A Daptomycin-Xylitol-loaded Polymethylmethacrylate Bone Cement: How Much Xylitol Should Be Used?

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Abstract

Background The rate of release of an antibiotic from an antibiotic-loaded polymethylmethacrylate (PMMA) bone cement is low. This may be increased by adding a particulate poragen (eg, xylitol) to the cement powder. However, the appropriate poragen amount is unclear.

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Questions/purposes We explored the appropriate amount of xylitol to use in a PMMA bone cement loaded with daptomycin and xylitol.

Methods We prepared four groups of cement, each comprising the same amount of daptomycin in the powder (1.36 g/40 g dry powder) but different amounts of xylitol (0, 0.7, 1.4, and 2.7 g); the xylitol mass ratio (X) (mass divided by mass of the final dry cement-daptomycin-xylitol mixture) ranged from 0 to 6.13 wt/wt%. Eight mechanical, antibiotic release, and bacterial inhibitory properties were determined using three to 22 specimens or replicates per test. We then used an optimization method to determine an appropriate value of X by (1) identifying the best-fit relationship between the value of each property and X, (2) defining a master objective function incorporating all of the best fits; and (3) determining the value of X at the maximum master objective function.

Results We found an appropriate xylitol amount to be 4.46 wt/wt% (equivalent to 1.93 g xylitol mixed with 1.36 g daptomycin and 40 g dry cement powder).

Conclusions We demonstrated a method that may be used to determine an appropriate xylitol amount for a daptomycin-xylitol-loaded PMMA bone cement. These findings will require in vivo confirmation.

Clinical Relevance While we identified an appropriate amount of xylitol in a daptomycin-xylitol-loaded PMMA bone cement as a prophylactic agent in total joint arthroplasties, clinical evaluations are needed to confirm the effectiveness of this cement.

Introduction

A recurring challenge with total joint arthroplasty (TJA) is periprosthetic joint infection (PJI) [13, 39, 49]. The

prevalence of this phenomenon is variable, ranging from 0.3% to 9% for primary cases [11, 48] and from 2% to 40% for revision cases [24, 49]. The ramifications are serious for the patient (in most cases, revision is necessary using, for example, the two-stage method [38]) and treatment costs are high (for example, in 2009, in the United States, the mean total charge for revision of an infected THA was approximately USD 94,000 [21]). Several investigators have recommended ways to reduce the likelihood of PJI [13, 24] and to improve treatment options [38, 45].

One approach to improving treatment is to use an antibiotic-loaded polymethylmethacrylate (PMMA) bone cement (ALABC) in which the cement powder is blended with a novel antibiotic [15, 31]. Novel antibiotics are considered those with potential to have rapid and effective bactericidal activity against pathogens, such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, that have become resistant to many of the common antibiotics used in current approved commercially available ALABC brands or orthopaedic surgeon-prepared/directed formulations (such as gentamicin, tobramycin, and vancomycin [13, 39, 45]). Although there is a growing number of these novel antibiotics (eg, dalbavancin, daptomycin, telavancin, and tigecycline) [4, 8, 10, 12, 15, 19, 31, 42, 45], only a few, such as daptomycin and telavancin, have been used in PJI treatment [15, 31].

Several studies have reported the in vitro properties of either low-dose daptomycin-loaded PMMA bone cement (≤ 1 g daptomycin per 40 g cement powder [13]) [5, 14, 26] or a higher-dose variant (≥ 2 g) [5, 14, 26, 41]. These reports show the daptomycin release profile in phosphate-buffered saline (PBS) solution at 37° C is characterized by a burst followed by a period of slow decrease [5, 14, 26]. At relatively low amounts (up to 7.5% of the mass of the dry cement powder-daptomycin mixture), daptomycin exerts a marginal influence on the cement's fatigue limit, tensile strength, yield strength, compressive strength, and compressive modulus (compared to the value for a cement that did not contain the antibiotic) [14, 26, 41], but with a higher amount of daptomycin (11 wt/wt%), there is an appreciable drop in the cement's fatigue limit [26]. There is a sizeable literature on methods to improve the release profile of antibiotics from ALABC cylinders, including incorporation of a particulate filler (such as lactose, mesoporous silica nanoparticles, or xylitol) in the cement powder [9, 37, 43] and subsection of cured cement specimens to continuous or intermittent watt-level low-frequency ultrasound [3]. However, none of these reported methods have been evaluated for daptomycin-loaded PMMA bone cement. Also, in the case of an ALABC loaded with a poragen, the question as to the appropriate poragen amount has not been fully investigated.

We therefore determined an appropriate amount of xylitol to be used in a daptomycin-xylitol-loaded PMMA bone cement.

Materials and Methods

The study design comprised three parts (Fig. 1). (1) We prepared four groups of cement, one not loaded with the xylitol and three having different xylitol amounts, namely, 0.7, 1.4, and 2.7 g. (2) For each of these four groups, we determined the following eight mechanical, antibiotic release, and bacterial inhibitory properties of the cement: fracture toughness (K_{IC}), fatigue limit, daptomycin release rate, coefficient of diffusion for outflow of daptomycin (D_{dapt}), index of activity of the daptomycin eluate against *S aureus* (inhibition index [I]), polymerization rate at 37° C (k'), coefficient of diffusion for intake of 1X PBS solution (D_{PBS}), and radiopacity (R') (Table 1) (see Fig. 1 for numbers of specimens or replicates used for each type of testing). These determined properties are of clinical relevance [17, 25, 29, 35, 41, 43, 44]. (3) Using our determined values for the cement properties and an optimization method, we determined an appropriate xylitol amount.

The materials used were a commercially available brand of PMMA bone cement (Orthoset® 1; Wright Medical Technology, Inc, Arlington, TN, USA), daptomycin (Cubicin®; Cubist Pharmaceuticals, Lexington, MA, USA), and xylitol (XyloSweet®; XLEAR, Orem, UT, USA). The daptomycin amount used (1.36 g/40 g dry cement powder) was determined, from our previous work [26], based on the fatigue life, rate of release of daptomycin, and activity of the released daptomycin against *S aureus* of daptomycin-loaded Orthoset® 1 cement. The xylitol mass ratios (X) (mass divided by mass of the final dry cement-daptomycin-xylitol mixture) in the four study groups were 0, 1.66, 3.27, and 6.13 wt/wt%. In a separate study, using a volumetric displacement method in a liquid in which the powder was not soluble, we determined the densities of the dry cement powder, daptomycin, and xylitol to be 1.25, 1.20, and 1.50 g cm⁻³, respectively. Thus, the corresponding xylitol amounts were 0, 1.39, 2.74, and 5.16 vol/vol% of the total dry cement powder mixture volume.

Before the preparation of the cement groups, the daptomycin was stored at 4° ± 1° C, while the xylitol and the cement powder and liquid monomer were stored in ambient laboratory conditions (temperature and relative humidity of 21° ± 1° C and 57% ± 2%, respectively). A commercially available cement powder mixer (OmoMix®; Tecres SpA, Verona, Italy) was used to mix the dry cement powder, daptomycin, and xylitol.

For the fracture toughness tests, the final powder mixture and the liquid monomer of the cement (18.37 mL)

Fig. 1 A flowchart shows the study design. The key features of the eight cement property characterization tests conducted are shown: fracture toughness (Y1), fatigue limit (Y2), daptomycin elution rate (Y3), diffusion coefficient for daptomycin egress (Y4), inhibition

index (Y5), polymerization rate at 37° C (Y6), diffusion coefficient for PBS intake (Y7), and normalized radiopacity (Y8). Study sets were no xylitol (XYL-00), 0.7 wt/wt% xylitol (XYL-07), 1.4 wt/wt% xylitol (XYL-14), and 2.7 wt/wt% xylitol (XYL-27).

Table 1. Summary of values of cement properties

Cement	K_{IC} (MPa $\sqrt{m}$)*	Fatigue limit (MPa) [†]	Daptomycin release rate ($\mu\text{g mL}^{-1} \text{d}^{-1} \text{g}^{-1}$) [‡]	D_{dapt} ($10^{-11} \text{m}^2 \text{s}^{-1}$)*	k' (10^{-3}s^{-1})*	D_{PBS} ($10^{-12} \text{m}^2 \text{s}^{-1}$)*	R' (equivalent Al% MR ⁻¹) ^{§,§}
XYL-00	1.92 ± 0.04	9.9 (8.3, 11.6)	45.2	3.75 ± 0.21	2.59 ± 2.10	4.49 ± 1.05	4.51
XYL-07	1.89 ± 0.06	9.8 (8.3, 11.3)	34.6	2.53 ± 0.63	2.21 ± 1.20	4.34 ± 1.60	3.30
XYL-14	1.96 ± 0.02	8.8 (6.2, 11.4)	50.0	2.72 ± 0.26	0.67 ± 0.32	3.37 ± 1.33	3.41
XYL-27	1.88 ± 0.04	9.7 (8.6, 10.8)	74.3	2.44 ± 0.29	0.61 ± 0.22	5.92 ± 1.15	3.63

* Values are expressed as mean $\pm$ SD; [†]values are expressed as mean, with 95% CIs in parentheses; [‡]values are expressed as mean only; [§]MR = mass ratio of the radiopacifier in the cement powder (9.1 wt/wt% BaSO₄); K_{IC} = fracture toughness; D_{dapt} = coefficient of diffusion for outflow of daptomycin; k' = cement polymerization rate at 37° C; D_{PBS} = coefficient of diffusion for intake of 1X phosphate-buffered saline solution; R' = normalized radiopacity; XYL-00 = no xylitol; XYL-07 = 0.7 wt/wt% xylitol; XYL-14 = 1.4 wt/wt% xylitol; XYL-27 = 2.7 wt/wt% xylitol.

were vacuum-mixed using a commercially available unit (MixeVac® II High-Vacuum System; Stryker Instruments, Kalamazoo, MI, USA), with an evacuation pressure of 74 ± 1 kPa. The prepared cement dough was injected into a stainless steel mold whose internal configuration and dimensions were of a compact tension test specimen with dimensions conforming to those stipulated in ASTM D5045 [2]: overall nominal height, width, and thickness were 35.7 mm, 37.2 mm, 14.9 mm, respectively. The

protocols used for finishing the specimens, accepting specimens for testing, conditioning the accepted specimens, performing the tests, and treating the results to obtain the fracture toughness (K_{IC}) of the cement were the same as those given in our previous work [30].

For the fatigue tests, the prepared cement dough was injected into a four-celled silicone mold, with each cell having the internal configuration and dimensions of a solid cylindrical dog bone, whose dimensions conformed to

Fig. 2 A graph summarizes the fatigue test results. Study sets were no xylitol (XYL-00), 0.7 wt/wt% xylitol (XYL-07), 1.4 wt/wt% xylitol (XYL-14), and 2.7 wt/wt% xylitol (XYL-27). Values are shown as mean with SD.

those stipulated in ASTM F2118-03 [1]: overall nominal diameter of the grip ends and length were 8.5 mm and 62 mm, respectively. Protocols for inspecting the fabricated specimens, selecting acceptable specimens, choosing the specimens to be tested from the acceptable ones, conditioning the test specimens, and performing the tests were the same as those given in our previous work [26]. Per ASTM F2118-03, acceptable specimens are those with no surface defects in the gage or transition sections and no internal defects with major diameter of larger than 1 mm in the gage section. The acceptable specimens were conditioned in 1X PBS at $37^{\circ} \pm 1^{\circ} \text{C}$ for 7 days before the tests. During the tests, the specimen was immersed in 1X PBS at $37^{\circ} \pm 1^{\circ} \text{C}$. For each cement group, the combination of applied stress (S) and number of specimens used were $\pm 20 \text{ MPa}$ (seven specimens), $\pm 15 \text{ MPa}$ (seven specimens), $\pm 12.5 \text{ MPa}$ (five specimens), and $\pm 10 \text{ MPa}$ (three specimens). These stresses have been used in many literature studies [7, 22, 26, 47], with $\pm 10 \text{ MPa}$ being of the order postulated to be experienced in the cement mantle in a cemented TJA [18]. The test results were presented in the form of S versus number of cycles to fracture N_f (as before [26], run-out was defined as no fracture after 1.557 million loading cycles) (Fig. 2). The method used for estimating the fatigue limit of the cement was the same as that used in our previous work [26].

For each cement group, 75% to 89% of the fabricated specimens were found acceptable for fatigue testing but not all were used. From among those not used, three were selected at random for the daptomycin release tests. These elution tests were performed using the protocols in our previous study [26]. The amount of daptomycin released was measured in 10 mL 1X PBS solution at $37^{\circ} \pm 1^{\circ} \text{C}$ at eight time points (t) of 1, 2, 5, 7, 10, 14, 21, and 28 days,

with complete PBS refreshment at each time point. The release results were compiled as current amount of daptomycin released (W) versus t (Fig. 3A) and cumulative amount (M) versus t (Fig. 3B). A commercially available software package (MATLAB® R2011b; The MathWorks, Natick, MA, USA) was used to obtain the best-fit relationship between W and t. The daptomycin release rate was then computed as the derivative of this W-versus-t relationship calculated at the value of t midway between the burst release point and the point at which the W-versus-t curve began to flatten.

The release of daptomycin from a test specimen was approximated using the expression for release of a drug from a specimen possessing aspects of both a long cylinder and a thin disc [40]. With the aid of a scientific calculator (TI-89 Titanium; Texas Instruments, Inc, Dallas, TX, USA), the M-versus-t results and the aforementioned expression were used to compute the coefficient of diffusion for outflow of daptomycin (D_{dapt}).

After the daptomycin release tests were completed, eluates at each of the eight time points were tested for an index of antimicrobial activity against *S aureus*, as described previously [26]. Thus, 200 μL of eluates was added to 1.75 mL Mueller Hinton II broth supplemented with 25 μg CaCl_2 per mL followed by the addition of an inoculum of *S aureus* ($\sim 10^4$ colony-forming units [CFU]) and an overnight incubation at 37°C . Growth was determined by measuring the optical density (OD) of the solutions, at 530 nm, using an ultraviolet spectrophotometer (GENESYS™ 20; Thermo Scientific, West Palm Beach, FL, USA). The inhibition index (I) was computed as before [26] (Table 2).

The differential scanning calorimetry work was performed at a heating rate of 15 K minute^{-1} (DuPont 910;

Fig. 3A–B Graphs show the daptomycin release profiles in (A) current amount and (B) cumulative amount. Study sets were no xylitol (XYL-00), 0.7 wt/wt% xylitol (XYL-07), 1.4 wt/wt% xylitol (XYL-14), and 2.7 wt/wt% xylitol (XYL-27).

Table 2. Summary of activity test results

Cement	Growth/inhibition of <i>Staphylococcus aureus</i> *								Mean inhibition index† (%)
	Elution time (days)								
	1	2	5	7	10	14	21	28	
XYL-00	—	—	—	—	—	+	+	+	62.5
XYL-07	—	—	—	—	—	—	—	—	100.0
XYL-14	—	—	—	—	—	—	−/+‡	—	95.8
XYL-27	—	—	—	—	—	—	—	—	100.0

* + = growth of *S. aureus* (inhibition index = 0); — = inhibition of *S. aureus* (inhibition index = 100%); [†]for a cement, index = [sum of inhibition indexes/total number of test specimens (= 24)]; [‡]of the three samples tested, two inhibited and one allowed growth of *S. aureus*; XYL-00 = no xylitol; XYL-07 = 0.7 wt/wt% xylitol; XYL-14 = 1.4 wt/wt% xylitol; XYL-27 = 2.7 wt/wt% xylitol.

Instrument Specialists, Inc, Spring Grove, IL, USA). The protocols used and the steps employed in treating the results to obtain the cement polymerization rate at 37° C (k') were as described in our previous work [30].

The PBS intake test involved measuring the mass gain of a circular cross-sectioned cement disc specimen cut from the end of an acceptable fatigue test specimen (nominal diameter and thickness of 8.50 mm and 3.00 mm, respectively) immersed in 15 mL 1X PBS solution at $37^\circ \pm 1^\circ$ C. Measurement continued until there was no observable increase in mass gain. Details of all the steps involved in using the mass gain versus time in PBS results to compute the PBS diffusion coefficient (D_{PBS}) were given in our previous work [30].

The experimental protocols followed for the determination of the radiopacity (R) of the cement were the same as those presented in our previous work [28]. However, in the present work, R was calculated as the ratio of the linear attenuation coefficient (μ) for the cement (slope of the linear regression plot of the logarithm of OD of cement disc versus cement disc thickness) to μ for Al (slope of the linear regression plot of OD of Al step-wedge versus Al step-wedge thickness) and expressed as equivalent Al% [36]. The normalized radiopacity (R') was computed as the ratio of R to the mass fraction of the radiopacifier in the cement powder (9.1 wt/wt% BaSO₄ for each cement group).

The optimization method used to assess the appropriate xylitol loading comprised seven steps: (1) for each of the eight cement properties, we obtained the best-fit relationship between our determined values and X; this relationship is called an objective function (Table 3); (2) for each cement property, we found the highest and lowest values, as given in previous literature; (3) for each cement property, we calculated its fractional objective function at a given value of X (within the range of 0 to 6.13 wt/wt%) as follows: (value of the objective function at X – lowest literature value of property) divided by (highest literature value of property – lowest literature value of property); (4) for a cement property that should be as high as possible (for example, fatigue limit), we calculated the quantity (fractional objective function)² at the X value and called this quantity Y1; for a cement property that should be as low as possible (for example, k'), we calculated the quantity $[1 - (\text{fractional objective function})]^2$ at the X level and called this quantity Y2; (5) we repeated Steps 3 and 4 for all the properties and hence calculated the master modified fractional objective function (MMFOF) as equal to (sum of all the values of Y2) – (sum of all the values of Y1); (6) we repeated Steps 3 to 5 at other values of X (within the range of 0 to 6.13 wt/wt%); and (7) we determined the appropriate value of X as that at which the maximum value of MMFOF, when plotted against X, occurred. Thus, this

Table 3. Summary of the objective functions (ie, best-fit relationships between each of the properties determined and the xylitol amount [X])

Cement property	Best-fit relationship	Adjusted R ^{2*}
Fracture toughness (Y1)	$Y1 = 1.92 - 0.0040X$	0.0829
Fatigue limit (Y2)	$Y2 = 9.70 - 0.0549X$	0.0799
Daptomycin elution rate (Y3)	$Y3 = 0.03583 + 0.000327 * X^2$	0.7163
Diffusion coefficient for daptomycin egress (Y4)	$Y4 = 2.587 \times 10^{-11} - 2.022 \times 10^{-13} * \ln(X)$	0.8933
Inhibition index (Y5)	$Y5 = 96.77 * X^{0.01578}$	0.9575
Polymerization rate, at 37° C (Y6)	$Y6 = 0.002656/(1.0 + (X/2.598)^{2.728})$	0.7776
Diffusion coefficient for PBS intake (Y7)	$Y7 = 4.036 \times 10^{-12} + 4.053 \times 10^{-15} * e^X$	0.2028
Normalized radiopacity (Y8)	$Y8 = 1/[0.292 - 0.069 * e^{-X}]$	0.5824

* Coefficient of multiple determination, adjusted for the degrees of freedom of the equation; PBS = phosphate-buffered saline.

appropriate value is the one that gives the best tradeoff among the cement properties. The calculations in Step 1 and those in Steps 3 to 7 were carried with the aid of two commercially available software packages (TableCurve 2D® v5.01; SYSTAT Software Inc, Chicago, IL, USA; and Microsoft® Excel® Solver; Microsoft Corp, Redmond, WA, USA), respectively.

Results

Using the objective functions (Table 3) and the optimization method, we determined the appropriate xylitol amount as 4.45 wt/wt% (equivalent to final dry powder mixture composition of 1.93 g xylitol, 1.36 g daptomycin, and 40 g cement powder).

Discussion

In TJAs, it is common practice to use either a commercially available brand or an orthopaedic surgeon-prepared/directed formulation of an ALABC as a prophylaxis agent against PJI [13, 39, 49]. There are many reports of substantial decrease in susceptibility of the pathogens involved in PJI (such as methicillin-resistant *S aureus*) to the antibiotic incorporated in the cement powder (usually, gentamicin, tobramycin, or vancomycin) [45]. This decrease in susceptibility provides opportunity to develop novel antibiotics. Among novel antibiotics, there are reports of the effectiveness of daptomycin and telavancin against these pathogens [15, 31]. Literature reports on

daptomycin-loaded cement cylinders show the daptomycin release rate is low [5, 14, 26]. When a poragen was added to the cement powder of an ALABC, antibiotic release rate from cylindrical specimens increased, but there are limited data on the impact on other cement properties [37]. The question as to what should be an appropriate poragen amount in an ALABC that contains a poragen has not been posed. In this study, we determined the appropriate amount of xylitol to be used in a daptomycin-xylitol-loaded PMMA bone cement.

We recognize several limitations of our study. First, although there are reports indicating xylitol-containing dental products may be useful in preventing or reducing the progression of dental caries [32]), the question as to the safety of xylitol as a constituent of an ALABC is an open one. However, there are reports of the beneficial effects of dietary xylitol against weakening of bone in aged animal models [33]. Thus, a xylitol-loaded ALABC may be viable for use in cemented TJAs. Second, we used one cement brand, one antibiotic, and one brand of xylitol. Given the large numbers of cement brands and antibiotics available, the present approach is justified from the perspective of study time and cost. Third, the conditions under which the daptomycin release tests were conducted are different in a number of respects, notably fluid dynamics and sink details, from the in vivo medium in which a TJA is located [6, 23]. However, these experimental conditions reportedly produce vancomycin elution levels that correlate with those obtained from the hip of an animal model [50]. Fourth, we used a generalized measure of the activity of the released daptomycin against *S aureus*, rather than a specific one, such as the number of bacteria on a cement specimen (CFU/surface area of the specimen). However, the former method was used in each of the other literature studies of daptomycin release from ALABC cylinders [5, 14, 26]; thus, we can compare present trends to those in these reports. We note two caveats regarding the appropriate xylitol amount we determined. (1) Even though a large and diverse array of cement properties was determined, there are other important ones that were not. One such property is resistance of a cement specimen to the formation of biofilm of a given pathogen implicated in PJI on its surface, a phenomenon that may result in a substantial reduction of the susceptibility of the pathogens to daptomycin [46]. Other undetermined properties are working time, which gives insight into the time window between packing the cement dough in the bone bed and placement of the prosthesis in that bed [20]; residual monomer content, which provides an indication of the potential for phenomena such as chemical necrosis of periprosthetic tissues [16]; and fatigue crack propagation rate in PBS at 37° C, which may

be related to the in situ life of a TJA [34]. (2) Weighting factors for the determined cement properties were not used; for example, considering the clinical application, in a head-to-head comparison between daptomycin release rate and fatigue limit, the former may be given a higher weighting factor. An investigation of the sensitivity of the assessed appropriate xylitol amount to the number of cement properties determined and the calculation of weighting factors for the cement properties determined are outside the confines of the present study. None of the above-mentioned limitations and caveats jeopardizes the assessed appropriate xylitol amount. However, these issues should be taken into account if our calculated xylitol amount is to be used in the clinical setting.

In a related study, Nugent et al. [37] investigated the influence of xylitol amount on the properties of ALABC cylindrical specimens and found antibiotic release increased progressively and compressive strength of the cement decreased with increase in xylitol amount. However, Nugent et al. [37] used a medium-viscosity cement [20], the antibiotic tobramycin, and xylitol amounts of 1 to 16 g/40 g cement powder; hand-mixed the final powder mixture with the cement liquid monomer; and did not assess an appropriate xylitol amount. In contrast, we used a high-viscosity cement brand [20], daptomycin, and xylitol amounts of 0.7 to 2.7 g/40 g cement powder; vacuum-mixed the final powder mixture with the cement liquid monomer; and assessed an appropriate xylitol amount. Two other relevant studies were by Lewis et al. [26, 27] on the computation of the appropriate amount of the antibiotic in an ALABC. Two methodologies were used in one study [27], each involving changes of each of the properties determined with antibiotic dose. The methodology used in the other study [26] was the same as that used in the present work, but many more cement properties were determined in the present study (eight versus three). Thus, there are no literature studies comparable to the present investigation.

In conclusion, we found an appropriate xylitol amount for a daptomycin-xylitol-loaded PMMA bone cement to be 4.46 wt/wt% (equivalent to 1.93 g xylitol mixed with 1.36 g daptomycin and 40 g cement powder). Since this amount is based on an analysis of a limited number of in vitro cement properties, including some properties, such as the local minimum inhibitory concentration of the eluted daptomycin, the findings will require confirmation from in vivo studies.

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