Effect of red propolis on hamster cheek pouch angiogenesis in a new sponge implant model[1]

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Abstract

Purpose: To evaluate the effects of red propolis on cheek pouch angiogenesis in a hamster new model sponge implant.

Methods: Forty eight animals divided into eight groups. (Groups I-IV), the animals were treated for 15 days before and 10 days after sponge implantation. (Groups V-VIII), the animals were treated for 10 days after sponge implantation (GI and GV: red propolis 100 mg/kg, GII and GVI: celecoxib 20 mg/kg, GIII and GVII: 1% gum arabic 5 mL/kg, GIV and GVIII: distilled water 5 mL/kg). On the 11th day of implantation, the animals were anesthetized for stereoscopic microscopic imaging and morphometric quantification of angiogenesis (SQAN), followed by histopathological evaluation (H&E).

Results: In the SQAN analysis, no significant difference was found between the groups. However, on histology, propolis was found reduce the population of mastocytes in the qualitative analyses (p = 0,013) in the quantitative analyses to reduce the number of blood vessels (p = 0,007), and increase the macrophage count (p = 0,001).

Conclusion: Red propolis inhibited inflammatory angiogenesis when administered before andcontinuously after sponge implant, and was shown to have immunomodulating effects on inflammatory cells (mastocytes and macrophages) in a new sponge implant hamster model.

Key words: Angiogenesis Inhibitors. Propolis. Inflammation. Mesocricetus. Cricetinae.

■   Introduction

Propolis is a water-insoluble resinous[1] mixture of bees saliva and vegetable resinous substance with a wide range of important biological properties (antioxidant, anti-inflammatory, antitumoral, immunomodulating, antibacterial, antiviral, antibiofilm, antifungal, analgesic, anesthetic and antiparasitic)[2].

In general, inflammatory processes are regulated by molecules capable of modulating vascular and cellular mechanismswhich promote angiogenesis[3]. Chronic inflammation results from persistent inflammatory stimuli (infectious, physical or chemical agents) triggering the release of inflammatory mediators[4] and the activation of endothelial cells and the immune system, leading to angiogenesis[5].

Angiogenesis is the formation of new blood vessels from existing ones[6]. The regulation of angiogenesisrequires a fine-tuned balance between molecules stimulating and inhibiting angiogenesis[7]. Pathological angiogenesis occurs through deficient or excessive neovascularization[8], one example that disorders is the inflammatory angiogenesis in psoriasis[9].

The purpose of this study was to evaluate the effect of red propolis on cheek pouch angiogenesis in a new sponge implant hamster model.

■   Methods

The study protocol followed the guidelines of the Brazilian Society of Animal Experimentation (COBEA) and was approved by the Animal Research Ethics Committee (CEUA), Universidade Federal do Ceará (UFC) (nº 05/2016).

The study had two arms with 4 groups of 6 animals each. In the first arm (Groups I-IV),

the animals were treated for 15 days before and 10 days after sponge implantation. In the second arm (Groups V-VIII), the animals were treated for 10 days after sponge implantation (GI and GV=red propolis 100 mg/5 mL/kg, GII and GVI=celecoxib 20 mg/kg, GIII and GVII=1% gum arabic 5 mL/kg, GIV and GVIII=distilled water 5 mL/kg) (Figure 1).
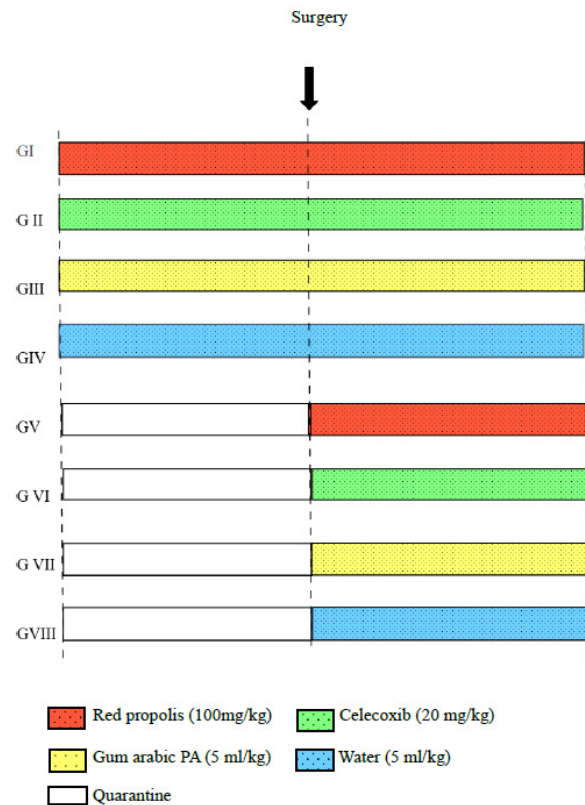

Figure1 - Desing of experiment.

Red propolis

Propolis in natura was acquired from a trusted supplier in Barra de Santo Antônio (Alagoas). Because propolis is water-insoluble, extraction was done with gum arabic, following the protocol described by Shulka, Bhaudaria and Jadon (2004).

Celecoxib (Celebra® 200 mg, Pfizer)

The anti-inflammatory agent celecoxibe ($C_{17H14F3N3O2S)}$ was diluted in sterilized distilled water and administered subcutaneously in the dorsocervical region at 20 mg/kg body weight.

Gum arabic 1%

Gum arabic AG 1% (Dinâmica Química Contemporânea Ltda) was diluted in sterilized distilled water andadministered subcutaneously in the dorsocervical region at 5 mL/kg body weight.

Sponge

The implant consisted of a fragment of polypropylene sponge measuring 3 mm (diameter) by 1 mm (thickness). The fragment was cut with a manual hollow hole punch cutter (Elizabeth Graziano EPP) and sterilized in an autoclave.

Surgical procedure

Following anesthesia with intraperitoneal administration of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight), the cheek pouches were cleansed for food residues with sterile saline using a sterilized (needleless) plastic syringe and, when necessary, sterilized gauze. The left pouch was then everted and stretched with forceps and two fingers, forming a symmetrical epithelial fold with exposure of the medial face. Using an insulin needle, 0.1 mL saline was injected into the subepithelium to make room for the implant (Figure 2A). Using colibri forceps, a 1-mm opening was made at the site of the needle perforation (Figure 2B) into which the sponge fragment was implanted (Figure 2C). The implant site was gently compressed with the finger and, following hydration with 0.9% saline, the pouch was inverted back into the oral cavity.

Figure 2 - Surgical procedure. **A**: Inoculation of saline to make room for the sponge implant. **B**: Creation of small opening with colibri forceps. **C**: Implantation of sponge.

Cheek pouch angiogenesis

On the 11th day of sponge implantation, the animals were anesthetized with ketamine (100 mg/kg body weight) and xylazine (20 mg/kg body weight) and submitted to a macroscopic cheek pouch evaluation. To do so, the left pouch was everted and cleansed for food residues with sterile saline. After verifying the presence of the implant and of possible purulent discharge, the base of the pouch was resected with a harmonic scalpel, prioritizing cauterization over resection. The resected pouch segment was fastened with insulin needles on a rubber surface and washed with saline to prepare the tissue for photography. Once photographed, the pouch segment was tied to acetate plates with nylon line and preserved in plastic vials containing 10% buffered formaldehyde.

Image acquisition and processing

The panoramic images of the cheek pouch were acquired at 16x magnification (Figure 3). The camera (PixelView® PV-TV304P, Prolink Microsystems Corp. Taiwan) was coupled to a surgical microscope (D.F. Vasconcellos M90, São Paulo) and connected to a microcomputer**.**
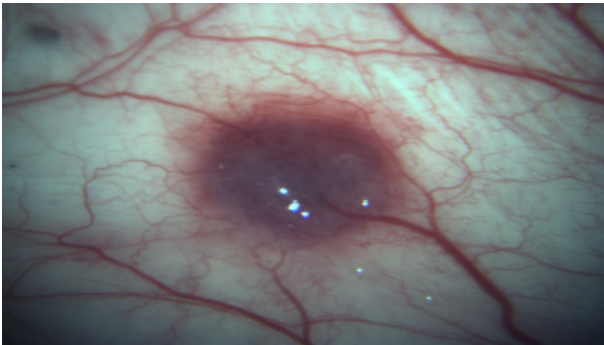

Figure 3 - Eleven days after sponge implantation in hamster cheek pouch. Angiogenesis at different levels towards the sponge in the four quadrants. Panoramic stereocopic microphotograph at x16 magnification.

Quantification of angiogenesis

The microphotographs were processed with a system (SQAN v. 1.00, 2005) 10 built specifically to quantify angiogensis (Figure 4).
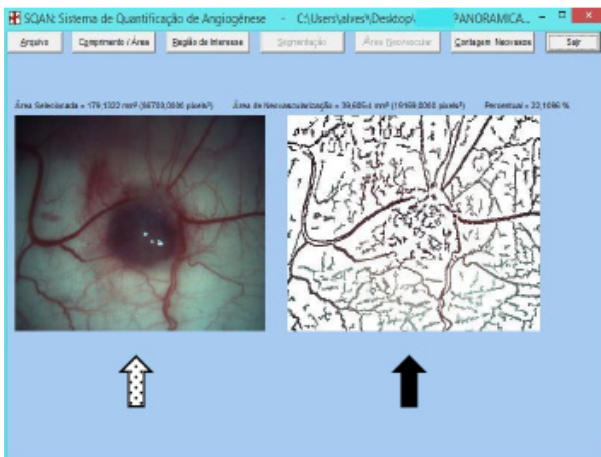

Figure 4 - Interface of the SQAN system. *White arrow*= panoramic stereocopic microphotograph of implant; *black arrow*=skeletonized area of neovascularization.

The system provides information on three variables: area of neovascularization, total vascular length, and number of blood vessels. The neovascular response was then quantified in the panoramic images (magnification: x16).

Staining with hematoxylin-eosin

The resected cheek pouch segments were cleaved, embedded in paraffin, processed, sliced and stained with H&E.

Statistical analysis

Continuous and discrete quantitative variables were submitted to the Kolmogorov-Smirnov test for normality of distribution. The descriptive statistics included mean values and standard deviation (parametric variables) or median, minimum and maximum values and interquartile intervals (nonparametric variables). The four groups in each study arm were compared pairwise with ANOVA associated with Tukey's multiple comparison test to detect differences between groups (parametric variables), or with the Kruskal-Wallis test followed by Dunn's multiple comparison test (nonparametric variables). Analyses and graphs of angiogenesis data were made with the software GraphPad Prism v. 5.00 (GraphPad Software, San Diego, California, USA). All tests were two-tailed, and the level of statistical significance was set at 5% ($p < 0.05$).

The groups were also compared with Student's t test or ANOVA with regard to inflammation, followed by Bonferroni's test associated with Fisher's multiple comparion test or Pearson's chi-square test. Inflammation levels were submitted to the Kolmogorov-Smirnov test of normality, expressed as mean values and standard error of the mean, and compared with Student's t test or ANOVA followed by Bonferroni's post-test (parametric variables). Analyses of inflammation data were made with IBM SPSS Statistics v. 20.0 for Windows, with a confidence interval of 95%**.**

■ Results

Quantification of angiogenesis (SQAN)

No significant difference was observed within the groups of each study arm (I, II, III and IV, and V, VI, VII and VIII) with regard to the amount of angiogenesis. The first arm of the experimente with the respective values expressed in pixels (F = 2.6712; p = 0.0752) and percentage (F = 2.6711; p = 0.0752) and the second arm of the experiment (treatment only) with the respective values expressed in pixels (F = 0.6255; p = 0.6069) and percentage (F=0.6255; p = 0.6069).

Quantification of inflammation (scoring system)

First arm of the experiment (prevention + treatment): Groups I, II III and IV

Groups I, II, III and IV did not differ significantly with regard to the following histological parameters: microabscess (p = 0.407), macrophages (p = 0.299), lymphocytes (p = 0.268), giant cells (p = 0.210), foreign body granuloma (p = 0.210) and fibrosis (p = 0.469). However, reduced mastocyte levels were more often observed in Groups I and II than in the water control group (p = 0.013), while moderate (33.3%) and mild (83.3%) mastocyte infiltration was more common in Groups III and IV.

Second arm of the experiment (treatment only): Groups V, VI, VII and VIII

Groups V, VI, VII and VIII did not differ significantly with regard to the following histological parameters: microabscess (p = 0.677), mastocytes (p = 0.877), macrophages (p = 0.863), lymphocytes (p = 0.918), giant cells (p = 0.212), foreign body granuloma (p = 0.439) and fibrosis (p = 0.608).

Quantitative evaluation of inflammation

First arm of the experiment (prevention + treatment): Groups I, II III and IV

The number of lymphocytes (p = 0.720) and giant cells (p = 0.103) did not differ significantly between Groups I, II, III and IV, but the number of blood vessels was significantly smaller in the groups treated with red propolis and celecoxib than in the control group (p= 0.007) (Figure 5), the number of mastocytes was significantly smaller in the group treated with celecoxib than in the control group (p = 0.002) (Figure 6), and the number of macrophages was significantly greater in Group I than in Groups I, II and III (p = 0.001) (Figure 7) (Table 1).
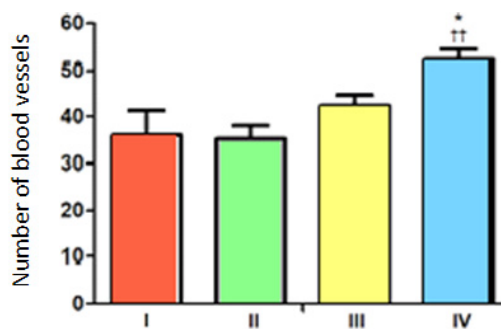
Figure 5 - Blood vessel count in the first arm of the experiment (prevention + treatment). *= p< 0.05 compared to Group I.; †† = p< 0.01 compared to Group II.
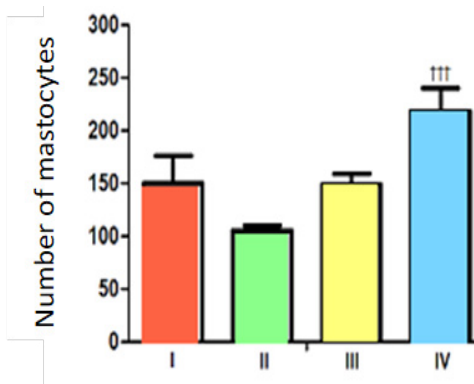
Figure 6 - Mastocyte count in the first arm of the experiment (prevention + treatment). ††† = p< 0.05 compared to Group II.
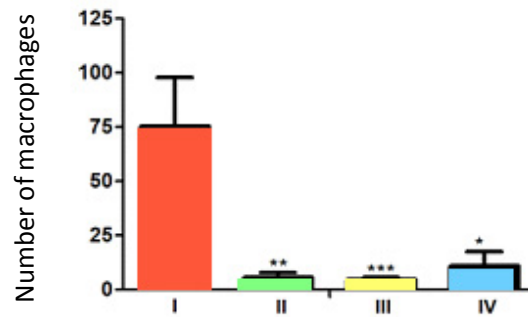
Figure 7 - Macrophage count in the first arm of the experiment (prevention + treatment). $* = p < 0.05$ compared to Group I; $** = p < 0.01$ compared to Group I; $*** = p < 0.001$ compared to Group I.

Table 1 - Quantitative evaluation of inflammation in the first arm of the experiment (prevention + treatment) expressed as mean values and standard error of the mean.

	Group				
	I	**II**	**III**	**IV**	***p*-value**
Vessels	36.0±5.4	35.3±2.8	42.5±2.3	52.3±2.2*††	*0.007*
Mastocytes	149.7±25.9	104.3±5.0	149.2±9.5	217.7±22.0†††	*0.002*
Macrophages	75.5±22.5	5.8±2.2**	4.8±0.8***	11.0±6.4**	*0.001*

$* = p < 0.05$ compared to Group I.
$** = p < 0.05$ compared to Group I.
$*** = p < 0.05$ compared to Group I.
$†† = p < 0.05$ compared to Group II.
$††† = p < 0.05$ compared to Group II.

Second arm of the experiment (treatment only): Groups V, VI, VII and VIII

Groups V, VI, VII and VIII did not differ significantly with regard to mastocytes ($p = 0.165$), macrophages ($p = 0.690$) or lymphocytes ($p = 0.079$), but the number of blood vessels was significantly greater in the group treated with celecoxib ($p = 0.008$) and the number of giant cells was significantly smaller in the groups treated with red propolis, celecoxib and gum arabic than in the water control group ($p < 0.001$) (Table 2).

Table 2 - Quantitative evaluation of inflammation in the second arm of the experiment (treatment only) expressed as mean values and standard error of the mean.

	Groups				
	V	**VI**	**VII**	**VIII**	***p*-value**
Vessels	49.2±4.0	56.7±3.9	43.5±3.9	37.8±1.9††	*0.008*
Giant cells	9.0±2.8	4.2±1.3	3.2±0.7	18.0±1.0**††‡‡‡	*<0.001*

$** = p < 0.05$ compared to Group V.
$†† = p < 0.05$ compared to Group VI.
$‡‡‡ = p < 0.05$ compared to Group VII.

■　　Discussion

Synthetic implants (such as sponge) may be used to study different components of inflammatory and angiogenic processes[11]. In addition to studies on inflammatory angiogenesis, such implants are increasingly employed in tissue engineering research due to the interrelatedness of biocompatibility, angiogenesis and inflammation[12].

The study of cheek pouch inflammatory angiogenesis induced by sponge implants relies on the use of new and reproducible models. In this study, we developed a model simulating a clinical setting of inflammation and angiogenesis caused by the presence of a foreign body (sponge) in hamster cheek pouch epithelium.

The hamster cheek pouch can be accessed easily by eversion, with little or no risk of trauma. The epithelial wall of the pouch is translucid; this facilitates visualization and helps avoid damage to blood vessels and potential antigenic contact through the blood. After inversion back into the oral cavity, the pouch wall accommodates the graft, protecting it against external trauma, making postoperative bandages unnecessary[13], despite the risk of trauma associated with food storage.

Nevertheless, complications may occur at the implant site. Some of the animals in Groups I, III, V, VI, VII and VIII displayed sponge extrusion, pus, ulcerations, infiltration, and adhesions (they were excluded from the analysis). In general, the presence of food residues in the pouch increases the risk of contamination.

The choice of the 11th postoperative day for the evaluation of angiogenesis was based on a pilot experiment in which absorbable and non-absorbable sponge implants were studied macroscopically every other day. Absorbable material was found to be inadequate since the short time of absorption (up to 15 days) compromised the standardization of the inflammatory stimulus (foreign body) and subsequent evaluation of inflammatory angiogenesis. This problem was avoided by using non-absorbable material.

In our model, the test substances were administered subcutaneously. Alternatively, the sponge implant might have been soaked in the test substance, a method which remains to be tested.

The inflammation induced by the presence of the implant promotes changes like vasodilation, increased vascular permeability, and increased leukocyte adhesion and migration through the walls of the blood vessels. Neutrophils are the most abundant leukocytes in acute inflammation; when activated, they produce reactive oxygen species (free radicals), in addition to monocytes, lymphocytes and macrophages, all of which are characteristic of chronic inflammation and produce free radicals[14].

When the level of reactive oxygen species overwhelms the antioxidant defense system, excess free radicals oxidate the membrane lipids, causing damage to nucleic acids—the molecular basis of several inflammatory conditions[15]. In this study, pouch tissues stained with H&E revealed increased levels of neutrophils, lymphocytes, mastocytes, macrophages and giant cells associated with inflammation.

The relationship between inflammatory angiogenesis and the production of free radicals by inflammatory cells highlights the need for the development of antioxidant compounds to prevent oxidative stress, which is etiopathogenic of a range of inflammatory conditions[16].

In the chemical analysis of the red propolis extract used in this study we identified the following compounds: 2'-hydroxy-4',7-dimethoxy-isoflavane; 2',7-dihydroxy-4'-methoxy-isoflavane;

2',4'-dihydroxy-7-methoxy-isoflavane; 4',7-dihydroxy-2'-methoxy-isoflavane; 2',4',4-trihydroxy-chalcone, and lup-20(29) -en-3-ol. In the antioxidant activity analysis, red propolis was comparable to the positive controls (vitamin C and trolox), and a significant level of anticholinesterase activity was observed[17].

The first study on the effect of propolis on inflammatory angiogenesis was published in 1999, suggesting propolis attenuates inflammation by inhibiting cyclooxygenase and lipoxygenase[18].Another study explained the anti-inflammatory effect of propolis as resulting from anti-angiogenic mechanisms[19]. Yet others have reported anti-angiogenic effects with green propolis[20].

However, in the morphometric quantification of angiogeneis (SQAN), which included an analysis of panoramic stereoscopic images of the vascular area around the implant and an analysis of quadrant mean scores, animals treated with propolis and controls displayed statistically similar results. In other words, in this analysis the use of red propolis did not inhibit angiogenesis.

The SQAN analysis of the tissues of animals treated with celecoxib revealed a small but non-significant level of inhibition in the vascular area around the implant when compared to controls. Thus, no significant inhibitory effect could be demonstrated for celecoxib.

The results of the SQAN analysis of the tissues of animals treated with gum arabic 1% were similar to the results for red propolis and celecoxib. In short, in this analysis none of the three substances significantly inhibited angiogenesis.

Mechanically extracted from the tree species *Acacia senegal*, gum arabic is known for its antioxidant properties[21]. Since red propolis was extracted using a solution of gum arabic at 1%, a positive control group treated with gum arabic was included in the study.

Angiogenesis was evaluated morphometrically, quantitatively and qualitatively, and on slides stained with H&E (scoring system), while the blood vessels were evaluated quantitatively only. In the quantitative analysis, the number of blood vessels was significantly smaller in the group treated preventively with red propolis than in the water control group ($p= 0,007$), indicating inhibition of angiogenesis. Interestingly, in a study using LDL gene knockout, red propolis was found to inhibit inflammatory angiogenesis related to atherosclerosis[22].

In the morphometric analysis, propolis did not present statistical significance in the inhibition of angiogenesis.SQAN analysis is after the 11th day of implant when the graft becomes pseudo-encapsulated (fibrosis) thereby limiting intra-sponge angiogenesis.The SQAN evaluates the periesponja angiogenesis. It should be kept in mind that the latter relies on stereoscopic microscopy at 16x magnification, while the histological analysis (H&E) uses optical microscopy at x400 magnification. Indeed, the significant findings of this study were in the microvascular setting.Thus, the vessels quantified in the panoramic view do not represent angiogenic vessels.

Quantitatively, our finding of significant anti-angiogenic activity for red propolis at the microvascular level supports the notion that propolis has immunomodulating properties, stimulating or inhibiting immunological events, as the case may be.

This immunomodulating effect was evidenced in our qualitative analysis: in the first arm of the experiment (prevention + treatment), a significant reduction was observed in the mastocyte levels of the propolis group in relation to the control group ($p = 0.013$). The same was observed in the quantitative analysis: mastocyte levels in the celecoxib group were significantly reduced compared, respectively, to the water control

group (*p* = 0.002).

Mastocytes are crucial to the development of inflammatory conditions as they alter vascular permeability by releasing vasoactive mediators and recruiting inflammatory cells[23]. The observed reduction in mastocyte levels matches the findings of a study on the effect of propolis on mastocytes in wound healing in the oral cavity of hamsters[24]. The relationship between mastocytes and vasculature and the high mastocyte levels observed in inflammatory conditions are associated with angiogenesis[25].Thus, based on the results of this study, it seems likely the anti-angiogenic effect of propolis is mediated by mastocytes.

Derived from monocytes, macrophages are the most important phagocytes in chronic inflammation. The persistence of the inflammatory stimulus (e.g. sponge implant) chronifies the inflammatory process. The condition is further consolidated by the presence of angiogenesis and macrophages which in turn release inflammatory cytokines, thereby prolonging it[26].

In the first arm of the experiment (prevention + treatment), the number of macrophages was significantly greater in tissues treated with red propolis (*p* = 0.001) in relation to Groups II, III and IV.

The observed increase in macrophages in the propolis group was unsurprising since propolis is known to promote macrophage activation. Phagocytosis plays a major role in organic defense, the production of free radicals, the mediation of inflammatory processes and the release of an array of substances, including enzymes, cytokines and components of the complement cascade. This may be accompanied by increased humoral and cellular immune response. Other researchers have shown that propolis can increase the phagocytic capacity of macrophages[27].

Giant cells (clusters of macrophages)

are a common finding in chronic inflammation, triggered by the presence of agents with low immune activity, such as non-absorbable materials[28].The water control group and the propolis group differed significantly with regard to the number of giant cells (*p*≤ 0.001), suggesting red propolis has anti-inflammatory activity.

The number of vessels was significantly smaller in the group treated preventively with celecoxib than in the water control group (*p* = 0.007), indicating inhibition of angiogenesis, but in the second arm of the experiment (treatment only) the number of vessels was significantly greater than in the control group (*p* = 0.008). In other words, celecoxib can both inhibit and promote angiogenessis[29].

In the morphometric analysis, celecoxib reduced angiogenesis very slightly (non-significantly). The results might have been significant, as shown in the literature, if a different dose and time of exposure had been used. Or perhaps two different mechanisms are involved, producing opposite effects (stimulatory and inhibitory).

In the qualitative analysis, the concentration of mastocytes was significantly smaller in the group treated preventively with celecoxib than in the water control group (*p* = 0.013), a finding subsequently confirmed in the quantitative analysis (*p* = 0.002). This is in agreement with the literature, which shows celecoxib to have immunomodulating effects[30].

In the second arm of the experiment (treatment only), the number of giant cells differed significantly between the control group and the celecoxib group (*p* ≤ 0.001), suggesting celecoxib has anti-inflammatory properties. A similar difference was observed between the control group and the gum arabic group with regard to giant cell count (*p* ≤ 0.001), suggesting gum arabic has anti-inflammatory properties as well.

■ Conclusion

Red propolis inhibited inflammatory angiogenesis when administered both before and after sponge implantation, and was shown to have immunomodulating effects on inflammatory cells (mastocytes and macrophages) in a new sponge implant hamster model.

■ References

1. Toreti VC, Sato HH, Pastore GM, Park YK. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med. 2013;2013:1-13. PMID: 23737843.

2. Sforcin JM. Biological properties and therapeutic applications of propolis. Phytother Res. 2016;30(6):894-905. PMID: 26988443.

3. Ridiandries A, Tan JT, Ravindran D, Williams H, Medbury HJ, Lindsay L, Hawkins C, Prosser HC, Bursill CA. CC-chemokine class inhibition attenuates pathological angiogenesis while preserving physiological angiogenesis. Faseb J. 2017;31(3):1179-92. PMID: 27998907.

4. Shrihari TG. Dual role of inflammatory mediators in cancer. Ecancermedicalscience. 2017;11:721. PMID: 28275390.

5. Arroyo AG, Iruela- Arispe ML. Extracellular matrix, inflammation, and the angiogenic response. Cardiovascular Res. 2010; 86(2):226-35. PMID: 20154066.

6. Guo L, Harari E, Virmani R, Finn AV. Linking hemorrhage, angiogenesis, macrophages, and iron metabolism in atherosclerotic vascular diseases. Arterioscler Thromb Vasc Biol. 2017;37(4):33-9. PMID: 28330947.

7. Folkman J, Klagsbrun M.Angiogenic factors. Science. 1987;235(4787):442-7. PMID: 2432664.

8. Li Y, Adur MK, Kannan A, Davila J, Zhao Y, Nowak RA, Bagchi MK, Bagchi IC, Li Q. Progesterone alleviates endometriosis via inhibition of uterine cell proliferation, inflammation and immunocompetent mouse model. Plos One. 2016;11(10):e0165347. PMID: 27776183.

9. Marina ME, Roman II, Constantin AM, Mihu CM, Tătaru AD. VEGF involvement in psoriasis. Clujul Med. 2015;88(3):247-52. PMID: 26609252.

10. Fechine Jamacaru FV. In vivo quantification of corneal angiogenesis using digital image processing [tese]. Departamento de Cirurgia, Universidade Federal do Ceará: Fortaleza; 2006. Available inhttp://www.repositorio.ufc.br/handle/riufc/7700.

11. Mendes JB, Rocha MA, Araújo FA, Moura SA, Ferreira MA, Andrade SP. Differential effects of rolipram on chronic subcutaneous inflammatory angiogenesis and on peritoneal adhesion in mice. Microvasc Res. 2009;78(3):265-71. PMID: 19732781.

12. Cassini-Vieira P, Araújo FA, da Costa Dias FL, Russo RC, Andrade SP, Teixeira MM, Barcelos LS. iNOS Activity modulates inflammation, angiogenesis, and tissue fibrosis in polyether-polyurethane synthetic implants. Mediators Inflamm. 2015;2015:138461. PMID: 26106257.

13. Hochman B, Vilas Bôas FC, Mariano M, Ferreiras LM. Keloid heterograft in the hamster (Mesocricetus auratus) cheek pouch, Brazil. Acta Cir Bras. 2005;20(3):200-12. PMID: 16033178.

14. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol. 2009;78(6):539-52. PMID: 19413999.

15. Chen YH, Wang P, Sriboonlue P, Chen YL. Antioxidative strategy for inflammatory diseases. Mediators Inflamm. 2015;2015:675816. PMID: 26063977.

16. Freires IA, de Alencar SM, Rosalen PL. A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. Eur J Med Chem. 2016;110:267-79. PMID: 26840367.

17. Aguiar GR, de Lemos TLG, Dornelas CA, da Silva AM, de Almeida MCS, Ferreira DA, Monte FJQ, Filho RB, de Oliveira IR, do Nascimento PGG. Estudo químico e avaliação biológica da propolis vermelha de Alagoas. Rev Virtual Quim. 2018;10(1). [Epub ahead of print]. Available from: http://rvq.sbq.org.br/imagebank/pdf/AguiarNoPrelo.pdf.

18. Hepsen IF, Er H, Cekiç O.Topically applied water extract of propolis to suppress corneal neovascularization in rabbits. Ophthalmic Res. 1999;31(6):426-31. PMID: 10474071.

19. Song YS, Park EH, Jung KJ, Jin C. Inhibition

of angiogenesis by propolis. Arch Pharmacal Res. 2002;25(4):500-4. PMID: 12214863.

20. Dornelas CA, Fechine-Jamacaru FV, Albuquerque IL, Magalhães HI, Dias TA, Faria MH, Alves MK, Rabenhorst SH, de Almeida PR, Lemos TL, Castro JD, Moraes ME, Moraes MO. Angiogenesis inhibition by green propolis and the angiogenic effect of L-lysine on bladder cancer in rats. Acta Cir Bras. 2012;27(8):529-36. PMID: 22850703.

21. Kaddam L, Fadl-Elmula I, Eisawi OA, Abdelrazig HA, Salih MA, Lang F, Saeed AM. Gum Arabic as novel anti-oxidant agent in sickle cell anemia, phase II trial. BMC Hematol. 2017;17:4. PMID: 28331623.

22. Daleprane JB, Freitas Vda S, Pacheco A, Rudnicki M, Faine LA, Dörr FA, Ikegaki M, Salazar LA, Ong TP, Abdalla DS. Anti-atherogenic and anti-angiogenic activities of polyphenols from propolis. J Nutr Biochem. 2012;23(6):557-66. PMID: 21764281.

23. Robuffo I, Toniato E, Tettamanti L, Mastrangelo F, Ronconi G, Frydas I, Caraffa A, Kritas SK, Conti P. Mast cell in innate immunity mediated by proinflammatory and antiinflammatory IL-1 family members. J Biol Regul Homeost Agents. 2017;31(4):837-42. PMID: 29254286.

24. Cruvinel WM, Júnior DM, Araújo JAP, Catelan TTT, de Souza AWS, da Silva NP, Andrade LEC. Immune system – Part I Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. Bras J Rheumatol. 2010;50(4):434-61. PMID: 21125178.

25. Barroso PR, Lopes-Rocha R, Pereira EM, Marinho SA, de Miranda JL, Lima NL, Verli FD. Effect of propolis on mast cells in wound healing. Inflammopharmacology. 2012;20(5):289-94. PMID: 22179947.

26. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med. 2011 Jul 11;13:e23. PMID: 21740602.

27. Fischer G, Conceição FR, Leite FP, Dummer LA, Vargas GD, Hübner Sde O, Dellagostin OA, Paulino N, Paulino AS, Vidor T. Immunomodulation produced by a green propolis extract on humoral and cellular responses of mice immunized with SuHV-1. Vaccine. 2007 Jan 26;25(7):1250-6. PMID:17084001.

28. Barbieri RL, Parreira SF, Studart SDV, DA-Silva AR, Duarte IDS, Leme PLS. Stem cells hematopoietic niches and inflammatory response to different synthetic prosthesis implanted in rat with incisional hérnias. Arq Bras Cir Dig. 2017;30(2):108-13. PMID: 29257845.

29. Xu K, Gao H, Shu HK. Celecoxib can induce vascular endothelial growth factor expression and tumor angiogenesis. Mol Cancer Ther. 2011;10(1):138-47. PMID: 21220497.

30. Chiba A, Mizuno M, Tomi C, Tajima R, Alloza I, di Penta A, Yamamura T, Vandenbroeck K, Miyake S. A 4-trifluoromethyl analogue of celecoxib inhibits arthritis by suppressing innate immune cell activation. Arthritis Res Ther. 2012;14(1):R9. PMID: 22251404.

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