J Clin Periodontol 2005; 32: 462–466 doi: 10.1111/j.1600-051X.2005.00698.x
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Journal of Clinical Periodontology

Interleukin-1 levels in gingival crevicular fluid adjacent to restorations of calcium aluminate cement and resin composite

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Konradsson K, van Dijken JWV. Interleukin-1 levels in gingival crevicular fluid adjacent to restorations of calcium aluminate cement and resin composite. J Clin Peridontol 2005; 32: 462–466. doi: 10.1111/j.1600–051X.2005.00698.x.
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Abstract

Objectives: The aim of this clinical study was to intra-individually compare Class V restorations of a calcium aluminate cement (CAC), resin composite and enamel with respect to the adjacent levels of interleukin (IL)-1α, IL-1β and IL-1 receptor antagonist (IL-1ra) in gingival crevicular fluid (GCF). The hypothesis was that there are higher IL-1 levels adjacent to resin composite, compared with CAC and enamel.

Materials and methods: In 15 subjects, at least one set of two Class V restorations with subgingival margins, one CAC and one universal hybrid resin composite, and one control surface of enamel were included. In a cross-sectional study and on days 0, 3 and 7 of an experimental gingivitis study, GCFs were collected with Periopaper for 30 s. The GCF concentrations of IL-1α, IL-1β and IL-1ra were quantified with enzyme linked immunosorbent assays.

Results: Neither the cross-sectional study nor the experimental gingivitis study showed any significant differences in the levels of IL-1α, IL-1β and IL-1ra between CAC, resin composite and enamel sites ($p > 0.05$). In the cross-sectional study, low IL-1 concentrations were observed. The IL-1 levels increased significantly during the experimental gingivitis.

Conclusion: Regardless of CAC or resin composite, the restorations *per se* did not affect the GCF levels of IL-1 and IL-1ra, neither at healthy gingiva, nor at initiation of plaque-related gingival inflammation.

Key words: cements; composites; experimental gingivitis; interleukin-1; restorations

Accepted for publication 8 September 2004

Release of components of dental materials and plaque accumulation on tooth restorations may influence their stability and biocompatibility (Ferracane 1994, Santerre et al. 2001). Many studies on the biocompatibility of restorative materials have focused on inflammatory responses and toxic effects on fibroblasts and epithelial cells (Wataha 1994, Hanks 1996, Schedle et al. 1998, Schmalz et al. 2000, Santerre et al. 2001). The intent of this clinical study was to evaluate the biocompatibility of calcium aluminate cement (CAC) in comparison with resin composite and enamel concerning the inflammatory response of interleukin-1 (IL-1). Recently, the CAC was developed as a ''bioceramic'' alternative to amalgam and resin composite. CAC is inorganic and non-metallic and thus falls within the definition of a ceramic material. It is based on two essential constituents, alumina and calcium oxides and a small amount of zirconium, ferric and silicone oxides. Resin composites are used frequently, in all cavity classes, owing to their aesthetics and tooth substance saving preparation technique. They contain a wide variety of monomers and additives, which can be released because of non-optimal conversion and degradation.

Cytokines are low-molecular-weight proteins synthesized in response to bacteria and their products, inducing and maintaining an inflammatory response. In addition, ''cell stressors'' of non-microbial origin may also stimulate synthesis of cytokines (Dinarello 2000). IL-1 is one of the major mediators of inflammatory response. Very different cells are producers of IL-1 (Seymour & Gemell 2001). IL-1 is produced as two structurally related

proteins, IL-1α and IL-1β. The IL-1 receptor antagonist (IL-1ra) exists as a unitary mechanism within the immune system to control IL-1 activity. This protein acts by binding with high affinity to the same receptors like IL-1α and IL-1β without inducing any of the biological effects (Weber & Iacono 1997).

Gingivitis develops within a couple of days of bacterial plaque accumulation (Löe et al. 1965). An early indication of gingival inflammation is an enhancement of the gingival crevicular fluid (GCF) flow. The content of the GCF reflects inflammatory reactions taking place in the gingival tissues (Cimasoni 1983, Embery & Weddington 1994, Eley & Cox 1998). Experimental gingivitis models, where gingivitis is deliberately induced by cessation of oral hygiene, have demonstrated that IL-1β in GCF increases during gingival inflammation before the onset of clinical signs of gingivitis (Heasmann et al. 1993, Zhang et al. 2002). To our knowledge, no reports have been published concerning the effect of dental restoratives on the contiguous GCF-levels of IL-1.

The specific aim of this clinical study was to compare, intra-individually, cervical restorations of a CAC and an universal hybrid resin composite, and non-restored enamel, with respect to the adjacent GCF levels of IL-1α, IL-1β and IL-1ra from healthy gingiva and at initiation of gingivitis.

The hypothesis was that there are higher IL-1 levels adjacent to resin composite, compared with CAC and enamel.

Material and Methods

During a 6 months period, all recall patients in an ongoing clinical follow-up of Class V restorations of a CAC were evaluated (Konradsson & van Dijken 2002). Exclusion criteria were use of antibiotics, anti-inflammatory drugs and/or oral anti-microbial agents within the preceding 3 months. Fifteen subjects (four females and 11 males; mean age 63.0 years, range 40–85) were included. Inclusion criteria: At least one set of two Class V restorations (>3 months old) with sub-gingival margins, one CAC (Doxa Certex, Uppsala, Sweden) and one hybrid resin composite, and one non-filled enamel surface, localized in the same region. A defined site of each surface selected was chosen for GCF sampling. Probing depth of the site, assessed after sampling, did not exceed 3 mm. Clinical indices of plaque and gingivitis were low (Konradsson & van Dijken 2002). The individuals gave informed consent to participate. The ethics committee of the University of Umeå approved this study.

Cross-sectional and experimental gingivitis study

Cross-sectional study

In conditions of oral hygiene on a regular basis, the GCF levels of IL-1α, IL-1β and IL-1ra adjacent to the CAC, resin composite and enamel were evaluated. The GCF, obtained from 20 sets, were analysed and intra-individually compared. No performance of the oral hygiene was permitted in the 2 h before sampling to minimize gingival irritation.

Experimental gingivitis study

During a week of neglected oral hygiene, in order to induce gingivitis, the GCF levels of IL-1α, IL-1β and IL-1ra adjacent to the CAC, resin composite and enamel were evaluated. The GCF obtained from 18 sets, on days 0, 3, and 7 of the study were analysed and intra-individually compared. On day 0, without polishing, plaque and gingival index approached zero. After measuring, non-test surfaces in the opposite jaw were polished, except on day 0.

Sampling and analysis of GCF

The sites to be sampled were isolated with cotton rolls and carefully sprayed with water to remove saliva. Each site was gently dried using an air syringe during 10 s. A paper strip (Periopaper, ProFlow, Amityville, NY, USA) was inserted at the orifice of the gingival crevice and left there for 30 s. The fluid volume was determined from standard curves using a Periotron 6000 (Periotron, Pro Flow). The absorbing part of the strip was cut off and transferred to a coded plastic tube. The sample was eluted twice by adding 50 µl 0.9% sodium chloride and centrifuged at 3000 rpm and 5°C for 20 min. The 100 µl supernatant was transferred to a tube containing 100 µl sodium chloride and stored frozen at −80°C (Rasmussen et al. 2000).

The levels of IL-1α, IL-1β and IL-1ra were quantified with enzyme linked immunosorbent assays (ELISA, Quantikine, R&D Systems Europe, Abingdon, Oxon, England) according to the instructions provided by the manufacturer. The optical density converting to concentrations was measured using a microtiter plate computerized reader. The supernatants from each individual set of the three sites were similarly diluted and run with the same assay. The supernatants were diluted 1:5 with sodium chloride. If a given value exceeded the standard curve at analysis, the supernatants were further diluted. To calculate IL-1 in the GCF, the concentration measured was multiplied with the quotient of the sodium chloride solution added and divided through the volume sampled.

Statistical analysis

The data were processed in SPSS (Statistical Package for the Social Sciences, version 10.0). Frequency distributions of the IL-1 values were described. Data were analysed with Kolmogorov–Smirnov goodness of fit tests for normality. Wilcoxon's signed rank test was used for the intra-individual comparisons. p-values < 0.05 were considered statistically significant.

Results

The GCF concentrations of IL-1α, IL-1β and IL-1ra in both the cross-sectional study and the experimental gingivitis study are given in Table 1. No statistically significant differences in GCF levels of IL-1α and IL-1ra were found between the sites adjacent to CAC, resin composite and enamel.

One fourth of all sites analysed in the cross-sectional study showed detectable levels of IL-1α and IL-1β (>0.99 ng/ml supernatant). IL-1ra (>3.4 µg/ml supernatant) was detected in all sites except three. Two enamel sites showed high outlying values of IL-1α (>100 ng/ml sample). The number of sites compared was reduced because of deficient supply of the supernatants within the laboratory procedures.

In the experimental gingivitis study, the amount of the supernatants was inadequate to measure IL-1β on day 0. For the same reason, missing data during the period resulted in exclusion of two sets of IL-1α, three of IL-1β, and

Table 1. The median concentration (range) of IL-1α (ng/ml), IL-1β (ng/ml) and IL-1ra (μg/ml) in samples of gingival crevicular fluid adjacent to calcium aluminate cement (CAC), resin composite and enamel in the cross-sectional study (CSS) and on days 0, 3, and 7 of the experimental gingivitis study

	CAC	*n*	Composite	*n*	Enamel	*n*
CSS						
IL-1α	0 (0–64.8)	19	0 (0–30.2)	19	0 (0–201.3)	19
IL-1β	0 (0–17.8)	6	0 (0–7.9)	6	0 (0–20.5)	5
IL-1ra	1.6 (0.1–8.7)	17	1.3 (0–4.8)	17	1.3 (0–7.5)	16
Day 0						
IL-1α	0 (0–211.1)	16	0 (0–134.1)	16	0 (0–168.2)	16
IL-1β	–	–	–	–	–	–
IL-1ra	0.8 (0–24.9)	15	1.0 (0–9.4)	15	1.4 (0–29.9)	15
Day 3						
IL-1α	9.8 (0–287.1)	16	5.8 (0–151.9)	16	14.1 (0–142.6)	16
IL-1β	0 (0–7.0)	15	0 (0–47.0)	15	0 (0–8.5)	15
IL-1ra	2.6 (0.3–17.2)	15	1.6 (0.2–31.0)	15	2.4 (0.7–5.3)	15
Day 7						
IL-1α	28.1 (0–401.0)	16	9.1 (0–153.9)	16	12.5 (0–147.6)	16
IL-1β	0 (0–12.1)	15	0 (0–25.4)	15	0 (0–27.6)	15
IL-1ra	6.0 (1.2–22.2)	15	8.6 (2.8–33.7)	15	5.6 (0–70.7)	15

n, number of sites; –, missing values; IL-1ra, interleukin-1 receptor antagonist.

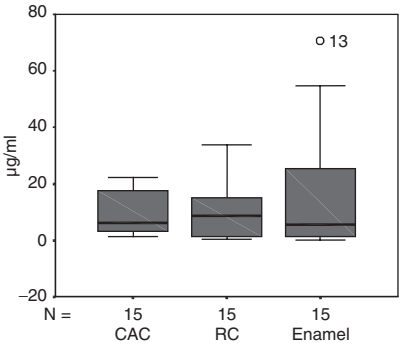

Fig. 3. Box-plots of the interleukin (IL)-1 ra concentrations (μg/ml) in samples of gingival crevicular fluid adjacent to calcium aluminate cement (CAC), resin composite (RC) and enamel on day 7 of the experimental gingivitis study ($N = 16$), median, 25th and 75th percentiles in box-plots. Whiskers include the minimum or maximum, except outliers (○), which are marked with their subject codes.

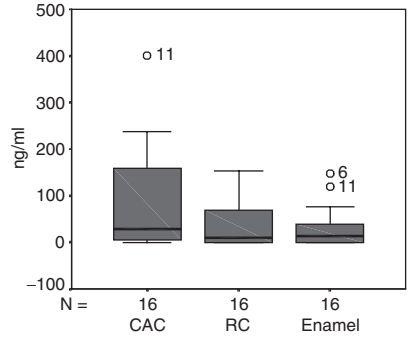

Fig. 1. Box-plots of the interleukin (IL)-1 α concentrations (ng/ml) in samples of gingival crevicular fluid adjacent to calcium aluminate cement (CAC), resin composite (RC) and enamel on day 7 of the experimental gingivitis study ($N = 16$), median, 25th and 75th percentiles in box-plots. Whiskers include the minimum or maximum, except outlier (○), which are marked with their subject codes.

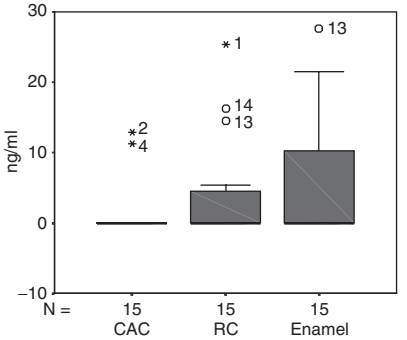

Fig. 2. Box-plots of the interleukin (IL)-1 β concentrations (ng/ml) in samples of gingival crevicular fluid adjacent to calcium aluminate cement (CAC), resin composite (RC) and enamel on day 7 of the experimental gingivitis study ($N = 15$), median, 25th and 75th percentiles in box-plots. Whiskers include the minimum or maximum, except outlier (○) and extreme outliers (*) which are marked with their subject codes.

three of IL-1ra. From day 0 to day 7, an increase of the GCF levels of IL-1α was observed adjacent to CAC ($p = 0.011$), resin composite ($p = 0.041$) and enamel ($p = 0.117$). The increase of IL-1ra was only significant adjacent to resin composite ($p = 0.02$). The peak values on day 7 are described for IL-1α in Fig. 1, Il-1β in Fig. 2, and IL-1ra in Fig. 3.

Discussion

Aesthetic features have driven the choice of the restoratives in dentistry and polymeric materials have been used extensively during the last years as replacement of amalgam. Degradation of restorative materials containing polymeric resins and their effect on durability and biocompatibility have been of interest (Santerre et al. 2001). During the first days after curing, not polymerized components from dental composites are eluted in a diffusion process. The degradation of CAC is not known. Aged restoratives were studied to avoid the influence of the initial peak elution. Nevertheless, hydrolysis and/or chemical degradation of the restoratives in the oral environment may probably result in further leaching of material components (Wataha 1999, Santerre et al. 2001).

Dental materials may favour initiation of gingivitis by facilitating local plaque accumulation and/or, which is different from sound tooth tissues, by releasing toxic substances (Wataha 1994). In a previous study (Konradsson & van Dijken 2002), low plaque and gingival indices were observed at buccal surfaces of CAC and resin composite in individuals with customary oral hygiene. After 10 days of undisturbed plaque growth, a larger amount of plaque was found on the restoratives compared with the enamel, as well as a tendency of an increase of the contiguous GCF flow.

The cytokine IL-1 is one of the primary mediators of the initial human host response to microbial challenge and plays a multifunctional role in the initiation of the host inflammatory response. The level of this pro-inflammatory cytokine can act as a marker of an initial gingival inflammatory response (Eley & Cox 1998).

The present study was designed to intra-individually compare the GCF levels of IL-1 adjacent to CAC, resin composite and enamel surfaces. In general, low IL-1 levels were observed. This demarcated part of the sub-clinical gingival inflammatory response contiguous to CAC and resin composite, was comparable with the non-restored enamel. Therefore, the hypothesis was not accepted.

An extensive inter-individual variation of the IL-1 concentrations was noticed. Distinguishing features of responses in health and gingivitis may be regulated as a characteristic of individual subject differences (Ebersole et al. 1993). Environmental and genetic factors underlying such differences, inclusive the IL-1 genotype, are discussed in a review of Tatakis et al. 2004. To enable elimination of these individual differences at interpretation of the results, the IL-levels were compared within each subject.

Previous experimental gingivitis studies have demonstrated elevated IL-1 levels in GCF during gingival inflammation. These findings were further supported in the present study. Biesbrock & Yeh (2000) observed increased surface epithelium concentrations of IL-1α over a 14-days experimental gingivitis. Heasmann et al. (1993) showed that the IL-1β levels of GCF augmented sharply within the first week of undisturbed plaque accumulation. In contrast, Gonzáles et al. (2001) found no rise of the IL-1β concentrations after 7 days, still, a significant increase was observed at the end of 18 days experimental gingivitis including healthy subjects.

Clinical trials in animal models and humans have shown that IL-1ra ameliorates the inflammatory responses (Dinarello 2000). Ishihara et al. (1997) reported a concurrent enhancement of IL-1α, IL-1β and IL-1ra in GCF from sites with periodontal disease compared with sites from non-inflamed gingiva. These findings were divergent from Rawlinson et al. (2000) who found a relationship between increasing levels of IL-1β and decreasing levels of IL-1ra.

IL-1 was not detected in GCF from all the sites, which is also reported by others (Ishihara et al. 1997). Contrary to findings by Rawlinson et al. (2000, 2003), no detectable levels of IL-1β were found in a considerable part of healthy or control sites by Gamonal et al. 2000, Wilton et al. 1992 and Faizuddin et al. 2003. Regarding IL-1α, Mathur et al. 1996 did not state any measurable amount in slightly less of the half of healthy sites sampled.

The appropriate dilution factors can be discussed. A lower dilution of the supernatants might have reduced the number of undetectable levels of IL-1α and IL-1β in GCF. However, the pilot study did not indicate such a condition. A disadvantage of a lower dilution would have implied a decreased quantity of sample available. In a part of the samples, a sampling time of 30 s was not enough to collect IL-1β in a detectable amount. Longer sampling times and/or pooled samples from repeated sampling could have been used to collect more GCF. However, lengthy sampling periods may sample mainly inflammatory exudates from vessels rather than the contents of the crevice (Eley & Cox 1998). Furthermore, prolonged sampling may result in enzymic degradation of cytokines in GCF (Rawlinson et al. 2003) and repeated insertion of paper strips into the gingival crevice may cause irritation of the epithelium and stimulate the GCF fluid flow (Cimasoni 1983).

In conclusion, regardless of the restorative material, CAC or resin composite, the restorations *per se* did not affect the levels of IL-1α, IL-1β and IL-1ra, neither at healthy gingiva, nor at initiation of plaque-related gingival inflammation.

Acknowledgements

This study was partly supported by the County of Västerbotten, Swedish Dental Association and Doxa Certex, Sweden.

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