Original Article

The Influence of Galvanic Currents and Voltage on the Proliferation Activity of Lymphocytes and Expression of Cell Surface Molecules

(metal / galvanism / lymphocyte / CD molecules / oral discomfort)

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Abstract. Release of metal ions from dental metal fillings supported by galvanism can cause local or general pathological problems in sensitive and genetically susceptible individuals. We aimed to investigate *in vitro* lymphocyte responses and expression of surface molecules influenced by galvanic currents and voltage. Human peripheral blood lymphocytes were influenced by galvanic currents and voltages and lymphocyte proliferation was measured. Control samples were not exposed to the influence of galvanism. We also studied the expression of surface molecules by the FACS analysis. A 15-h and shorter influence of almost all tested currents and voltages caused a significant decrease in lymphocyte proliferation and the 15-h influence of 20 μA currents significantly increased expression of surface molecules CD 19, 11a/18, 19/69 and 19/95. An influence of 10 and 3 μA currents led to a significant decrease in the expression of surface molecules CD 3, 11a/18, 3/69 and 3/95 and to a significant increase in CD 19 expression. An 80 mV voltage influence led to a significant decrease in the expression of surface molecules CD 3, 11a/18, 3/69, 3/95, 19/69 and 19/95, and 200 and 300 mV voltages significantly decreased the expression of surface molecules CD 3, 19, 11a/18, 3/95 and 19/95 and significantly increased CD 19/69 expression. A long-lasting influence of galvanism can, in sensitive and genetically susceptible individuals, influence lymphocyte proliferation and surface molecule expression. The threshold for pathological values of 5 μA for galvanic currents and 100 mV for galvanic voltage was confirmed.

Introduction

A release of metal ions from dental metal fillings supported by galvanism can cause local or general pathological problems in sensitive and genetically susceptible individuals.

All alloys used in dentistry undergo, more or less, mechanical and electrochemical changes, which may cause oral discomfort (Wataha, 2000). The intensity of galvanic effect is determined by the difference of electrode potentials between the casual metals (Gjerdet, 1980; Bergman et al., 1982). This effect is further influenced by creation and function of passivation layers on the metal-electrolyte interface (Syrjanen et al., 1984).

Release of metal ions from the dental alloys depends not only on their composition, but also very significantly on the quality of their processing (Bergman et al., 1978; Nilner et al., 1982a; Johansson, 1986; Wataha, 2000; Walker et al., 2003; Valentine-Thon et al., 2006).

Protecting passivation layers are continuously damaged by abrasion (Watson and Wolcott, 1976). Neither abrasion nor corrosion can be completely eliminated but can be minimized by the choice of suitable materials and strict observance of the optimal technology (Wataha, 2000).

The presence of different metal alloys in the oral cavity may influence induction and adverse effects of elec-

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Abbreviations: AB+ – variety of human blood serum, CD – cell surface molecule, Con A – concanavalin A, FACS – fluorescence-activated cell sorting, PWM – pokeweed mitogen, mitogen from Phytolacca americana.

trochemical corrosion, in which the metallic materials act as electrodes and the liquids in the oral cavity, such as saliva and crevicular fluid, act as electrolytes (Holland, 1980; Arvidson and Johansson, 1985; Yontchev et al., 1989).

Electrodes with different potentials have the tendency to change the potential difference. This occurs by an electric current passing from one electrode to the other in the conductive environment of the oral cavity (Sutow et al., 2004).

An objective characterization of the galvanic effects may be attained by detection of metal elements in saliva and/or by measurement of galvanic potentials and currents (Nilner et al., 1982a, b; Nogi, 1989; Wataha, 2002; Sutow et al., 2004; Prochazkova et al. 2006).

A galvanic current of 5 μA was considered as the limit of pathological values (Nilner et al., 1982a; Axell et al., 1983; Hampf et al., 1987; Kucerova et al., 2002; Prochazkova et al. 2006).

These processes may affect the development of inflammation of the oral mucosa and the tongue, paresthesia, glossodynia, stomatodynia, hyperaemia of the pulp, neuralgia, etc. An electric current may also manifest its effects in mucosal changes.

In vitro studies of low-frequency electromagnetic field effects have revealed a variety of sensitive cell-physiologic end-points. Effects have been reported on DNA, RNA and protein synthesis, cell proliferation, cation fluxes and binding, immune responses and membrane signal transduction. Typically, such effects occurred as a short-term exposure result of cells to electromagnetic fields at frequencies of 100 Hz or less and at low field intensities (Cleary, 1993).

Long-term electromagnetic field exposure leads to a chronically increased level of free radicals and could lead to a higher incidence of DNA damage and therefore to an increased risk of tumour development (Simko and Mattsson, 2004).

The aim of this study was to investigate the influence of galvanic currents and voltage on lymphocyte activity *in vitro* using pokeweed mitogen (PWM)- and concanavalin A (Con A)-stimulated proliferation and using expression of the surface molecules CD 3, 19, 11a, 18, 69 and 95.

Material and Methods

Lymphocytes

Human peripheral blood lymphocytes gained from the buffy coat and diluted to the concentration of 10^6 cells/ml were influenced by galvanic currents (3, 10 and 20 μA) and voltages (80, 200 and 300 mV) for 30, 60, 120 min and for 15 h as well in a specifically designed micro-titration plate.

Human peripheral blood lymphocytes used in the experiments were gained from the buffy coat received from Institute of Haematology and Blood Transfusion, Charles University, Prague. This experimental material was received without any information about the donor and no identification of the donor was thus possible.

Instruments

To study the problem the researchers of the Institute of Biomedical Engineering, Czech Technical University, Prague, Czech Republic designed, developed and created two sets of voltage generators for values of 80, 200 and 300 mV and two sets of current generators for values of 3, 10 and 20 μA. The devices allow influencing peripheral blood cells by different values of currents and voltage within one experiment.

The generator of voltage represents a fusion of three independent sections formed by voltage sources with fixed values of 80, 200 and 300 mV.

The generator of currents also represents a fusion of three independent sections formed by current sources with fixed values of 3, 10 and 20 μA.

The devices can be connected by connectors with micro-titration 48-well culture plates.

Procedure

The buffy coat was layered on Ficoll-Paque gradient and centrifuged at 600 *g* for 30 min. Mononuclear cells were collected from the interface, washed twice and mixed with 5 ml of cell culture medium RPMI 1640 (**vyrobce, sidlo, stat**) containing 20 % of AB+ serum (variety of human blood serum, **dodavatel, sidlo, stat**). Plastic-adherent cells were partly depleted from leukocyte suspension by incubation on plastic surfaces for 40 min at 37 °C. After incubation, the lymphocytes were counted and diluted with RPMI 1640 medium enriched with 10 % of AB+ serum and glutamine to a final dilution of $1x10^6$ cells/ml. The cells were influenced by galvanic currents and voltages in a 48-well culture plate for different time periods, they were stimulated by PWM (**vyrobce, sidlo, stat**) and Con A (**vyrobce, sidlo, stat**) and cultivated five days in an atmosphere of 5 % CO_2 in humidified air at 37 °C. Samples with no influence of galvanism and no stimulation were observed as well as samples with influence of galvanism and no stimulation. We also studied the expression of surface molecules after the galvanic action for 24 h by the FACS analysis.

Five experiments were performed with 30, 60 and 120 min exposure to galvanic currents of 3, 10 and 20 μA and galvanic voltages of 80, 200 and 300 mV, and because we had enough experimental cell material, an experiment with a 15-h exposure was performed as well. From the results of these experiments it was estimated that a short-term exposure to galvanic currents and voltages would have lower impact on the lymphocytes than a long-term exposure.

The expression of cell surface molecules after a 24-h exposure to galvanic currents of 3, 10 and 20 μA and galvanic voltages of 80, 200 and 300 mV was studied. The expression of cell surface molecules CD 3, CD 19, CD 11a, CD 18, CD 69 and CD 95 was studied using a method of indirect immunofluorescence with labelled monoclonal antibodies CD 3, CD 19, CD 11a, CD 18,

CD 69 and CD 95. After the incubation with monoclonal antibody, cells were prepared for FACS analysis performed in the FACScan (**vyrobce, sidlo, stat**).

Statistics

Data (counts per minute, CPM) were transformed to normality by logarithmic transformation. Differences among groups (different treatments) were evaluated by one-way ANOVA followed by Dunnett's post-hoc test for estimating the difference between the particular group and control. Homoscedasticity and normality of residuals were assessed by modified Levene equal variance test and omnibus normality of residuals test, respectively; potential outliers were removed. The NCSS 2007 (Number Cruncher Statistical Software, Kaysville, UT) statistical package was used.

Results

The results of all experiments are shown in Fig. 1 (results are shown as follows: median: lower to upper quartile range: non-outlier range, significant differences are shown by a star symbol next to the right upper corner of the lower to the upper quartile range bar). In the first row, results of non-stimulated culture of lymphocytes are shown. In the second and third rows, results of lymphocytes stimulated by PWM and Con A are shown. In first three columns, results of influence of galvanic currents of 3, 10 and 20 μA are shown. In the fourth, fifth and sixth columns, results of influence of galvanic voltages of 80, 200 and 300 mV are shown.

The short-term galvanic current action (30, 60 and 120 minutes) did not lead to such a high decrease in lymphocyte proliferation as compared to 15-h influence. Almost all differences between the groups influenced by currents or voltage for 15 h and the control culture group were statistically significant. Generally, the highest significant decrease in lymphocyte proliferation was found after 15-h influence of 20 μA current on cells stimulated by PWM.

The highest significant decreases in lymphocyte proliferation in non-stimulated culture were found after 15-h influence of 80, 200 and 300 mV and 10 and 20 μA as well as after only 30-min influence of 80 mV.

The 15-h influence of 20 μA currents on tested cells stimulated by PWM led to the highest significant decrease of cell proliferation in general; a highly significant decrease of lymphocyte proliferation was also found after the 15-h influence of 10 μA currents. Galvanic voltages did not influence cells stimulated by PWM so markedly as galvanic currents, although all de-

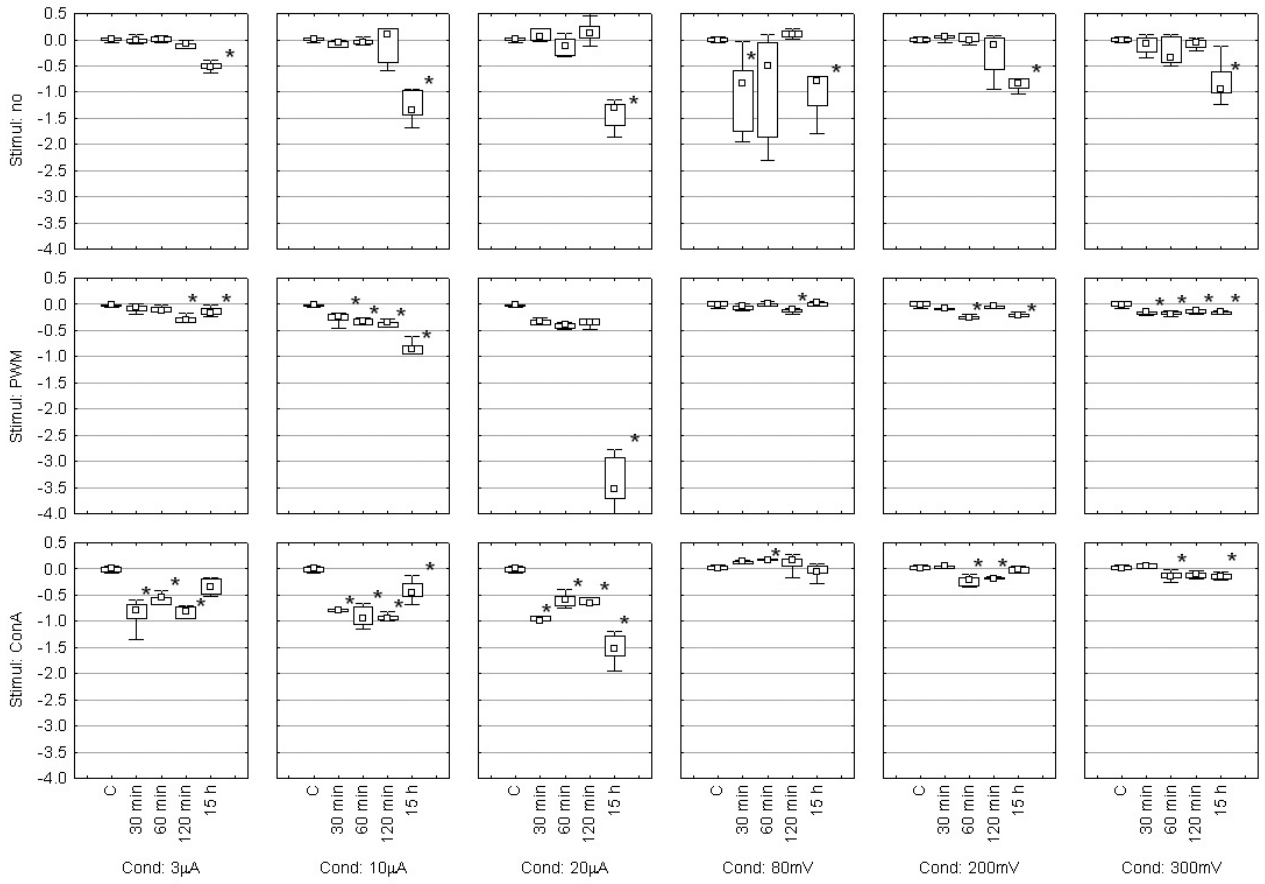

Fig. 1. The influence of galvanic currents and voltages on lymphocyte proliferation (x-axis: control culture (C), influence of 30, 60, 120 min and 15 h, y-axis: logarithmic transformation of CPM values, * – significant difference, $P < 0.05$)

Table 1. The influence of various currents and voltages on the cell surface CD marker expression (in percentage, statistically significant differences ($P < 0.05$) are shown by stars).

	CD 3	CD 19	CD11a/18	CD 3/95	CD 3/69	CD 19/95	CD 19/69
control	66.8	11.4	49.7	25.8	2.6	1.9	0.6
3 μA	54.2 *	12.9 *	40.9 *	14.7 *	1.2 *	1.9	0.6
10 μA	56.6 *	12.3 *	44.0 *	17.0 *	1.6 *	2.0 *	0.9 *
20 μA	65.5	16.1 *	75.8	25.4	2.2 *	3.1 *	1.5 *
80 mV	53.7 *	11.9	32.7 *	12.3 *	1.1 *	1.7 *	0.5 *
200 mV	26.7 *	6.6 *	16.4 *	10.3 *	3.4 *	1.5 *	1.9 *
300 mV	38.0 *	8.5 *	21.6 *	11.2 *	2.2 *	1.7 *	1.2 *

creases after the influence of 300 mV voltage were significant.

The 15-h influence of 20 μA currents to cells stimulated with Con A led to the highest significant decrease in cell proliferation; a significant decrease in this group was also found after shorter than 15-h influence of 3, 10 and 20 μA currents. The results of voltage influence on the cells stimulated with Con A show that, on the contrary, there were no such large differences in cell proliferation after the influence of all tested voltages, although some changes were significant.

We have investigated how the galvanic currents and voltage influence the expression of cell surface markers CD 3 (T lymphocytes), CD 19 (B lymphocytes), CD 11a/18 (adhesive molecules), CD 95 (apoptosis) and CD 69 (activation). The influence of galvanic currents and voltage on CD marker expression was compared to control samples, which were influenced neither by currents nor by voltage. The results of changes in CD marker expression are shown in Table 1.

The expression of surface molecules CD 19, 11a/18, 19/69 and 19/95 was significantly increased by the action of 20 μA current. The expression of surface molecules CD 3, 11a/18, 3/69 and 3/95 was significantly decreased and CD 19 expression was significantly increased by the action of 10 and 3 μA currents. The expression of surface molecules CD 3, 11a/18, 3/69, 3/95, 19/69 and 19/95 was significantly decreased by the action of 80 mV voltage and the expression of surface molecules CD 3, 19, 11a/18, 3/95 and 19/95 was significantly decreased by the action of 200 and 300 mV voltages.

The most important result from these experiments is the trend of increased surface marker expression by the current of 20 μA and on the contrary, the trend of decreased surface marker expression by the voltages of 200 and 300 mV.

Discussion

Most of the patients with oral discomfort problems have a galvanic cell in their oral cavity for a long period of time.

The first results of our study showed that short-term galvanic currents and voltage did not influence the lymphocyte proliferation to such extent as the long-term influence, and these results supported our hypothesis that we should rather focus on long-term influence, which is more similar to *in vivo* galvanic conditions.

The long-term influence of most tested galvanic currents and voltages caused significantly decreased cell proliferation.

Both pathologic values of galvanic currents and voltage therefore led to disturbances in cell functions. These results are in concordance with previously published data (Cleary, 1993).

Proliferation of T lymphocytes was significantly decreased by long-term action of most of the tested galvanic currents and voltages *in vitro* and this decrease could be enhanced *in vivo* by metal ions released from dental metal restorations, which are known to influence the lymphocyte membrane potentials and therefore reduce or block K^+ and Ca^{2+} conductance in the ion channels (DeCoursey et al., 1985) or have dramatic effects on cell functions (Bental and Deutsch, 1994).

Functional K^+ channels are necessary for T-lymphocyte proliferation (Chandy et al., 1985; DeCoursey et al., 1985) and it is therefore possible that pathologic values of galvanic currents and voltage can affect potentials in these channels; this can lead to their block or malfunction and in such a way result in disturbed proliferative activity of T lymphocytes.

Proliferation of B lymphocytes was significantly decreased by galvanic voltages 200 and 300 mV; on the other hand, it was significantly increased by all tested currents; these results point to different ways of action by which cells can be influenced.

Apoptosis of T lymphocytes was significantly decreased by almost all tested galvanic currents and voltages (only after 20 μA influence the decrease was not significant); on the other hand, apoptosis of B lymphocytes was significantly decreased by all tested voltages and there was a significant increase in apoptosis by action of 10 and 20 μA currents.

Activation of T lymphocytes was significantly decreased by all tested currents and voltages; only 200 mV voltage caused a significant increase in the activation. Activation of B lymphocytes was significantly increased by higher values of galvanic currents (10 and 20 μA) and voltages (200 and 300 mV).

Galvanic currents and voltage had a much more profound effect on T-lymphocyte proliferation and functions as compared to B lymphocytes, and this can explain why the patients with oral discomfort and galvanic

cells in their oral cavity improved their health after removal of causal dental metal restorations (Prochazkova et al., 2006).

The values of galvanic currents and voltage intensity that could be considered to be pathological are still discussed and they range from 3 to 10 μA and from 80 to 200 mV (Simsa, 1955; Skach et al., 1963; Kobayashi, 1989). Therefore, we have studied *in vitro* the influence of these and higher values of galvanic currents and voltage on human cell functions.

The long-term effect of galvanism can, in sensitive and genetically susceptible individuals, influence the lymphocyte proliferation and surface molecule expression. Our findings confirm the hypothesis that suffering from the oral discomfort is not only a subjective feeling of the patient but that it is based on real cell discomfort due to the release of metal ions from dental metal fillings and due to galvanism.

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