Published in final edited form as:

J Endod. 2008 November ; 34(11): 1360–1363. doi:10.1016/j.joen.2008.08.020.

The Effect of Calcium Hydroxide on Pro-Inflammatory Cytokines and Neuropeptides

Asma A. Khan, BDS, PhD, **Xiaoling Sun, DDS, PhD**, and **Kenneth M. Hargreaves, DDS, PhD**

Abstract

Calcium hydroxide, a widely used intracanal medicament, is known to exert an antimicrobial effect and degrade bacterial-derived lipopolysaccharides. However, little is known about the effect of $Ca(OH)_2$ on endogenous inflammatory mediators such as interleukin-1α (IL-1α), tumor necrosis factorα (TNFα) and calcitonin gene-related peptide (CGRP). This is an important gap in knowledge since these inflammatory mediators play an important role in mediating the pathogenesis of periradicular periodontitis. We tested the hypothesis that $Ca(OH)_2$ denatures IL-1α, TNFα and CGRP. Human IL-1α (0.125ng/ml), TNFα (0.2ng/ml) and CGRP (0.25ng/mL) were incubated with $Ca(OH)_2$ (0.035mg/mL) for 1 to 7 days. At the end of the incubation period, the pH of the samples was neutralized and the concentrations of the mediators measured by immunoassays. Data were analyzed using one-way ANOVA and Bonferroni's multiple comparison tests. The results indicate that $Ca(OH)_2$ denatures IL-1α, TNFα and CGRP by 50–100% over the testing periods ($p<0.001$). We conclude that denaturation of these pro-inflammatory mediators is a potential mechanism by which $Ca(OH)_2$ contributes to the resolution of periradicular periodontitis.

Introduction

Periradicular periodontitis is thought to represent an immunological and inflammatory response to microorganisms in infected root canal systems (1,2). In treating teeth with periradicular periodontitis, the goal of endodontic therapy is to eliminate or reduce intracanal microorganisms and promote healing of periradicular tissues. Antimicrobial intracanal medicaments such as calcium hydroxide are widely used in order to reduce the microbial load present in the root canal systems. In addition to its antimicrobial activity (3–7), calcium hydroxide is also known for its ability to dissolve tissues (8–10), inhibit tooth resorption (11) and induce hard tissue formation (12). It reduces lipopolysaccharide (LPS)-stimulated osteoclast formation (13) and attenuates the effect of LPS on expression of matrix metalloproteinase-1 (14).

Correspondence: Asma A. Khan, BDS, PhD, Department of Endodontics, University of North Carolina, 1098 Old Dental Building, Chapel Hill, NC 27599-7450, Telephone: 919 966 2707, Fax: 919 966 6344, Asma_Khan@dentistry.unc.edu.

While most endodontic studies evaluating the role of calcium hydroxide as an intracanal medicament have focused on its effects on bacteria and their byproducts, the effects of calcium hydroxide on endogenous inflammatory mediators such as interleukin-1α (IL-1α), tumor necrosis factorα (TNFα) and calcitonin-gene related peptide (CGRP) are unknown. This is an important gap in knowledge since pro-inflammatory cytokines play an important role in regulating tissue inflammation**,** leading to the destruction of connective tissue matrices in inflammatory conditions such as periradicular periodontitis (15,16). These cytokines are also known to activate/sensitize nociceptors and contribute to the development of hyperalgesia (a stronger pain response to noxious stimuli) and allodynia (a decrease in pain threshold) (17–19).

Other mediators which are known to modulate tissue inflammation include neuropeptides such as CGRP. Inflamed pulpal and periradicular tissues contain higher levels of CGRP as compared to normal tissues (20). The release of CGRP into the peripheral tissues is known to cause vasodilation which results in increased plasma extravasation (21, 22) and to enhance the chemotactic activities of neutrophils (23, 24).

The antibacterial effect of calcium hydroxide is attributed to the release of its hydroxyl ions and several studies have demonstrated that after placement of calcium hydroxide in the root canal system, the hydroxyl ions diffuse through the dentinal tubules to the outer surface of the root (25–27). Based upon these reports, it is possible that calcium hydroxide exerts an immunomodulatory effect by local denaturation of inflammatory mediators, possibly via alkaline hydrolysis of amide bonds (28). This could potentially constitute a mechanism by which calcium hydroxide, when used as an intracanal medicament, contributes to the healing of inflamed periradicular tissues. Therefore, the present study tested that hypothesis that calcium hydroxide reduces levels of the inflammatory mediators IL-1α, TNFα and CGRP.

Materials and Methods

Human recombinant IL-1α and TNFα were purchased from R&D systems (Minneapolis, MN, USA) and were diluted using sterile phosphate buffered saline (PBS) with 1% bovine serum albumin to 0.125ng/mL and 0.2ng/ml respectively. Human CGRP was purchased from SPI-BIO (Montigny le Bretonneux, France) and was diluted to 0.25ng/ml. The concentrations of these inflammatory mediators are within the physiologic range found in pulpal and periradicular tissues as well as other orofacial tissues (31–33). IL-1α (0.125ng/mL) was incubated with calcium hydroxide (0.035%) (UltraCal XS™, Ultradent products Inc. South Jordan, UT, USA) for 1, 3 and 7 days at 37°C. TNFα (0.2ng/mL) was incubated with calcium hydroxide (0.035%) for 1 and 7 days at 37°C. CGRP (0.25ng/ml) was incubated with calcium hydroxide (0.035%) at 37°C or at 2°C for 1 and 3 days. At the end of the incubation period, the samples were centrifuged and the aspirates collected. The pH of the aspirates was neutralized using 0.1N HCl and the concentrations of IL-1α, TNFα and CGRP were measured using ELISA (R&D systems, Minneapolis, MN, USA and SPIBio Montigny le Bretonneux, France). Controls were samples prepared under identical conditions but which were incubated with PBS instead of calcium hydroxide. All samples were run in duplicates. Data were analyzed using one-way ANOVA and Bonferroni's

multiple comparison tests. The significance level was set at 0.05. Data are presented as mean ± SD.

Results

Incubation with calcium hydroxide resulted in a complete loss of detectable IL-1α at all time points examined ($p<0.001$) (Figure 1). A time-dependent decrease in levels of IL-1α was noted in the controls. Levels of IL-1α were lower in the 7 day controls as compared to the 1 day controls ($p<0.05$).

Calcium hydroxide had a similar effect on TNFα. Incubation of TNFα with calcium hydroxide for 1 or 7 days resulted in 66–100% loss of TNFα ($p<0.001$) (Figure 2). Seven days of incubation with calcium hydroxide resulted in decrease in TNFα below the levels detectable by the ELISA. The levels of TNFα in the 7 day control sample were significantly lower than those in the 1 day control sample ($p<0.001$).

For the CGRP experiments, incubation at 37°C resulted in a significant and nearly complete denaturation of CGRP in the controls as well as the experimental samples (data not shown) and as such we were unable to draw any comparisons between the samples and the controls. We then repeated the experiment at 2°C. Incubation of CGRP with calcium hydroxide resulted in a decrease in levels of CGRP ($p<0.001$) (Figure 3). The level of CGRP in the 3 day controls was lower than that in the 1 day control ($p<0.001$).

Discussion

The results of this study demonstrate that under the experimental conditions used, calcium hydroxide effectively denatures immunoreactive IL-1α, TNFα and CGRP. The time points at which the effects of calcium hydroxide were examined were based on previous studies which reported diffusion of hydroxyl ions through the dentinal tubules to the outer root surface as early as 1 hr after placement of calcium hydroxide in the root canal system and were sustained for over 7 days (28–30). Additionally, when used as an intracanal medicament during non-surgical root canal therapy, calcium hydroxide dressing is usually left in the root canal space for a minimum of 7 days.

The concentration of calcium hydroxide in most commercial preparations ranges from 35 to 70% and the pH is approximately 12.5 (29). A number of studies have examined the diffusion of hydroxyl ions across the dentinal tubules following placement of calcium hydroxide in the root canal systems (25,33). A recent *in vitro* study examined the diffusion of different preparations of calcium hydroxide (including the preparation used in this study UltraCal) in a simulated periradicular environment (34). The increase in pH in the periradicular environment following placement of UltraCal was in the range of 11.1±0.83. However, this study did not take into consideration the known buffering capacity of dentin. As such we diluted our samples a 1000 fold (ie, final concentration of 0.035%) and found that, even at this dilution, calcium hydroxide retained a significant and substantial effect on the mediators examined.

The diffusion of hydroxyl ions across dentinal tubules can be evaluated using different experimental methods. A commonly used method involves placing the samples in tubes/vials of distilled water and then measuring changes in the pH of the water (34). Another approach is to place microelectrodes into cavities prepared on the root surface in order to measure changes in pH (25, 33). Both these methods are not truly reflective of the concentration of hydroxyl ions in the periradicular space in vivo as extracellular fluids contain buffers which maintain the pH within a range of 7.35 to 7.45. As such it is unlikely that the increase in pH which occurs following diffusion of hydroxyl ions into the periradicular space is sustained for long periods of time.

Clinical as well as preclinical studies have demonstrated increased expression of IL-1α and TNFα in periradicular periodontitis and in periapical granulomas (15, 16, 30, 35–39). In addition, levels of endogenous IL-1α and TNFα are positively correlated with pain reports in several clinical conditions (40–42). Administration of an IL-1 receptor antagonist or antibodies to TNFα in patients with chronic inflammatory conditions significantly reduced the pain experienced by these patients (43, 44). Under the experimental conditions used in this study, IL-1α and TNFα, both at physiologic levels, were effectively denatured by calcium hydroxide.

A characteristic feature of chronic apical periodontitis is extensive sprouting of peripheral peptidergic nerve fibers into the periradicular tissue. This extensive neuronal arborization occurs during the onset of periapical lesions with a selective increase in the neuropeptides CGRP and substance P (SP) (20, 45, 46). CGRP exerts a vasodilative effect on the microvascular system (22) which enhances plasma extravasation induced by other inflammatory mediators such as SP (21). The exudation of fluids and blood borne mediators such as kininogen and albumin results in the edema formation and in the liberation of bradykinin which is a highly potent stimulator of nociceptors. Thus it appears that CGRP contributes to both pain and inflammation.

In the present study, CGRP was incubated with calcium hydroxide at 2°C and not at body temperature as incubation at 37°C resulted in a significant denaturation of CGRP in the controls as well as the experimental samples. Under these conditions CGRP was effectively denatured by calcium hydroxide. It is likely that at higher temperatures calcium hydroxide denatures CGRP in an even more effective manner.

A limitation of this study is that it is conducted *in vitro*. The ideal way to evaluate the effect of calcium hydroxide on inflammatory mediators in the periradicular tissues would be to first collect exudates from teeth with periradicular periodontitis and then place calcium hydroxide as an intracanal medicament. Comparison of the initial periapical samples with those collected at subsequent appointments would be partially reflective of the therapeutic effect of calcium hydroxide on inflammatory mediators. However, it is also likely that the reduction in levels of inflammatory mediators at subsequent appointments would be partly due to a decrease in the microorganisms present in the root canal system. Thus this *in vitro* approach serves to evaluate the effect of calcium hydroxide on inflammatory mediators alone without any confounding additional effects.

We do not advocate placing calcium hydroxide past the apex into the periradicular tissues. As calcium hydroxide is extremely alkaline it will cause severe tissue injury. Taken together, the data support the hypothesis that calcium hydroxide leads to substantial (50–100%) and significant reduction in detectable levels of endogenous inflammatory mediators.

Acknowledgments

Funded in part by the AAE Foundation, NIDCR K23 DE14864, P01 DA16719 and R01 NS45186

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965; 20:340–349. [PubMed: 14342926]
2. Stashenko P. Role of immune cytokines in the pathogenesis of periapical lesions. Endod Dent Traumatol. 1990; 6:89–96. [PubMed: 2079017]
3. Georgopoulou M, Kontakiotis E, Nakou M. In vitro evaluation of the effectiveness of calcium hydroxide and paramonochlorophenol on anaerobic bacteria from the root canal. Endod Dent Traumatol. 1993; 9:249–253. [PubMed: 8143576]
4. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J. 1991; 24:119–125. [PubMed: 1778624]
5. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol. 1985; 1:170–175. [PubMed: 3865763]
6. Stuart KG, Miller CH, Brown CE Jr, Newton CW. The comparative antimicrobial effect of calcium hydroxide. Oral Surg Oral Med Oral Pathol. 1991; 72:101–104. [PubMed: 1891227]
7. Law A, Messer H. An evidence-based analysis of the antibacterial effectiveness of intracanal medicaments. J Endod. 2004; 30:689–694. [PubMed: 15448460]
8. Yang SF, Rivera EM, Baumgardner KR, Walton RE, Stanford C. Anaerobic tissue-dissolving abilities of calcium hydroxide and sodium hypochlorite. J Endod. 1995; 21:613–616. [PubMed: 8596083]
9. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod. 1988; 14:125–127. [PubMed: 3268627]
10. Andersen M, Lund A, Andreasen JO, Andreasen FM. In vitro solubility of human pulp tissue in calcium hydroxide and sodium hypochlorite. Endod Dent Traumatol. 1992; 8:104–108. [PubMed: 1289067]
11. Heithersay GS. Replantation of avulsed teeth. A review Aust Dent J. 1975; 20:63–72. [PubMed: 240345]
12. Foreman PC, Barnes IE. Review of calcium hydroxide. Int Endod J. 1990; 23:283–297. [PubMed: 2098345]
13. Jiang J, Zuo J, Chen SH, Holliday LS. Calcium hydroxide reduces lipopolysaccharide-stimulated osteoclast formation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 95:348–354. [PubMed: 12627109]
14. Yang WK, Kim MR, Lee Y, Son HH, Lee W. Effect of calcium hydroxide-treated Prevotella nigrescens on the gene expression of matrix metalloproteinase and its inhibitor in MG63 cells. J Endod. 2006; 32:1142–1145. [PubMed: 17174669]
15. Wang CY, Tani-Ishii N, Stashenko P. Bone-resorptive cytokine gene expression in periapical lesions in the rat. Oral Microbiol Immunol. 1997; 12:65–71. [PubMed: 9227128]
16. Tani-Ishii N, Wang CY, Stashenko P. Immunolocalization of bone-resorptive cytokines in rat pulp and periapical lesions following surgical pulp exposure. Oral Microbiol Immunol. 1995; 10:213–219. [PubMed: 8602333]
17. Junger H, Sorkin LS. Nociceptive and inflammatory effects of subcutaneous TNFalpha. Pain. 2000; 85:145–151. [PubMed: 10692613]

18. Follenfant RL, Nakamura-Craig M, Henderson B, Higgs GA. Inhibition by neuropeptides of interleukin-1 beta-induced, prostaglandin-independent hyperalgesia. Br J Pharmacol. 1989; 98:41–43. [PubMed: 2804553]
19. Khan AA, Diogenes A, Jeske NA, Henry MA, Akopian A, Hargreaves KM. Tumor Necrosis Factor α enhances the sensitivity of rat trigeminal neurons to capsaicin. Neuroscience. 2008 In Press.
20. Kimberly CL, Byers MR. Inflammation of rat molar pulp and periodontium causes increased calcitonin gene-related peptide and axonal sprouting. Anat Rec. 1988; 222:289–300. [PubMed: 3265042]
21. Brain SD, Williams TJ. Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br J Pharmacol. 1985; 86:855–860. [PubMed: 2416378]
22. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. Nature. 1985; 313:54–56. [PubMed: 3917554]
23. Buckley TL, Brain SD, Collins PD, Williams TJ. Inflammatory edema induced by interactions between IL-1 and the neuropeptide calcitonin gene-related peptide. J Immunol. 1991; 146:3424–3430. [PubMed: 1673985]
24. Buckley TL, Brain SD, Rampart M, Williams TJ. Time-dependent synergistic interactions between the vasodilator neuropeptide, calcitonin gene-related peptide (CGRP) and mediators of inflammation. Br J Pharmacol. 1991; 103:1515–1519. [PubMed: 1884106]
25. Esberard RM, Carnes DL Jr, del Rio CE. Changes in pH at the dentin surface in roots obturated with calcium hydroxide pastes. J Endod. 1996; 22:402–405. [PubMed: 8941747]
26. Nerwich A, Figdor D, Messer HH. pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. J Endod. 1993; 19:302–306. [PubMed: 8228751]
27. Hosoya N, Takahashi G, Arai T, Nakamura J. Calcium concentration and pH of the periapical environment after applying calcium hydroxide into root canals in vitro. J Endod. 2001; 27:343–346. [PubMed: 11485253]
28. Voel, Donald; Voel, Judith G.; Pratt, Charlotte W. Fundamentals of biochemistry. 1. Hoboken NJ: John Wiley & Sons; 1999. p. 93-123.
29. Siqueira JF Jr, Lopes HP. Mechansims of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999; 32:361–369. [PubMed: 10551109]
30. Ataoglu T, Ungor M, Serpek B, Haliloglu S, Ataoglu H, Ari H. Interleukin-1beta and tumour necrosis factor-alpha levels in periapical exudates. Int Endod J. 2002; 35:181–185. [PubMed: 11843974]
31. Pezelj-Ribaric S, Magasic K, Prpic J, Miletic I, Karlovic Z. Tumor necrosis factor-alpha in peripical tissue exudates of teeth with apical periodontitis. Mediators Inflamm. 2007; 2007:69416. [PubMed: 18320014]
32. Awawdeh LA, Lundy FT, Linden GJ, Shaw C, Kennedy JG, Lamey PJ. Quantitative analysis of substance P, neurokinin A and calcitonin gene-related peptide in gingival crevicular fluid associated with painful human teeth. Eur J Oral Sci. 2002; 110:185–191. [PubMed: 12120702]
33. Perez F, Franchi M, Peli JF. Effect of calcium hydroxide form and placement on root dentine pH. Int Endod J. 2001; 34:417–423. [PubMed: 11556506]
34. Zmener O, Pameijer CH, Banegas G. An in vitro study of the pH of three calcium hydroxide dressing materials. Dent Traumatol. 2007; 23:21–25. [PubMed: 17227376]
35. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol. 1999; 44:55–66. [PubMed: 10075151]
36. Kabashima H, Nagata K, Maeda K, Iijima T. Involvement of substance P, mast cells, TNF-alpha and ICAM-1 in the infiltration of inflammatory cells in human periapical granulomas. J Oral Pathol Med. 2002; 31:175–180. [PubMed: 11903825]
37. Shimauchi H, Takayama S, Imai-Tanaka T, Okada H. Balance of interleukin-1 beta and interleukin-1 receptor antagonist in human periapical lesions. J Endod. 1998; 24:116–119. [PubMed: 9641143]
38. Safavi KE, Rossomando EF. Tumor necrosis factor identified in periapical tissue exudates of teeth with apical periodontitis. J Endod. 1991; 17:12–14. [PubMed: 1895033]

39. Takeichi O, Saito I, Tsurumachi T, Moro I, Saito T. Expression of inflammatory cytokine genes in vivo by human alveolar bone-derived polymorphonuclear leukocytes isolated from chronically inflamed sites of bone resorption. Calcif Tissue Int. 1996; 58:244–248. [PubMed: 8661955]

40. Takahashi T, Kondoh T, Fukuda M, Yamazaki Y, Toyosaki T, Suzuki R. Proinflammatory cytokines detectable in synovial fluids from patients with temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85:135–141. [PubMed: 9503445]

41. Nordahl S, Alstergren P, Kopp S. Tumor necrosis factor-alpha in synovial fluid and plasma from patients with chronic connective tissue disease and its relation to temporomandibular joint pain. J Oral Maxillofac Surg. 2000; 58:525–530. [PubMed: 10800908]

42. Alstergren P, Kopp S. Insufficient endogenous control of tumor necrosis factor-alpha contributes to temporomandibular joint pain and tissue destruction in rheumatoid arthritis. J Rheumatol. 2006; 33:1734–1739. [PubMed: 16960936]

43. Markham A, Lamb HM. Infliximab: a review of its use in the management of rheumatoid arthritis. Drugs. 2000; 59:1341–1359. [PubMed: 10882166]

44. Bresnihan B, Cunnane G. Interleukin-1 receptor antagonist. Rheum Dis Clin North Am. 1998; 24:615–628. [PubMed: 9710890]

45. Khayat BG, Byers MR, Taylor PE, Mecifi K, Kimberly CL. Responses of nerve fibers to pulpal inflammation and periapical lesions in rat molars demonstrated by calcitonin gene-related peptide immunocytochemistry. J Endod. 1988; 14:577–587.

46. Byers MR, Taylor PE, Khayat BG, Kimberly CL. Effects of injury and inflammation on pulpal and periapical nerves. J Endod. 1990; 16:78–84. [PubMed: 2388022]

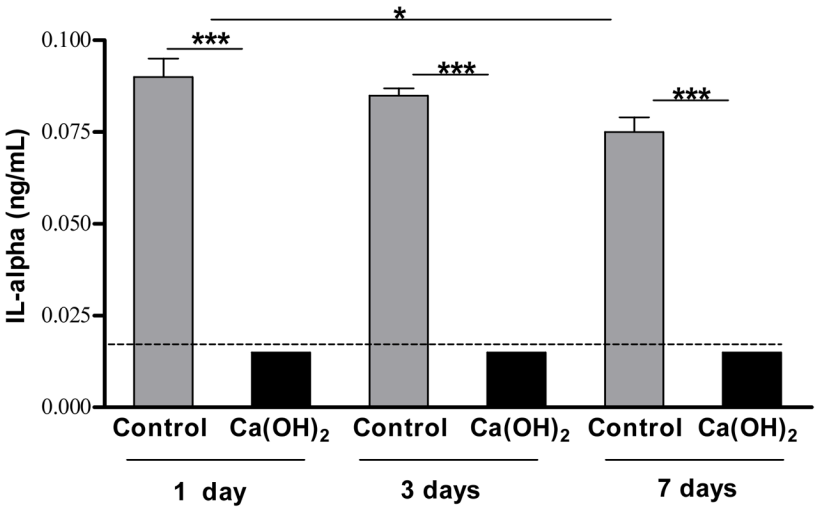

Figure 1.

Calcium hydroxide denatures IL-1α in vitro. Human IL-1α (0.125 ng/ml) was incubated with calcium hydroxide (0.035%) for 1, 3 and 7 days at 37°C. Controls were samples prepared under identical conditions but which were incubated with sterile phosphate buffered saline. Dashed line across the graph represents the minimal detectable concentrations of IL-1α in the ELISA kit used. Data were analyzed using one-way ANOVA and Bonferroni's multiple comparison tests. ***$p<0.001$; *$p<0.05$

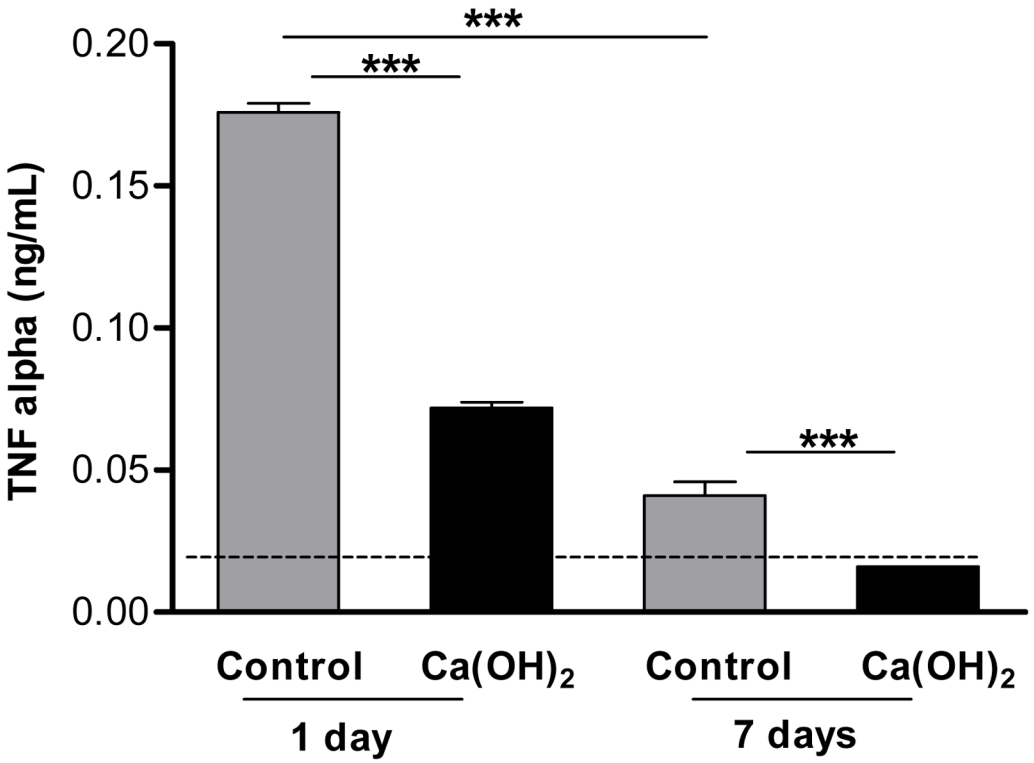

Figure 2.

Calcium hydroxide denaturesTNFα in vitro. Human TNFα (0.2 ng/ml) was incubated with calcium hydroxide (0.035%) for 1 and 7 days at 37°C. Controls were samples prepared under identical conditions but which were incubated with sterile phosphate buffered saline. Dashed line across the graph represents the minimal detectable concentrations of TNFα in the ELISA kit used. Data were analyzed using one-way ANOVA and Bonferroni's multiple comparison tests. ***$p<0.001$

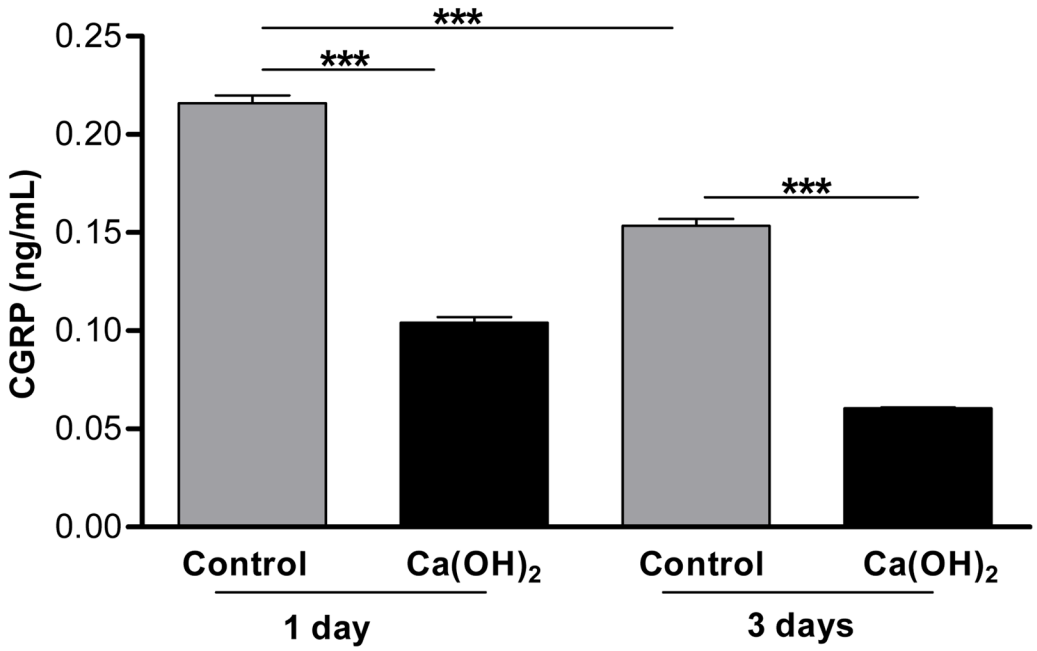

Figure 3.

Calcium hydroxide denatures CGRP in vitro. CGRP was incubated with calcium hydroxide (0.035%) for 1 and 3 days at 2°C. Controls were samples prepared under identical conditions but which were incubated with sterile phosphate buffered saline. Data were analyzed using one-way ANOVA and Bonferroni's multiple comparison tests. ***$p<0.001$