Effect of Er:YAG Laser on Bond Strength to Dentin of a Self-Etching Primer and Two Single-Bottle Adhesive Systems

Renata P. Ramos, DDS,* **Daniela T. Chimello,** DDS, **Michelle A. Chinelatti,** DDS, **Tomio Nonaka,** DDS, MS, PhD, **Jesus D. Pécora,** DDS, MS, PhD, **and Regina G. Palma Dibb,** DDS, MS, PhD
Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo (USP), Av. do Café, S/N Monte Alegre, Cep: 14040-904, Ribeirão Preto-SP, Brasil

Background and Objectives: To assess the effect of erbium:yttrium aluminium garnet (Er:YAG) laser on bond strength to dentin of a self-etching primer (Clearfil Liner Bond 2V, CL2V) and two single-bottle agents (Excite, EX; Gluma One Bond, GB).
Study Design/Materials and Methods: Thirty human molars were selected, roots were removed and crowns were bisected, providing 60 halves. Specimens were included and ground to expose dentin. Bonding site was limited and samples were assigned to three groups: I, CL2V; II, EX; III, GB. Dentin was either conventionally treated or submitted to laser conditioning + conventional treatment. The adhesive protocol was performed, samples were stored for 24 hours and bond strength was tested to failure (0.5 mm/min).
Results: Statistical analysis showed a decrease in bond strength for lased subgroups and this drop was more evident for EX. CL2V provided the best overall results, regardless of the surface treatment.
Conclusions: Er:YAG laser may adversely affect bond strength in higher or lesser degree, depending on the adhesive system used. Lasers Surg. Med. 31:164–170, 2002.

Key words: adhesion; laser irradiation; surface treatment; dentin

INTRODUCTION

In the last years, an expressive number of studies [1–8] have been focused on investigating the efficiency of erbium:yttrium aluminium garnet (Er:YAG) laser for potential dental applications, such as removal of carious lesions, cavity preparation, surface conditioning, endodontic procedures, and other. Emitted at a 2.94-μm wavelength, Er laser is strongly absorbed by water and is well absorbed by hydroxyapatite. The ablation of tooth structure is achieved via a thermomechanical interaction and since the tissue is not completely vaporized, but only disintegrated into fragments, the majority of incident radiation is consumed in the ablation process, leaving very little residual energy for adverse thermal interactions to the pulp and surrounding soft and/or hard structures [1,9].

Additionally, the irradiation of enamel and dentin surfaces with Er:YAG laser prior to the accomplishment of adhesive procedures has been widely investigated [2,6,9–14]. However, the review of existing literature shows controversial results as to the effect of lasing tooth structure before bonding of composite materials. Several parameters, such as output energy, frequency, pulse mode, focal length, irradiation time, and refrigeration during irradiation, play significant roles in the response of dental tissues to Er:YAG laser, determining the intensity of ablation process and hence the ability of laser to roughen dental surface and produce the microretentive structure which will be further infiltrated by the resin bonding agent.

In great part of currently available adhesive systems, primer and bonding agent are combined in a single bottle, in an attempt to reduce the number of handling steps and make the applicability of such products more practical than that of multiple-bottle bonding agents [15]. Although commonly marketed as "one-step" systems, indeed most single-bottle agents require a separate acid conditioning/rinsing step and the application of several coats of primer/adhesive. Recent developments in adhesive dentistry have reintroduced the concept of utilizing the smear layer as a bonding substrate, but this time with novel insights and improved formulations that can etch beyond the smear layer into the underlying dentin matrix [16]. This promising approach to adhesion in dentin substrate involves the use of acidic primers, which combine the acid etching step with the priming procedure. The goal of the so-called self-etching primers adhesive systems was to simplify the bonding procedure and to reduce the sensitivity technique of adhesive protocol by eliminating the acid-conditioning step [17–19].

Renata P. Ramos is a post-graduate student; Daniela T. Chimello, a post-graduate student; Michelle A. Chinelatti, a post-graduate student; Tomio Nonaka, a professor; Jesus D. Pécora, Professor and Chairman of the Department of Restorative Dentistry; and Regina G. Palma Dibb; Professor at Ribeirão Preto School of Dentistry University of São Paulo.
Grant sponsor: CAPES; Grant sponsor: CNPq; Grant number: 300.886/98-5.
*Correspondence to: Renata P. Ramos, DDS, Faculdade de Odontologia de Ribeirão Preto/USP, Departamento Odontologia Restauradora (Mestrado-Dentística), Av. do Café, S/N Monte Alegre, Cep: 14040-904, Ribeirão Preto-SP, Brasil.
E-mail: virtualr@netsite.com.br
Accepted 28 June 2002
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.10106

However, it is important to emphasize that all adhesive materials and procedures were originally developed to act on tooth substrate prepared and treated by rotatory cutting instruments and conventional techniques. Therefore, in view of the increasing use of laser devices in dental practice, it is mandatory to assess the performance of the different adhesive systems (taking into account intrinsic features inherent to composition, adhering mechanism, and required pretreatment) on irradiated surfaces, particularly on dentin substrate due to its well-known physiological dynamism, heterogeneous composition, and complex structure. Since the appearance of lased dentin surfaces has been shown to differ morphologically from that of conventionally treated and considering the great variability in formulation, characteristics and acting mode of commercially available bonding agents, it is of extreme interest for adhesive dentistry to investigate the interaction of laser-treated surface with the adhesive materials in order to yield an optimized bonding protocol.

Considering that, the aim of this study was to assess the effect of Er:YAG laser irradiation on bond strength to dentin of a self-etching primer adhesive system and two single-bottle bonding agents.

MATERIALS AND METHODS

Thirty sound human molars, extracted within a 6-month period and stored in thymolized 0.9% saline solution at 4°C, were selected and cleaned with scaler and water/pumice slurry in dental prophylactic cups. Roots were sectioned 2 mm below the amelocemental junction and crowns were bisected longitudinally in a mesiodistal direction with a water-cooled diamond saw in a sectioning machine (Minitom, Struers A/S, Copenhagen, DK-2610, Denmark), providing a total of 60 halves. Surfaces were identified to avoid that both buccal and lingual halves of the tooth were assigned to the same experimental group. Halves were individually embedded in polyester resin using polyvinyl chloride (PVC) cylinders (2.1 cm diameter; 1.1 cm high) and, after polymerization, specimens were ground under water refrigeration in a polishing machine (Politriz, Struers A/S, Copenhagen, DK-2610, Denmark) using #120- to #400-grit silicon carbide (SiC) paper to remove the overlying enamel and expose flattened, smooth middle dentin surface. Complementary grounding was accomplished with #600-grit SiC paper for 30 seconds to produce and standardize the smear layer.

To delimit the dentin-bonding site, a small piece of insulating tape with a central hole was attached to specimen surface. The tape perforation was made by means of an Ainsworth rubber-dam punch modified to provide 3-mm diameter holes. The limitation of bonding area has a double aim: to define a fixed test surface, so that calculations of bond strength will be solely related to the evaluated area; to ensure that the restorative material would be further correctly placed thus forming the resin cone exactly on the desired site since it is difficult to visualize the area to be evaluated, once the matrix is placed.

The 60 specimens were randomly assigned to three groups of equal size, according to the adhesive system used: I, Clearfil Liner Bond 2V (CL2V); II, Excite (EX); III, Gluma One Bond (GB). The tested materials with their compositions, specifications, and manufacturers are listed on Table 1. Then, each main group was divided into two subgroups ($n = 10$), according to the surface treatment accomplished: (A), in which the bonding site was conventionally treated; (B), in which dentin was previously treated by a short pulsed Er:YAG laser emitted at a 2.94 μm wavelength (Kavo Key Laser 2, Kavo Co., Biberach, Germany) and then the conventional treatment was accomplished. The irradiation was performed for 10 seconds on defocused mode, with 80 mJ output energy and 2 Hz frequency, at a distance of 17 mm, with fine water mist at 15 ml/min. Laser beam spot size was 0.63 mm and a handpiece (2051) with a removable tip attached to flexible fiber delivery system was used. The experimental groups are displayed on Table 2.

The adhesive protocol was performed following the manufacturers' instructions. The tested adhesive systems were carefully applied on the limited dentin surface with disposable brush tips to avoid excess and pooling of adhesive along the edges of the insulating tape, which could compromise the distribution of tensions during the test and hence the validity of results. For group I (A and B), CL2V self-etching primer adhesive system was used. Equal drops of primer liquids A and B were dispensed into a well of the mixing dish and mixed immediately before application. The resultant mixture was applied to dentin, left undisturbed for 30 seconds and gently air-dried to evaporate the volatile ingredients. No subsequent rinsing was accomplished. Then, bond liquid A was applied to the entire surface, slightly thinned with a mild oil-free air stream and light-cured for 20 seconds, with a visible light curing-unit (XL 3000, 3M Dental Products, St. Paul, MN) with a 450 mW/cm^2 output. Group II (A and B) was bonded with EX, an ethanol-based, single-bottle bonding agent. Dentin surface was etched with a 37% phosphoric acid gel (Total Etch, Vivadent Ets., FL-9494, Schaan, Liechtenstein) for 10 seconds, rinsed and gently dried with absorbing paper to remove excess water and keep tooth surface moist. EX was applied to dentin with a light scrubbing motion for 10 seconds. Next, the remaining solvent was evaporated with a brief, mild air-blast and bonding agent was light-cured for 20 seconds. For group III (A and B), the adhesive protocol was accomplished using an acetone-based, single-bottle bonding agent (GB). The limited bonding area was entirely etched with a 20% phosphoric acid gel (Gluma Etch 20 gel, Heraeus Kulzer, Inc., South Bend, IN) for 20 seconds, rinsed thoroughly for 20 seconds and gently dried with absorbing paper. Bonding agent was applied to etched surface, left for 10 seconds, and a second coat was richly applied. GB was spread over with a gentle air-stream to evaporate the solvent and residual moisture and light-cured for 20 seconds.

After bonding procedure, specimens were individually fixed in a metallic clamping device (developed by Houston Biomaterial Research) in such way that the dentin site remained parallel to a flat surface. A split bisected Teflon® matrix was positioned over the tooth/resin block resulting in an inverted conical cavity with the smaller diameter

TABLE 1. Tested Materials

Material	Gluma One Bond	Excite	Clearfil Liner Bond 2V	Filtek-Z250
Type	Light-curing, single component bonding agent	Filled, light-curing, single component bonding agent	Filled, light-curing self-etching primer adhesive system	Light-curing, hybrid composite resin
Principle ingredients	HEMA, 4-META, UDMA, acetone	HEMA, dimethacrylates, phosphonic acid acrylate, highly dispersed silicon dioxide, alcohol solution	Primer-HEMA, MDP[a], water; Bond A-MDP[a], BIS-GMA, HEMA; silanated colloidal silica	BIS-GMA, UDMA, BIS-EMA, aluminum oxide, zircon/silica
Batch number	135603	C08332	Pr A-00064B, Pr B-00065B, Bond A-00105B	1LY
Manufacturer	Heraeus Kulzer, Inc., South Bend, IN, 46614	Vivadent Ets, FL-9494, Schaan, Liechtenstein	Kuraray Co. Ltd., 530-8611, Osaka, Japan	3M, Dental Products, St. Paul, MN

[a]10-methacryloyloxydecyl dihydrogen phosphate.

coincident to the delimited bonding area (Ø 3 mm). A hybrid light-curing composite resin (Filtek Z250, 3M Dental Products) was inserted into the matrix in three increments, polymerized for 40 seconds each. As the matrix cavity was completely filled, the specimen was removed from the clamping device and the matrix was opened and separated, leaving adhered to the limited dentin surface an inverted, composite resin cone with a 6 mm diameter tapering to a 3 mm diameter and 4 mm high.

After 24-hour storage in distilled water at 37°C, the cone-shaped composite/polyester resin block was placed into an apparatus with an internal taper, which corresponds to the shape of the specimen. This configuration was loaded in tension bond using an Universal Testing Machine (Mod. MEM 2000, EMIC Ltd., SJ Pinhais-PR, Brazil), at a cross-head speed of 0.5 mm/min and a 50 kgf load cell until fracture. Bond strength values were calculated in kgf/cm^2 and converted into MPa. Means and standard deviation were determined. Bond strength data were analyzed by two-way analysis of variance (ANOVA) using a factorial design with bonding agent and surface treatment as independent variables. Means were compared to Tukey interval calculated at the 0.05 significance level.

Dentin-bonding areas were viewed under a stereoscopic optical magnifier (40 ×) to assess the type of failure. *Adhesive* failure was considered the one at the specimen/adhesive interface, *cohesive* failure occurred in the material or the substrate, with no damage to the interface, and finally *mixed* failure was the one involving at the same time the interface and the material. Bond failure sites were not statistically analyzed.

RESULTS

Bond strength means and standard deviation are shown on Table 3.

The analysis of data showed a decrease in bond strength when dentin surface was treated by Er:YAG laser irradiation prior to accomplishing the adhesive procedure ($P < 0.05$), and it was more evident for EX.

Comparing the tested adhesive systems, regardless of the surface treatment, CL2V yielded the highest bond strength mean and was statistically superior to the other groups ($P < 0.05$). Considering the interaction between the bonding agents and the surface treatment accomplished, it was observed that CL2V applied on conventionally treated dentin reached the best overall results while the use of EX on Er:YAG lased dentin resulted in the lowest bond strength mean.

Failure after testing mostly occurred involving at the same time the bonding interface and the adhesive system/composite resin (*mixed* failure) except for the non-lased CL2V subgroup, which showed *cohesive* failures in composite for the great majority of specimens (60%).

DISCUSSION

The ability of Er:YAG laser to effectively ablate dental hard tissues is ascribed to its 2.94-μm wavelength emission, which is coincident to the main absorption band of water

TABLE 2. Experimental Groups

	Adhesive systems		
	CL2V (I)	EX (II)	GB (III)
Surface treatment	(IA): self-etching primer (IB): Er:YAG laser + self-etching primer	(IIA): acid etching (IIB): Er:YAG laser + acid etching	(IIIA): acid etching (IIIB): Er:YAG laser + acid etching

(~3.0 μm) and is also well absorbed by OH^- groups in hydroxyapatite [1,9,10]. Due to the great water content in its composition, dentinal substrate is a target tissue with a stronger interaction with Er:YAG laser irradiation. The incident radiation is highly absorbed by water molecules in dentin components and structures, mainly the intratubular fluid and collagen network, leading to sudden heating and water evaporation. The resulting high-stream pressure leads to the occurrence of successive microexplosions with ejection of tissue particles, which are characteristic of the ablation process and determine the microcrater-like appearance of lased surfaces.

Nevertheless, the potential impact of the Er:YAG laser on collagen network has not yet been clearly disclosed. It remains unclear if the microstructural alteration and microrupture of collagen fibers caused by laser irradiation could actually compromise the interaction of adhesive systems with lased dentin substrate, which would inherently affect the resulting bond strength. This speculation is based on the fact that the major mechanism of bonding to dentin surface relies directly upon the entanglement of hydrophilic monomers to the exposed collagen web and thereby depends on the availability and integrity of the fiber mesh. Therefore, as the literature has strongly emphasized [20,21], if the structure of the collagen net somehow collapses or is altered, the penetration of primer monomers and hence the adhesive protocol is hampered and incomplete.

Furthermore, it is also important to highlight the action of laser irradiation on the mineral components of dentinal substrate. Although SEM observations have revealed that dentin surface treated by Er:YAG laser shows very little or absent smear layer and open dentinal tubules, the laser does not effectively act on peritubular dentin and hence is not able to enlarge tubules' openings [4,7,22–24]. Additionally, several studies refer to zones that show a significant change in the structure or morphology of mineral crystal following laser exposure. There have been reports on various patterns of surface microirregularities (often accompanied by microfissure propagation), besides few fusion and recrystalization areas [2,7,14,22,24–26]. That peculiar morphology of lased surface, resulting from the thermomechanical ablation process, could interfere negatively with the acid-reactivity of dentin substrate, as the etchant agents or acidic monomers may not be as efficient at dissolving the mineral component of the superficial layer of lased dentin.

In this context, the findings of the conducted research seem to corroborate the fore-mentioned assumption that the Er:YAG laser may exert an adverse effect on both the mineral and collagen components of dentin and would thereby impair or affect to some degree the optimal interaction of adhesive systems to laser-etched substrate. The results of the present study disclosed that conditioning dentin surface with Er:YAG laser prior to the accomplishment of the adhesive protocol resulted in decreased bond strength, as compared to the conventional treatment. Indeed, the real benefits and possible disadvantages of Er:YAG laser etching remain inconclusive and controversial. Bond strength to dentin has been found to be higher after Er:YAG laser etching than after acid conditioning [2]. However, hydroxyapatite fusion and sealing at craters surface (probably resulting from fragments that were not ejected during ablation and got fused to tooth structure) are reported to give rise to poor adhesion [27]. Furthermore, it has been claimed that dentin treated by laser shows an extensively cracked and slightly denatured subsurface layer, which does not appear to be favorable to adhesion [22].

An earlier investigation, in which the differences in bonding to acid-etched and Er:YAG-lased tooth surface were analyzed, draw the conclusion that the research hypothesis of an expected similar or better adhesive force after laser irradiation was not valid and that laser treatment provided some deleterious effects on tissue surface that could have weakened the adhesion [14]. According to those authors, when bonding to laser-etched dentin, the scattering of values around the mean was very marked. A

TABLE 3. Bond Strength Means (MPa) to Dentin for the Experimental Groups and Subgroups

	Adhesive systems		
Subgroups	CL2V (I)	EX (II)	GB (III)
Non-lased (A) means (MPa) and SD	21.62 (±5.28)**	19.45 (±3.84)**	16.30 (±3.56)*
Lased (B) means (MPa) and SD	19.20 (±2.99)**	15.02 (±2.21)*	15.52 (±5.28)*

Same symbols indicate statistical similarity.

possible explanation for such results would be that the greater heterogeneity of lased substrate (mostly arising from the union of microcraters derived from laser procedure) coupled with the less uniform etching pattern produced by irradiation, resulted in a structure unsuitable for adhesion. Previous studies reported that Er:YAG lasing of dentin leads to fractures and fissures not fully impregnated by the bonding resin during the adhesive technique, thus creating a weakened zone just below the interface [22,28].

The concept of infiltrating the hydrated demineralized dentin matrix with a hydrophilic resin capable of interweaving with the exposed collagen network has been supported by several studies [20,21,29] as being the principle to achieve appropriate dentin bonding. Acid etching opens and widens the orifices of dentinal tubules, increases intratubular dentin exposure/permeability, and demineralizes intertubular dentin, reportedly to a depth of 7 μm [30]. The subsequent infiltration and polymerization of the monomer results in a resin–dentin inter-diffusion zone or hybrid layer, which is believed to be the source of bonding to acid-etched dentin [20,30]. Unfortunately, there is still little information available concerning the mechanism of resin adhesion to lased dentin. The formation of a well-defined inter-diffusion zone similar to that described for conventionally acid-etched dentin seems to be unlikely. In view of the anfractuous nature of laser-etched surface and the presence of open dentinal tubules, a micromechanical retentive mechanism is likely to be more effective [2]. According to Aoki et al. [22], this approach raises the assumption that bond strength to lased dentin appears to develop mainly from penetration of resin tags into dentinal tubules.

The findings of an earlier investigation [31] showed that Er:YAG laser irradiation followed by acid-etching led to the formation of hybrid layer and resin tags equivalent in size and depth to those obtained with acid-conditioning only. Dentin surfaces solely lased showed no hybrid layer formation and the tags were much thinner as compared to other groups. Nevertheless, no significant difference was observed among the tested groups regarding bond strength to dentin. According to the authors, a reasonable explanation for this similarity would be that, although laser did not lead to a hybrid layer formation, the adhesive system reached the open dentin tubules and superficial micro-cracks resulting from irradiation, thus providing good micromechanical retention even without subsequent acid conditioning.

It has been reported that the depth of the demineralized intact dentin is related to the concentration of the acidic monomer in the primer and the application time of primer [32]. The overall acidity of a primer mixture will determine its self-etching potential. In this regard, a minimum pH value would be required for any primer solution to counteract the buffering capacity of both the smear layer and intact dentin before it can etch beyond those substrates. The self-etching primer system tested in the conducted study exhibits in its composition 10-methacryloyloxydecyl dihydrogen phosphate (MDP), an acidic phosphate resin monomer that replaced the phenyl-*P* from the original formulation [16]. The acidity of priming solution (with a 2.8 pH), although less than that of 37% phosphoric acid gels, is sufficient to etch through the smear layer into the underlying dentin.

That improved etching potential of the non-rinsed, self-etching primers coupled with the application of a polymerizable adhesive layer resulted in the formation of a thin hybridized complex, comprising a hybridized smear *layer* and a true hybrid layer. Although the *hybridized complex* is usually thinner than the hybrid layer formed by adhesive agents using phosphoric acid etching (generally less than 2-μm thick) studies have reported that self-etching primer systems produce relatively high bond strength to dentin [33,34]. Tay et al. [16], in a recent investigation with a self-etching primer (Clearfil SE Bond), showed that the thickness of the underlying true hybrid layer remained reasonably consistent, despite great variation in thickness of the surface hybridized smear layer (0–4.1 μm). The forementioned authors [16] support that this thin layer is possibly mainly responsible for the high bond strength produced by this adhesive system, which may indicate that hybrid layers do not have to be thick to be strong, as long as the collagen fibrils are optimally infiltrated by resin. No correlation between hybrid layer thickness/morphology and bond strength could be found [15,35].

Nevertheless, in the present work, we did not evaluate the presence or absence of a hybrid layer in any of the groups. Indeed, the optical technique used for characterizing bond failure does not provide the resolution necessary to observe a hybrid layer and assess its thickness and quality. Due to this limitation, drawing any conclusion about the role of hybrid layer as contributing factor to the bond strength values recorded in this investigation would be imprecise and unreliable.

In the reported research, the self-etching primer adhesive system (CL2V) showed overall improved bond strength values over the self-priming adhesives (EX and GB) on both lased and non-lased subgroups. Additionally, an expressive amount of *cohesive* failures (80%) was observed for conventionally treated specimens. A suitable explanation for such performance would be that self-etching primers are generally less technique-sensitive than the bonding agents that require a separate acid conditioning/rising step. The acidic monomer of self-conditioning systems demineralizes superficially dentin surface by partially dissolving mineral crystals around the collagen fibrils and simultaneously permitting monomer infiltration, as the demineralizing component of the primer is also the infiltrating resin [16]. Since the tooth surface is not rinsed following treatment with acidic primers, there is no loss of mass from dentin. This is thought to minimize the susceptibility to shrinkage or collapse of the collagen network [36], thereby providing optimal bonding.

In the conducted research, GB showed lower bond strength values than EX for the conventionally treated subgroup, but provided superior results to those of EX when dentin surface was previously conditioned by Er:YAG laser. The lower concentration of GB etchant gel (20%) may have possibly led to the lower bond strength means observed for the non-lased specimens. On the other hand, the use of weaker etchant agents would be more favorable for dentinal substrate previously treated by laser irradiation, since the action of Er:YAG itself already results in absence of smear layer and opening of dentinal tubules [4,7,22,23]. Therefore, the role of acid conditioning would be to "refine" the surface treatment through the selective removal of mineral contents from the intertubular dentin besides the amplification of tubules' openings. The use of an etchant gel with higher concentration (32–37%) would possibly result in "overetching" of dentin surface, thus compromising its ultrastructure and affecting the adhesive procedure.

In conclusion, based on the reported results and within the limitations of an in vitro investigation, it may be stated that the pretreatment of dentin surface with Er:YAG laser, under the tested parameters, influenced the performance of the adhesive systems evaluated in this study. However, it seems appropriate to emphasize that, despite the notable and unquestionable advances in dental research, there is too much to be disclosed and defined concerning the ultimate effect of lasing on dental substrate. Focusing specifically on Er:YAG laser, long-term studies have yet to determine, for instance, how the morphological changes, typically provided by irradiation, could affect the performance of adhesive materials. Indeed, most conclusions derived from researches on the mechanism of bonding to lased tooth surfaces (specially dentin) are still based on empiric observations and speculations. This status may be easily understood, taking into account the fact that the investigators are dealing with three extremely complex matters, which are subjected to several and variable factors: laser irradiation, adhesive systems, and dental substrate. Successful interaction of these features is at the same time the most critical point and the key for providing optimal bonding.

Further investigation is certainly required to determine which adhesive protocol should be preferred to yield an optimal bonding to laser-treated dental substrate, as well as to establish the basis for rational assessment and applicability of this technology in restorative dentistry.

ACKNOWLEDGMENT

This work was partially supported by CAPES and CNPq (process no. 300.886/98-5).

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