Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article ANTI-INFLAMMATORY EFFECT OF AMALGAM ON PERIAPICAL LESION CELLS IN CULTURE

ANTI-INFLAMACIJSKI EFEKAT AMALGAMA NA ĆELIJE IZ PERIAPEKSNE LEZIJE U KULTURI

Authors Mile Eraković*, Miloš Duka*, Marina Bekić†, Marijana Milanović‡, Sergej Tomić†, Dragana Vučević‡, Miodrag Ćolić†‡ ||, Vojnosanitetski pregled (2019); Online First April, 2019.

UDC:

DOI: https://doi.org/10.2298/VSP190225043E

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
ANTI-INFLAMMATORY EFFECT OF AMALGAM ON PERIAPICAL LESION CELLS IN CULTURE

ANTI-INFLAMACIJSKI EFEKAT AMALGAMA NA ĆELIJE IZ PERIAPEKSNE LEZIJE U KULTURI

Mile Eraković*, Miloš Duka*, Marina Bekić†, Marijana Milanović‡, Sergej Tomić†, Dragana Vučević‡, Miodrag Ćolić†‡||

* Clinic for Stomatology, Military Medical Academy, Belgrade, Serbia
† Institute for the Application of Nuclear Energy, Zemun, Serbia
‡ University of Defense, Medical Faculty of the Military Medical Academy, Belgrade, Serbia
|| University of East Sarajevo, Medical Faculty Foča, R.Srpska, BiH

Correspondence: Miodrag Ćolić, University of Defense, Medical Faculty of the Military Medical Academy, Crnotravska 17, Belgrade, Serbia

e-mail: mjolic@eunet.rs
Abstract

Background/Aim. Amalgam has been used for years in dentistry, but controversy on its adverse effects both on local oral / dental tissues and systemic health still exists. When used for retrograde filling in apical surgery, amalgam comes in close contact with periapical tissue and sometimes is responsible for induction of periapical lesion (PL) or its exacerbation. Therefore, our aim was to examine the effect of amalgam on cytotoxicity and production of pro-inflammatory cytokine by cells isolated from PL. Methods. Conditioned medium from freshly prepared amalgam (ACM) was performed according to ISO 10993-12, by incubating the alloy in RPMI medium (0.2g/ml) for 3 days at 37°C. Cells were isolated from 20 human PLs after apicotomy by collagenase/DNA-ase digestion and cultured with different dilutions of ACM. Cytotoxicity was determined by MTT (n=7 cultures) and apoptosis/necrosis assays (n=8 cultures), whereas cytokine production was measured by a Flow Cytomix Microbeads Assay (n= 8 cultures). Results. Undiluted (100%) and 75% ACM was cytotoxic due to induction of apoptosis of PL cells. Non-cytotoxic concentrations of ACM (50% and 25%) inhibited the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8), concentration dependently. Conclusion. Our results showed for the first time an unexpected anti-inflammatory property of amalgam on PL cells, which could be beneficial for PL healing after apicotomy.

Key words: amalgam, periapical lesion, cytotoxicity, cytokines, inflammation.

Apstrakt

Uvod/Cilj. Amalgam se godinama koristi u stomatologiji, ali i dalje postoje kontroverze o njegovim neželjenim efektima na lokalno oralno/dentalno tkivo i sistemsko zdravlje. Kada se koristi za retrogradno punjenje u apikalnoj hirurgiji, amalgam dolazi u blizak kontakt sa periapeksnim tkivom, što je ponekad povezano sa indukcijom periapeksne lezije (PL) ili njenom egzacerbacijom. Zato je cilj ovog rada bio da se ispita efekat amalgama na
citotoksičnost i produkciju pro-inflamacijskih citokina od strane ćelija izolovanih iz PL. 

Metode. Od sveže napravljenog amalgama je pripremljen kondicionirani medijum (ACM) inkubiranjem legure na 37°C u RPMI medijumu u toku 3 dana (0.2g/ml) kako je predloženo standardom ISO 10993-12. Ćelije su izolovane iz 20 humanih PL nakon apikotomije, digestijom tkiva pomoću kolagenaze/DNA-aze, a zatim korišćene za kulturu u prisustvu različitih razblaženja ACM. Citotoksičnost je rađena pomoću MTT testa (n= 7 kultura) i detekcijom apoptoze/ nekroze (n= 8), dok je nivo produkovanih citokina meren simultano pomoću eseja sa mikroguglicama uz pomoć protočne citometrije (n= 8).

Rezultati. Nerazblažen (100%) i 75% ACM su pokazali citotoksični efekat, indukujući apoptozu PL ćelija. Necitotoksične koncentracije ACM (50% i 25%) su inhibirale produkciju pro-inflamacijskih citokina (TNF-α, IL-1β, IL-6 i IL-8) na dozno-zavisan način.

Zaključak. Naši rezultati po prvi put pokazuju neočekivano anti-inflamacijsko svojstvo amalgama na PL ćelije, što može biti korisno za zarastanje lezije nakon apikotomije.

Ključne reči: amalgam, periapeksna lezija, citotoksičnost, citokini, zapaljenje.

Introduction

Dental amalgam is one of the most versatile restorative materials used in dentistry for about 170 years, particularly as a first choice for restoring posterior teeth. However, it has a myriad of uses, including root end filling in periapical surgery. This procedure prevents the invasion of irritants from infected root canals into the periapical tissues. The advantage of using amalgam for retrograde filling for so long period of time is its self-sealing capacity, easy manipulation, radio-opacity and insolubility in tissue fluids. The preferred amalgam is high copper-zinc free amalgam, composed of silver 40–70%, tin 12–30% and copper 12–24%. However, it has many disadvantages such as production of corrosive by products, cytotoxicity of mercury and other dissolved metal ions, moisture sensitivity and staining of hard and soft tissues. There is possibility to release of non-resorbable scattered particles during amalgam manipulation, which may be difficult to retrieve. Also, amalgam does not seal properly the root end three-dimensionally, has poor marginal adaptation and does not prevent the leakage of microorganisms and their products in the
peri-radicular tissue\(^2\). However, in spite of these disadvantages and evidence of a decrease in its use, amalgam’s cost, durability and ease of manipulation have persuaded many dentists to continue to use it, and amalgam remains as a standard to which other materials are compared\(^2,8\).

The major concern of using amalgam in dentistry is its cytotoxic effect, which has been documented in many human and animal cells as well as established cell lines in vitro\(^6,9-11\). In the past few decades, however, potential systemic and local toxic effects have been described in vivo\(^2,3,12,13\). Patients may suffer from hypersensitivity reactions to mercury or other amalgam components. Other reactions to amalgam with a variety of clinical symptoms, collectively termed “amalgam disease,” have been reported, including adverse immunological effects and autoimmune phenomena\(^12,14,15\).

Clinical and histopathological studies show that amalgam, implanted subcutaneously or in the bone is well tolerated\(^16,17\). This is in contrast with some studies showing the capability of amalgam particles to cause periapical lesion\(^18\) and to cause cytotoxic effect on periodontal ligament cells and periodontal fibroblasts\(^19-21\). However, there is no study investigating the effect of amalgam on human PL cells in vitro, which was the main goal of our study. This knowledge is important since the alloy communicates with the periapical tissue for a long period of time. Our results showed for the first time an unexpected anti-inflammatory effect of amalgam on PL cells which could be beneficial for PL healing.

**Methods**

*Periapical lesion samples*

Human PLs (n =20) were extracted during apicotomy at the Department for Oral Surgery, Clinic for Stomatology, Military Medical Academy (MMA), Belgrade, Serbia. The study was approved by the Ethical Committee of MMA, followed by an informed consent from patients. The exclusion criteria were patients with malignant, autoimmune and other chronic inflammatory diseases as well as those on immunosuppressive/immunomodulatory therapy. The patients included had not been treated with antibiotics for one month before PLs excision. PLs were diagnosed by clinical and radiographic criteria. No distinction between age, sex, tooth type, size and clinical presentation of PLs was made. After extraction, PLs were immediately placed in a medium consisting of RPMI-1640 (Sigma, Munich, Germany) and antibiotics/antimycotics, and transported to the laboratory.
Isolation of cells from PLs

The cells from PLs were isolated by a procedure which has been previously introduced by our research group\textsuperscript{22}. Briefly, periapical tissue was placed in a Petri dish containing 1 ml RPMI-1640 medium and cut into 2–3 mm diameter pieces using a scalpel. The tissue was then digested for 20 min with 0.05% collagenase type IV (Sigma) and 0.02% DNAse (Sigma) dissolved in RPMI-1640 medium in a cell incubator at 37°C. After that, the tissue was pressed through a stainless-steel mesh using a syringe plunger, filtered and resuspended in RPMI-1640 medium containing 1 mM EDTA. The released cells were pooled, washed twice by centrifugation in the RPMI medium at room temperature (400 g for 10 min), and counted. The viability of cells, determined by Trypan Blue dye, was 93±3%. The cells were used for \textit{in vitro} experiments. Eight periapical lesions were used to study cytokine production and apoptosis/necrosis. Twelve PLs containing either larger number of cells (higher than 2.0×10^6 cells; n= 4 PLs) or pooled PLs from the same donors (n=8 PLs from 3 patients) were used for MTT assay. The total number of individual cultures for this assay was 7.

Preparation of conditioned medium

Amalgam, consisting of encapsulated alloy (Extracap) and mercury was purchased from Galenika, Belgrade, Serbia. One-gram (g) powder of the alloy contained silver (500 mg), tin (299 mg) and cooper (201 mg). The alloy mass was 0.360 g and mercury mass was 0.400 g. Amalgam specimens were prepared by triturating amalgam alloy powder with pure mercury in an amalgamator, and after mixture, disc-form specimens, diameter around 10 mm, thickness about 1-2 mm, were prepared. The freshly prepared amalgam discs were used for preparation of amalgam conditioned medium (ACM) by placing the amalgam disc in a glass tube containing RPMI-1640 medium with addition of antibiotics/antimycotics. The mass of amalgam to volume of RPMI medium was 0.2 g/ml according to ISO 10993:5 and ISO 10993:12. Conditioning was lasted for 3 days. Control CM was prepared by incubating control inert material, polystyrene, under same conditions. ACM and control
(C)-CM were supplemented with 10% FCS. There was no need for pH adjustment which was remained 7.4. Such prepared CM were further used for PL cell culture experiments.

**Cell cultures**

The cells isolated from PLs were cultivated in 96-wells, with round-bottomed plates (ICN, Costa Mesa, CA) (1×10^5 cells/well, 200 µl) in the complete culture medium consisted of RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) (Sigma) and standard culture solutions of antibiotics^22. The cultures were treated with different dilutions of ACM or C-CM. Undiluted CM is considered as 100% CM. After 24 h, the cell supernatants were collected, centrifuged and frozen at −70 °C until the levels of cytokines were determined. The cells were used for apoptosis/necrosis assay.

**MTT assay**

PL cells were cultivated in 96-well plates (1×10^5/well; triplicates), in either fresh complete RPMI medium, different dilutions of ACM or C-CM. After a 24-hour incubation period, the plates were centrifuged and the medium was carefully removed. The solution of 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) (100 µl/well, final concentration 100 µg/ml), was added. Wells with a MTT solution without cells served as blank controls. The plates were incubated with MTT for 3 hours in an incubator at 37ºC. Dissolution of formazan was done by incubated the MTT-treated cultures with 0.1N HCl / 10% SDS (sodium dodecyl sulphate) (100µl/well) overnight. Next day the optical density (OD) of the developed colour was read at 570/650 nm (ELISA reader, Behring II). The results were expressed as the relative metabolic activity compared to the metabolic activity of control cultures.

The relative metabolic activity was calculated as follows: metabolic activity (%) = (OD of cultures with ACM / OD of cultures with control fresh medium) x 100.

**Apoptosis/necrosis assay**

Apoptosis/necrosis was detected by Annexin-V–fluorescein isothiocyanate (FITC) and Propidium Iodide (PI) staining kit (R&D), following the manufacturer’s protocol. Briefly, cultivated PL cells were collected, washed with binding buffer, followed by incubation with Annexin-V–FITC and PI. The labeled cells were analyzed on a flow cytometer.
Annexin-V-FITC$^+$ cells were recognized as primary apoptotic cells (early phase of apoptosis), PI$^+$ cells were primary necrotic cells, whereas double positive cells were apoptotic/secondary necrotic cells (late phase of apoptosis).

**Cytokine assays**

The concentrations of IL-1β, IL-6, IL-8 and TNF-α in culture supernatants were detected by a FlowCytomix Microbeads Assay. This is a bead-based ELISA-like assay optimized for flow cytometry, allowing for the simultaneous detection of several cytokines in a volume of sample (50µl). The inflammation kit containing microbeads coupled with antibodies to pro-inflammatory cytokines was purchased from Biolegend. The levels of cytokines were determined by constructing standard curves based on known concentration of these cytokines.

**Statistical analysis**

Student t-test was used for comparison of parametric variables between two groups. Friedman test (paired one-way ANOVA) was used for comparison between groups for non-parametric variables with Dunn's multiple comparison post-test. The values of p<0.05 were considered to be statistically significant. Software SPSS version 23.0 (IBM, Armonk, New York, USA) was used to analyze the data.

**Results**

The first aim of this study was to examine the cytotoxicity of ACM on PL cells in culture. By using the MTT test (Fig.1), we showed that only concentrated (100%) and 75% ACM significantly reduced the viability of PL cells (p<0.001 and p<0.01, respectively). The cytotoxicity was due to the induction of apoptosis (Fig.2A and 2B). Fig. 2B shows that ACM increased the proportion of late apoptotic/secondary necrotic cells.

The second aim was to investigate the effect of ACM on the production of pro-inflammatory cytokines (IL-1β, TNFα, IL-6 and IL-8) by PL cells. We used non-cytotoxic concentrations (50%, 25% and 12.5%) of ACM. The 50% and 25% concentrations of ACM suppressed the production of all four cytokines dose-dependently (Fig. 3), whereas 12.5% concentration did not show any modulatory effect (data not shown).
Discussion

The first aim of this study was to examine the cytotoxicity *in vitro* of a copper, zinc-free amalgam, which is the oldest root end filling material in apical surgery. Apicotomy is a common procedure to remove periapical lesions (granuloma or cysts) when the conventional endodontic treatment is not efficacious. Amalgam has been still used for this purpose because of its self-sealing capacity, radio-opacity, insolubility in tissue fluids and low price. However, since amalgam does not seal properly the root end three-dimensionally, has poor marginal adaptation and does not prevent successfully the leakage of microorganisms in the peri-radicular tissue\(^{1-3, 23}\), our hypothesis was that amalgam, due to their cytotoxic effect, could aggravate periapical inflammation. Therefore, cells isolated from PLs, which are dominantly composed of infiltrating inflammatory cells\(^{22, 24}\), were the most suitable target to test this hypothesis and this was our original approach.

Before starting with crucial experiments, it was necessary to determine the cytotoxicity of amalgam by using this culture model. Up to now, many different tests have been used for assessment of amalgam cytotoxicity, but MTT, which is based on the evaluation of cellular metabolic activity, is the most acceptable as first screening assay\(^6\). It is known that amalgam causes cytotoxicity either in direct contact with examined cells or indirectly by metallic ions released from the alloy\(^2, 6, 9\). We decided to study the effect of amalgam indirectly by analyzing the effect of ACM in which its leachable products are present and which are considered as the dominant cytotoxic factors\(^9, 25\). The study was conducted exactly as recommended by the ISO 10993:5 standard. We showed that only high concentrations of ACM (concentrated and 75%) were cytotoxic for PL cells due to apoptosis induction, suggesting that amalgam is generally cytotoxic alloy similarly as shown on other target cells. A relatively high proportion of apoptotic cells were also observed in control PL cell cultures and the most sensitive cells were granulocytes, followed by macrophages, whereas lymphoid cells were more resistant (data not shown). These observations are in line with already known facts about high apoptotic rate of extravasated neutrophils, as terminally differentiated cells\(^26\).

We did not examine the concentrations of released ions from amalgam because this has been extensively investigated and published\(^5, 6\). Actually, all metal ions can be released in
CM from amalgam such as mercury, silver, cooper and thin. Of them, cooper is the most cytotoxic, but it can be hypothesized that other ions act synergistically in inducing cytotoxicity. This hypothesis was based on previous publications which investigated thoroughly the release and cytotoxicity of metal ions from amalgams of different composition. In this context, Kaga et al. have demonstrated that pure copper showed the highest cytotoxicity among the metals tested in zinc-free amalgams. Silver and mercury showed a reduced cytotoxicity, and tin was non-cytotoxic. In contrast, zinc-containing amalgams are more cytotoxic due to easy release of Zn ions. The toxic effects of mercury are believed to be due to the high reactivity of mercury species toward thiol-groups and other functional groups, notably in proteins. It has been shown that both organic and inorganic mercury induce apoptosis of different cells including human lymphocytes.

The second part of this work was related to the effect of ACM on the production of pro-inflammatory cytokines by PL cells. We tested non-cytotoxic concentrations of ACM because toxic concentrations could not be relevant for a proper conclusion, partly due to the spontaneous release of cytokines from dead cells. We observed an unexpected result that ACM at non-cytotoxic concentrations significantly inhibited the secretion of pro-inflammatory cytokines (IL-1β, TNFα, IL-6 and IL-8). Therefore, our hypothesis was rejected.

The anti-inflammatory effect of ACM is contrary to published data about the pro-inflammatory effect of amalgam particles which could be able to induce the PL development if released into periapical tissue during endodontic surgery. Similarly, amalgam has been found to cause inflammatory response in the dental pulp, which is transitory and significantly decreased in due time. These differences (pro-inflammatory versus anti-inflammatory properties of amalgam) can be explained by the difference in setting experiments. Namely, cytotoxic effects of amalgam on periodontal tissue in vivo can provoke an inflammatory reaction due to the direct contact, where in the vicinity of the alloy relatively high concentrations of cytotoxic metallic ions can be released. This effect dominates over anti-inflammatory effects seen at non-cytotoxic concentrations of leachable amalgam components.
No one published study related to amalgam examined the changes of multiple pro-inflammatory and other cytokines. The most relevant paper is that published by Shendle et al.\textsuperscript{30} who investigated the effects of dental amalgam on cytokine production by human peripheral blood mononuclear cells (PBMC) from healthy donors. To induce cytokine production, they stimulated PBMC in culture with lipopolysaccharide, phytohemagglutinin, or staphylococcal enterotoxin A in the presence of fresh amalgam, aged amalgam or ACM prepared from fresh amalgam. They showed that freshly prepared amalgam as well as ACM reduced the production of interferon-\(\gamma\) (IFN-\(\gamma\)) and IL-10, but increased the levels of TNF-\(\alpha\). Both fresh amalgam and ACM had no effects on the levels of IL-2, IL-6, or granulocyte-macrophage colony-stimulating factor. Amalgam aged for 6 weeks did not modulate the concentrations of any of the above cytokines. To investigate which heavy metal cations released from amalgam caused the observed immunomodulatory effects, Cu\textsuperscript{2+}, Hg\textsuperscript{2+}, and Sn\textsuperscript{2+}, which were detected in ACM, were added as salts to PBMC cultures. Cu\textsuperscript{2+} and Hg\textsuperscript{2+} decreased the IFN-\(\gamma\) and IL-10 levels. However, Hg\textsuperscript{2+} increased TNF-\(\alpha\) concentrations, whereas Sn\textsuperscript{2+} had no modulatory effect.

It is evident that our results, showing a decrease of TNF-\(\alpha\) production, are opposite. The difference could be due to the following reasons, respectively: different concentrations of ACM (concentrated \textit{versus} diluted ACM); different cells (stimulated PBMC \textit{versus} non-stimulated PL cells); different mass/volume ratio for ACM preparation (1.92g/ml \textit{versus} 0.2g/ml); different incubation time for cell cultures (48 h \textit{versus} 24 h). Some other studies investigated the effect of mercury. In this context, Soleo \textit{et al.}\textsuperscript{31} showed an increase in the number of CD4\textsuperscript{+} cells in peripheral blood of subjects exposed to mercury from dental amalgam together with a decrease of serum IL-8 levels. Podzimek \textit{et al.}\textsuperscript{32} examined cytokine production (IL-1\(\beta\), IL-4, IL-6, TNF-\(\alpha\) and IFN-\(\gamma\)) by human lymphocytes in cultures treated with mercury and found increased production of TNF-\(\alpha\) and IFN-\(\gamma\). Ilday \textit{et al.}\textsuperscript{33} observed reduced clinical periodontal findings in patients after overhang amalgam restoration removal but these findings did not correlate with the changes in the levels of IL-6, IL-8 and TNF-\(\alpha\) in gingival crevicular fluid. This is in contrast with another study, published previously, which showed that removal of dental amalgam restorations was associated with decreased concentrations of Th1-type pro-inflammatory cytokines in serum, supporting the hypothesis that amalgam could be responsible for stimulation of the Th1-type response \textit{in vivo}\textsuperscript{34}.
It is known that cytokines play a key role in the pathogenesis of PLs. Pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α, orchestrate the recruitment and activation of innate immune cells, presumably neutrophil granulocytes and monocytes in the early inflammatory phase and T and B cells in the later inflammatory phase, respectively. In this context cytokines of T cells are main controllers of the immune/inflammatory reactions. T-helper 1 (Th1) cells and Th-17 cells, by producing interferon-γ (IFN-γ) and IL-17, respectively, are involved in the progression of PLs and bone destruction, whereas T-helper 2 (Th2) cytokines, such as interleukin 4 (IL-4), IL-5, IL-10 and IL-33, are involved in the humoral immune response and attenuation of the tissue damage. Therefore, further experiments investigating the effect of amalgam on this panel of cytokines could make much better conclusion.

Conclusion

By using inflammatory cells isolated from human PL, we showed for the first time a potent anti-inflammatory effect of non-cytotoxic concentrations of ACM. This finding is in contrast with previous findings that particulate amalgam particles, released during retrograde filling, can cause chronic apical periodontitis. Our results suggest that, in contrast to high release of toxic ions from amalgam, slow release of leachable components from this amalgam, by down-modulating the production of pro-inflammatory cytokines, may control an excessive inflammation and promote PL healing.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development, R.Serbia (grant No:OI 175102) and grants from Ministry of Defence, R.Serbia (MFVMA/7/17-19 and MFVMA/9/16-18).

Fondacije / institucije — Ministarstvo za prosvetu, nauku i tehnološki razvoj, R.Srbije.

Naziv projekta — Primena funkcionalizovanih ugljeničnih nanocevi i nabočestica zlata za pripremu dendritiških ĉelija u imnoterapiji tumora.

Oznaka projekta — OI 175102.

Fondacije / institucije — Ministarstvo odbrane, R.Srbije.
Naziv projekta — Biokompatibilnost i imunomodulacijska svojstva biomaterijala.

Oznaka projekta — MFVMA/7/17-19.

Fondacije / institucije — Ministarstvo odbrane, R.Srbije.

Naziv projekta — Regulatorni mehanizmi u zapaljenskim i imunskim reakcijama.

Oznaka projekta — MFVMA/9/16-18.

References


Figure legends

Fig. 1– Cytotoxicity effect of amalgam on periapical lesion cells in culture. Periapical lesion (PL) cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The viability of PL cells was determined by the MTT test, as described. Values are given as mean±SD (n= 7 cultures) of relative metabolic activity of cells. **p<0.01; ***p<0.001 compared to control cultures. FM= Fresh Medium; C-CM= Control Conditioning Medium
Fig. 2 – Effect of amalgam on apoptosis of periapical lesion cells in culture. Periapical lesion (PL) cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The apoptosis of PL cells was determined by the Annexin V-FITC/PI assay, as described. A) Values are given as mean±SD (n=8 cultures) of apoptotic cells. *p<0.05; **p<0.01 compared to control cultures. B) Representative histograms showing that ACM accelerate apoptosis of PL cells, manifested by an increase of late apoptotic/secondary necrotic cells. CM= Control Medium
Fig. 3 – Effect of amalgam on the levels of pro-inflammatory cytokines in culture of PL cells. Periapical lesion (PL) cells, prepared as described in Materials and methods, were cultured with different dilutions of amalgam conditioned medium (ACM) for 24 hours. The levels of pro-inflammatory cytokines in culture supernatants were determined by Flow Cytomix Microbeads Assay. Values are given as mean±SD (n= 8 cultures) levels of cytokines; * = p<0.05; ** = p<0.01; *** = p<0.001 compared to control cultures or compared to 50% ACM (indicated by corresponding bars). CM= Control Medium.

Received on February 25, 2019.
Accepted March 19, 2019.
Online First April, 2019.