/ml) were determined from concentration-response curves (1, 6). Nitrite concentrations are expressed as μM/g of tissue weight. Data are reported as mean ± SEM. Two-way ANOVA was employed to determine differences between concentration-response curves for aortas with and without endothelium (1, 6). The data between groups were compared using Student's *t* test (1, 6, 9) and differences were considered significant when $p < 0.05$.

Results

Relaxant Responses to 3MSB and PBNT

3MSB and PBNT did not alter basal tension of the rat aortic rings with or without endothelium, up to concentrations that produced maximum vasorelaxation in NE-contracted tissues ($n = 4$; data not shown). In the presence of L-NAME, indomethacin or glybenclamide, the dentin bonding agents did not also change basal tension of the tissues ($n = 4$; data not shown).

In NE-contracted endothelium-intact rings, both 3MSB and PBNT elicited concentration-dependent relaxation, with 100% maximum response (Fig. 1*A*, *B*). As assessed by EC_{50} values and the maximum relaxation, in the presence of endothelium, both products produced effects that were not significantly different ($p < 0.05$) from each other (Table 2).

Removal of the endothelium resulted in lack of relaxation to Ach, demonstrating absence of functional endothelium ($n = 12–18$; data not shown). Both 3MSB and PBNT relaxed the endothelium-denuded rings in a concentration-dependent manner with a maximum relaxation of nearly 100% (Fig. 1*A*, *B*). The responses to sub-maximum concentrations of both agents were reduced compared to the corresponding responses obtained in the intact vessels (Fig. 1*A*, *B*; Table 2). Comparison of responses revealed that while the EC_{50} values and maximum relaxation of the denuded aortas to 3MSB and PBNT were similar (Table 2), responses to several lower concentrations (≤0.01 μl/ml) of 3MSB were more pronounced compared to corresponding responses to PBNT (percent of relaxation to 3MSB versus PBNT for 0.0005, 0.001, 0.005, 0.01 μl/ml = 5.3 ± 1.7, 15.7 ± 2.5, 23.4 ± 4.3, 35.8 ± 4.3 versus 0.9 ± 0.3, 3.8 ± 0.8, 15.1 ± 1.2, 25.6 ± 1.6, respectively; $n = 6–8$; $p < 0.05$).

The nonmethacrylate compounds, ethanol, camphorquinone, 4-methoxyphenol, acetone and water, did not produce measurable vascular effects individually or in combination ($n = 4$; data not shown).

Effects of L-NAME, Indomethacin and Glybenclamide on Relaxant Responses to 3MSB and PBNT

The relaxant responses of endothelium-intact aortas to sub-maximum concentrations of both 3MSB and PBNT were significantly inhibited ($p < 0.05$) by L-NAME (10^{-5}M) (Fig. 1*C*, *D*). However, tissue responses to both bonding agents were not significantly affected by indomethacin (10^{-5}M) or glybenclamide (10^{-6}M) in the presence as well as absence of the endothelium ($n = 4$; data not shown).

3MSB and PBNT-Induced Nitrite Production

Both 3MSB and PBNT at 0.04 μl/ml concentration caused significant ($p < 0.05$) enhancement in nitrite production in endothelium-intact aortic rings compared to basal values (Fig. 1*E*). However, no significant differences were observed between the dentin bonding products in nitrite production.

Discussion

The results demonstrate that both 3MSB and PBNT induced concentration-dependent relaxation of NE-contracted rat aortas in presence and absence of endothelium, supporting our hypothesis. However, the responses to several sub-maximum concentrations of the products were greater in presence of endothelium, indicating that the bonding agents produce endothelium-dependent and -independent relaxations of this vascular preparation. The greater relaxations in endothelium-denuded aortas in response to lower concentrations (≤0.01 μl/ml) of 3MSB relative to the effect of PBNT suggest that the aortic smooth muscle is more sensitive to the action of 3MSB. It is believed that the vasorelaxant effects generated by both bonding agents were not caused by toxicity because (a) the responses of the tissues were reproducible and (b) the responses to NE, Ach or SNP were not altered by exposure of tissues to both products up to three hours or after washout. Neither 3MSB nor PBNT altered basal tension of the aortas suggesting that these products do not cause contraction of the tissue on their own nor do they relax it under basal conditions.

The nitric oxide synthase inhibitor, L-NAME, attenuated the relaxant responses of endothelium-intact, but not denuded, rings to 3MSB and PBNT. This observation suggests that at least part of the responses produced by these agents in intact tissues is mediated via endothelium-derived nitric oxide (1, 10–12). This is supported by our finding of increased production of nitrite in response to both products. On the other hand, lack of inhibition by L-NAME of the relaxation elicited by the bonding products in denuded rings and absence of increased nitrite production in these tissues indicate that smooth muscle cell-derived nitric oxide is not involved in the relaxant responses of the rat aorta to these dental products. We also observed that neither indomethacin nor glybenclamide altered the vasorelaxant effects of 3MBS and PSNT. This finding suggests that the responses to both products are not mediated via prostanoids or K_{ATP} channels (13, 14).

The underlying mechanism for the action of nitric oxide released from the endothelium is believed to involve activation of soluble guanylate cyclase in the smooth muscle. This process generates cGMP that results in reduced intracellular calcium and dephosphorylation of myosin light chain kinase (15, 16). Thus, the endothelium-dependent responses observed with 3MSB and PSNT were likely to be related to this mechanism. In the absence of prostanoid generation and K_{ATP} channel activation, relaxation of the aortic smooth muscle induced by the bond-