UNIVERSIDADE FEDERAL DE UBERLÂNDIA

FACULDADE DE ODONTOLOGIA

THAÍS DE CARVALHO FREITAS

EFFECT OF SYNADENIUM CARINATUM LATEX LECTIN (ScLL) ON VIABILITY OF PERIPHERAL BLOOD MONUCLEAR CELLS STIMULATED WITH LIPOPOLYSACCHARIDE

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Trabalho de conclusão de curso apresentado a Faculdade de Odontologia da UFU, como requisito parcial para obtenção do título de Graduado em Odontologia

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"O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínino fará coisas admiráveis."

José de Alencar

SUMMARY

Effect of *Synadenium carinatum* Latex Lectin (ScLL) on viability of peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide (LPS).

Efeito da Lectina *Synadenium Carinatum* (ScLL) na viabilidade de células mononucleares (PBMC) estimulados com lipopolissacarideo (LPS).

ABSTRACT

The *Synadenium carinatum* Latex Lectin (ScLL) is a protein extracted from the latex of a plant. Recently, the anti-inflammatory activity of ScLL protein was evaluated in the oral cavity, suggesting a possible immunomodulatory potential of this substance. It is known that several cell types are present in the gingival and periapical tissues, among them monocytes/macrophages. Monocytes are cells that play essential role in the control of inflammatory responses and wound healing. Therefore, this study evaluated the effect of ScLL on viability of peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide (LPS). The PBMCs were isolated, stimulated with 1µg/ml of LPS and treated with ScLL 10 µg/ml and ScLL 5 µg/ml for 1 h and 5 h. After, the cell viability was evaluated by 3-(4,5-dimeth¬ylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data were analyzed for normality and homoscedasticity using Shapiro-Wilk's and Levene's tests and compared by one-way ANOVA, Kruskal-Wallis test ($p < 0.05$) and Dunnett's test ($\alpha=0.05$). The results showed that ScLL 5 µg/ml had no cytotoxic effect on PBMCs and the groups treated with ScLL showed highest viability than negative control group (LPS). In this way, more studies should be performed to evaluate the potential of ScLL as an immunomodulator and anti-resorption agent for avulsion therapy and periodontal inflammation.

KEYWORDS: Lectin (ScLL), Cell Viability, PBMC

RESUMO

A Lectina Synadenium carinatum (ScLL) é uma proteína extraída do látex de uma planta de cerrado. Recentemente, a atividade anti-inflamatória da proteína ScLL foi avaliada na cavidade bucal, sugerindo um possível potencial imunomodulador dessa substância. Sabe-se que vários tipos celulares estão presentes nos tecidos gengival e periapical, entre eles monócitos / macrófagos. Os monócitos são células que desempenham um papel essencial no controle de respostas inflamatórias e cicatrização de feridas. Portanto, este estudo avaliou o efeito da ScLL na viabilidade de células mononucleares do sangue periférico (PBMC) estimuladas com lipopolissacarídeo (LPS). As PBMCs foram isoladas, estimuladas com 1μg / ml de LPS e tratadas com 10 μg / ml e 5 μg / ml com ScLL por 1 he 5 h. Depois, avaliou-se a viabilidade celular por ensaio de brometo de 3- (4,5-dimetiltiazol-2-il) -2,5-difeniltetrazólio (MTT). Os dados foram analisados quanto à normalidade e homocedasticidade utilizando os testes de Shapiro-Wilk e Levene e comparados por ANOVA one-way, teste de Kruskal-Wallis ($p < 0,05$) e teste de Dunnett ($\alpha = 0,05$). Os resultados mostraram que ScLL 5 μg / ml não teve efeito citotóxico nas PBMCs e os grupos tratados com o ScLL mostraram maior viabilidade do que o grupo controle negativo (LPS). Desta forma, mais estudos devem ser realizados para avaliar o potencial da ScLL como agente imunomodulador e anti-reabsorção para a terapia de avulsão e inflamação periodontal.

PALAVRAS - CHAVES : Lectina (SCLL), Viabilidade Celular, PBMC

INTRODUCTION

Protein-carbohydrate interactions are considered to be involved in the regulation of diverse cellular activities. Lectins are carbohydrate-binding proteins which interact with receptor-linked glycans on cell surface. Having first been detected in plants, these proteins can be found both in animal and plant kingdom (Moreira *et al.* 1991; Kayeshta *et al.* 1993; Sharon *et al.* 1995; Gabius *et al.* 1997).

Lectins are involved in a wide variety of biochemical processes including intra- and intercellular trafficking, initiation of signal transduction, cell growth regulation, migration and adhesion (Rüdiger et al. 2000; Souza et al. 2013). The D-galactose binding lectin (ScLL) is a protein extracted from the Synadenium carinatum specie, whose anti-inflammatory and immunomodulatory properties has been proven in several studies enrolling leishmaniasis (Afonso-Cardoso et al. 2007); asthma (Rogério et al. 2007) and neosporosis models (Cardoso et al. 2012).

Recently, ScLL was evaluated in the oral cavity in the presence of Porphyromonas gingivalis lipopolysaccharides (LPS) (Reis et al. 2016). The referred study evaluated the effect of viability, proliferation and IL-10 release in gingival fibroblasts, showing that ScLL did not affect viability, proliferation and had no cytotoxic effects in the cells. These promising results suggest its potential use in dentistry as immunomodulator. However, other cell types are present in the gingival and periapical tissues, among them monocytes/macrophages which exert essential role in the control of inflammatory responses and wound healing (McNally et al 1995).

Monocytes precursors are present on bone marrow, and by leaving the peripheral blood towards the tissues, they are called macrophages, participating in several processes such as phagocytosis, regulation of the function of other cells, inflammatory reactions (Junqueira,l.c./Carneiro, josé_12°Ed.2013). Therefore, the study of possible immunomodulatory agents should evaluate the effect of the product on viability of this cellular model.

In view of the functional relevance of lectins, they may offers an attractive source for the development of new drugs (Rüdiger et al. 2000). Considering the promising results of ScLL, and the need for research using different cellular models, this study evaluated the effect of ScLL on viability of peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide (LPS). It was hypothesized that the treatment with different concentrations of ScLL would not affect the cell viability of monocyte.

MATERIAL AND METHODS

CULTURE OF PBMC CELLS

PBMCs were isolated from 5 healthy volunteers (Dentistry School of Federal University of Uberlândia), after written informed consent obtained. The study was approved by the Ethics Committee (Protocol No. 1,480,043).

Blood was diluted 1: 1 in Roswell Park Memorial Institute (RPMI) medium (Vitrocell Embriolife , Campinas, SP, Brazil), layered over Ficoll PM 400 (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 650 x g for 20 min. The interface layer was re-suspended in RPMI and then centrifuged (600 × g) for a further 10 min after which the cells were re-suspended in medium supplemented with 10% fetal bovine serum (FBS) (Vitrocell Embriolife). Cells were plated in 96-well plates at a density of 5×10^5 cells per well to a final volume of 100 µl of RPMI containing 10% FBS and 1% penicillin- streptomycin (complete medium) (Vitrocell Embriolife). After 2 h of incubation, cultures were vigorously rinsed RPMI to remove non-adherent cells, and then maintained in complete medium. After 24 h, cells were stimulated with 1 µg/ml of LPS from *P. gingivalis* (Invivogen, San Diego, CA, USA) in complete medium for 24 h. Then, the LPS - containing medium was removed and the cells were treated with 5 and 10 µg/ml of ScLL (Uberlandia Federal University Herbarium) in a fresh medium for 1 and 5 h. The positive control group contained the cells maintained in no stimulated culture medium, representing their physiological behavior. The negative control group contained the cells kept in LPS, mimicking the inflammatory condition. After the period of incubation, the cells were prepared for analysis of cell viability by 3- (4, 5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay. The tests were performed with five samples per group.

CELL VIABILITY ASSAY

The cell viability was evaluated using the 3-(4,5-dimeth¬ylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay. After removing the medium, the cells were rinsed with a phosphate-buffer solution (PBS), and MTT solution (5 mg/ml) was added to the cells and incubated at 37ºC for 4 h to allow development of the formazan crystals. The supernatant of each well was replaced with the same volume of dimethyl sulfoxide (Sigma-Aldrich) to dissolve the crystals. Optical density was measured at 570 nm using a microplate reader (Biochrom, Cambridge, UK, England).

STATISTICAL ANALYSIS

Data of cell viability were analyzed for normality and homoscedasticity using Shapiro-Wilk's and Levene's. The Kruskal-Wallis test was used to compare the data. Statistical significance was set at $\alpha = 0.05$. Statistical analysis was performed using Sigma Plot 12.0 software (Systat Software, San Jose, CA, USA)."

RESULTS

Figure 1 shows cell viability and standard deviations. The levels of cell viability in PBMCs were statistically higher for the group treated with ScLL 5 μg / ml at 1 h, compared with all groups ($p < 0.05$). No significant differences were found among ScLL treated groups ($p = 0.437$) at 5 h.

No significant differences were found between ScLL 10 μg / ml and positive control group (RPMI) ($p > 0.05$). The levels of cell viability for ScLL 10 μg / ml were statistically higher than the positive control ($p = 0.036$) at 5 h. No significant differences were found between ScLL 5 μg / ml and positive control ($p > 0.05$) at 5 h. The values of viability for negative control group (LPS) were statistically lower compared to all groups ($p < 0.05$) at 1 h.

All the ScLL treated groups had significantly higher values of viability than the negative control ($p < 0.05$) at 5 h.

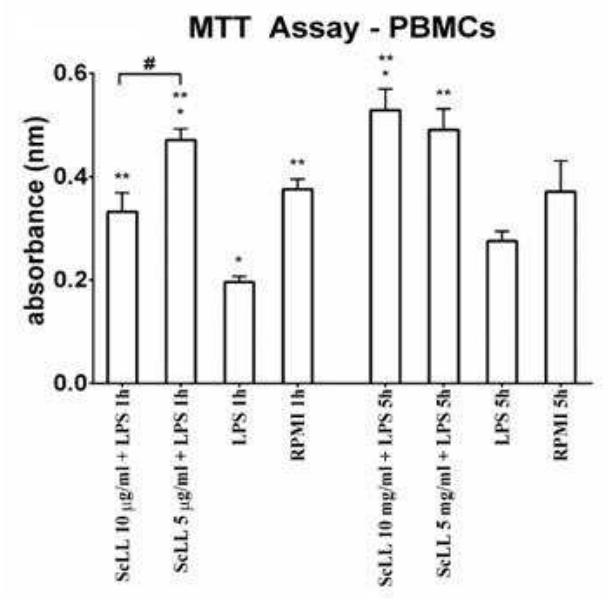

FIGURE 1: Effect of ScLL on cell viability of PBMC by MTT assay. Statistical significance was determined using Kruskal-Wallis and Dunnet's test. # $p < 0.05$ compared between the experimental groups. * $p < 0.05$ compared with positive control (RPMI). ** $p < 0.05$ compared with negative control (LPS).

DISCUSSION

The hypothesis of the present study was partially validated, since treatments with ScLL did not affect cell viability, but only the concentration of 5 µg/ml. This result i was not similar to previous study in immortalized fibroblasts, which independently of ScLL concentration the cell viability was not affected (Reis et al 2016). Diverge on results between the studies may be explained ddoe to the different cell types and the experimental methods.

In the present study the method used to cell viability was MTT-based colorimetric assay. MTT is reduced in metabolically active cell by a mitochondrial enzyme to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent. The resulting purple color can be quantified by

measuring the absorbance, which has a linear relationship with the cell numbers (Damas et al 2011). Compared with other feasibility studies, the MTT assay is simple, rapid, accurate, sensitive and reproducible (Sepet et al., 2009). Take into account these requirements, the fact at MTT assay is a standard method to determine the cytotoxicity of dental materials in cultured cells (Al Anezi et al 2010) and based on these findings, no significant toxicity was caused by ScLL 5ug / ml in cells previously stimulated with LPS.

Although the use of immortalized cell cultures in preliminary study assessing medicines is recommended in the literature (Martins et al 2015; Reis et al 2016) due to the reproducibility of the results, unlimited life span, and higher rate of cell multiplication compared to primary culture cells (Wang et al 2001), in this study the use of primary cultures was chosen in order to mimics the monocyte behavior *in vitro*. Furthermore, the PBMCs were used because are central in controlling wound healing inflammatory/ reparative responses (McNally et al 1995). On this way, the knowledge about the effect of ScLL on viability of these cells is extremely important.

In addition, the treatment with LPS to stimulate PBMCs aimed to induce cellular stress, mimicking the condition observed in the inflammatory response (Seo et al. 2012). Thus, in the presence of LPS is expected to adversely interfere with cell viability. In fact, the groups treated with ScLL, had higher viability than negative control group (LPS), which suggests that the ScLL can act in any point of the apoptotic pathway by blocking a molecular event in the apoptotic process. Although a previous study evaluated the effect of ScLL on macrophages, murine cells infected with Leishmania were used (Afonso-Cardoso et al. 2011), and therefore the results can not be compared to the present study.

Despite the limitations of the present study, the result presented here suggests that ScLL may be promising in dentistry, specifically in the lower concentrations. Therefore, more research using different models *in vitro* and *in vivo* are necessary to evaluate the potential of ScLL as an immunomodulator on periodontal and periapical disease and anti-resorption agent for avulsion therapy. Moreover, further studies should associate immunomodulatory agents with storage media, in order to bind the modulatory and nutritional properties, for use as a product for storage of avulsed teeth.

CONCLUSION

Based on the results of the present study, it can be concluded that 5 μg / ml ScLL did not alter the viability in PBMC cells and did not cause cytotoxic effects. At the 10 μg / ml ScLL concentration no significant differences were found between the positive control group (RPMI). Cell viability levels to 10 μg / ml were statistically higher than the positive control ($p = 0.036$) at 5 h.

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ATTACHMENT

JOURNAL RULES: BIOSCIENCE JOURNAL

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The essay must strive for clarity, brevity and conciseness. The text should be typed in Times New Roman, size 11, simple space and with a margin of at least2 cm. All lines must be numbered. Papers should be submitted without the identification of the authors. The authors' names, title and address of work must be presented to metadata submission and in the cover letter. Figures and tables must be inserted in the text, as close as possible to where cited.

The article will be sent to three (03) reviewers in the area in question, in the shortest possible time, without identifying the authors and will be considered as approved upon 02 favorable opinions.

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Paper title: The title must be brief and sufficiently specific and descriptive, containing the keywords that represent the contents of the text separated by colon, both accompanied by their translation into Portuguese.

Abstract: An informative summary must be prepared with about 200 to 400 words, including objective, method, results, conclusion, accompanied by its translation into Portuguese. Both must have 800 words at most.

Keywords: The keywords must not repeat words in the title, the scientific name of the species studied must be included. Words should be separated by a colon and begin with a capital letter. Authors must submit 3-6 terms, taking into consideration that a term may be composed of two or more words.

Acknowledgements: Acknowledgements as to help received in the preparation of the paper must be mentioned at the end of the article, before the references.

Notes: The notes contained in the article must be indicated with an asterisk immediately after the sentence to which they refer. The notes must be at the bottom of the corresponding page. Exceptionally, numbers may be adopted for the notes together with asterisks on the same page. In which case, the notes with asterisks precede the notes with numbers, regardless of the order of these notes in the text.

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Manuscripts, even if they present scientific relevance and are methodologically correct, may be refused if they are not properly organization and if they are outside the norms of the Bioscience Journal.

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2. The figures must have a maximum width of8.0 cmor16.0 cm.

3. The titles and the x and y axes scale must be in Times New Roman size 11. The axel lines and other lines (e.g., regression curves) must have a thickness of 0.3mm. All information contained inside the figure (e.g., equations, captions) must be in Times New Roman size 10 or at least 8. Right hand and top edges in graphs are not necessary.

4. All figures must be conveniently inserted into the text after being mentioned, consecutively and in Arabic numerals. The figures should be inserted in the text by means of the "Insert → Image/Figure→ File" command.

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