Effects of a polyglutamic solution on modulating the biological parameters of rosacea - Inflammation, hypervascularization and sensitive skin

Silva, Michelle Sabrina[1*]; Rocha,Tatiane [2]; Sousa, Rafaela Araujo[1]; Addor, Flávia Alvim Sant'Anna[1]; Schalka, Sérgio[1]; Silva, Maurício Baptista[3].

[1]MEDC*IN*VITRO – GRUPO MEDC*IN*, Brazil.

[2]SIMPLE ORGANIC, Brazil.

[3]INSTITUTE OF CHEMISTRY – UNIVERSITY OF SÃO PAULO, Brazil.

*Corresponding author: Michelle Sabrina da Silva

R. Atílio Delanina, 178 - Vila Campesina, Osasco - SP, 06381-095

+55 19-988113390

michelle.sabrina@medcinvitro.com.br

Abstract

Rosacea is a chronic inflammatory skin disease characterized by recurrent episodes of facial flushing, erythema, papules, pustules and telangiectasias on the central face. Although the exact pathophysiology of rosacea is debated, current theories implicate dysregulation of innate and adaptive immunity, aberrant neurovascular signaling, chronic inflammation, and overgrowth of skin commensal organisms. Sensitized *ex vivo* human skin fragments were treated with the polyglutamic solution for 4 consecutive days, once a day. 24 hours after the last treatment, the fragments and supernatants were collected for subsequent quantification of IL-1α, VEGF (both in the supernatant), LL-37 and TRPV-1. Our results demonstrated that the treatment of *ex vivo* sensitized human skin fragments modulated the exacerbation of the inflammatory response, reducing IL-1α production by 21% ($P<0.05$) and LL-37 cathelicidin production by 37% ($P<0.05$), when compared with the group exposed to SDS 5%. Additionally, we observed a potential for reducing skin hypersensitivity associated with rosacea decreasing TRPV-1 synthesis by 15%

(P<0.001), compared to the positive control group (sensitized skin). Finally, treatment with the polyglutamic solution controlled one of the main components of skin hypervascularization, reducing VEGF production by 15% (P<0.01) when compared to the positive control group (skin exposed to UV radiation without treatment).

Keywords: Rosacea; inflammation; hypervascularization; sensitive skin.

Introduction.

Rosacea is a chronic inflammatory skin disease characterized by recurrent episodes of facial flushing, erythema, papules, pustules and telangiectasias on the central face with possible ocular and phymatous involvement [1]. Although the exact pathophysiology of rosacea is debated, current theories implicate dysregulation of innate and adaptive immunity, aberrant neurovascular signaling, chronic inflammation, and overgrowth of skin commensal organisms [2-4]. Importantly, the generation of reactive oxygen species (ROS) due to an altered innate immune response appears to be a component of the rosacea disease mechanism, as studies have demonstrated higher levels of ROS in patients with this condition [5]. The innate response characteristic of rosacea skin involves the secretion of antimicrobial peptides (AMP) from keratinocytes, sebocytes and mast cells. The expression of these peptides is strictly regulated, as although it is an important line of defense, it can also cause tissue damage [9]. Cathelicidins are a family of cationic AMPs, from the same class as defensins and histatins, found in macrophages and polymorphonuclear leukocytes [7]. Cathelicidin LL-37 is an important molecule in innate metabolism, and in inflammatory diseases such as rosacea, there is a disturbance in its processing, which results in peptide fragments that cause inflammation, erythema and even telangiectasia [8-9].

This peptide has a pro-inflammatory action and an “alarm” function, affecting vascular growth, leading to angiogenesis and neovascularization [9]. In patients with the erythematous telangiectatic form, there is a strong presence of pro-inflammatory and vasoregulatory genes, even in the initial forms of the disease. There is also a predominantly perivascular inflammatory

infiltrate, consisting of lymphocytes, macrophages and mast cells, also with an increase in vascular endothelial growth factor (VEGF) [10]. Increased vascularization and dermal vasodilation contribute to increased transepidermal water loss, which acts as a stimulus for the proliferation and differentiation of keratinocytes [11].

There is a neural interaction with immunological and vascular changes, studies present evidence that the TRPV1 receptor (transient receptor potential cation channel 1), sensitive to capsaicin, has greater expression in patients with rosacea, resulting in greater discomfort to chemically similar substances, such as some perfumes and herbal extracts [12-13].

The present study aimed to evaluate the effect of polyglutamic solution on the regulation of inflammatory markers (LL-37 and IL-1α), skin hypersensitivity (TRPV-1) and tissue hypervascularization (VEGF) associated with rosacea, using a sensitized human *ex vivo* skin model.

Materials and Methods.

1. *Ex vivo* skin model

Ex vivo skin fragments obtained from elective plastic surgery (abdominoplasty) after approval by the human research ethics committee (CAAE: 32317520.4.0000.55). The fragments were collected from 1 female research participant aged 34 years.

Fragments were transported to the laboratory in saline solution. Then, the hypodermis was removed, and the fragments were subjected to standardized cuts with 8mm in diameter, for a subsequent asepsis procedure using DMEM high glucose supplemented with gentamicin, for 1 hour at 4ºC. After the procedure, the sections were mounted in transwell inserts for 24-well plates and cultured in an air-liquid interface.

To mimic the *ex vivo* sensitized human skin model, 25µL of a 5% SDS solution was applied, which was kept in contact with the epidermis for 15 minutes. Next, the fragments were washed 20 times with 1mL of PBS, then treatment with the polyglutamic solution began. To carry out the treatments, the product was applied to the surface of the epidermis (25mg/cm^2). The treatment

was carried out for 4 consecutive days, and the product was removed with a sterile cotton swab before new application and the culture medium was renewed every day. To quantify VEGF, from the second to fourth day of treatment, exposure to UV radiation (10J/cm2) was carried out. 24 hours after the last treatment, tissue lysates and supernatants were collected for subsequent quantification of the biological markers.

2. Quantification of biological markers – LL-37, IL-1α, TRPV-1, VEGF

Quantification of LL-37, TRPV1 (both in tissue lysates), IL-1α, and VEGF (both in supernatants) from cultures was carried out using the enzyme-linked immunosorbent assay (Elisa) technique, with commercially purchased kit, according to the manufacturer's instructions (Novus Biologicals, BT-LAB, R&D Systems, Invitrogen, respectively). The amount of LL-37 and TRPV-1 was normalized by the total amount of proteins present in the samples. In the statistical evaluation, the non-parametric ANOVA analysis of variance was used, followed by the Bonferoni post hoc test. A significance level of 5% was used (GraphPad Prism v9).

Results.

The results obtained demonstrated that exposure to SDS 5% increased the synthesis of LL-37 by 68% (*P<0.01*), IL-1α by 43% (*P<0.01*) and TRPV1 by 36% (*P<0.001*), when compared to the basal control group.

On the other hand, treatment of *ex vivo* sensitized skin with the polyglutamic solution reduced LL-37 synthesis by 37% (*P<0.05*), IL-1α by 21% (*P<0.05*) and TRPV1 by 15% (*P<0.001*), when compared to the group exposed to 5% SDS. These results are presented in figure 1.

Additionally, we can observe that the product showed the ability to protect the skin against damage caused by exposure to 5% SDS (sensitized skin) in relation to the LL-37 synthesis of 91%, 70% for IL-1α, and 5% for TRPV1. This parameter was calculated considering the maximum increase in biomarkers synthesis in the SDS 5% group as 100% damage, correlating with the ability of treatment with the product to return to the baseline state (normal, non-sensitized skin).

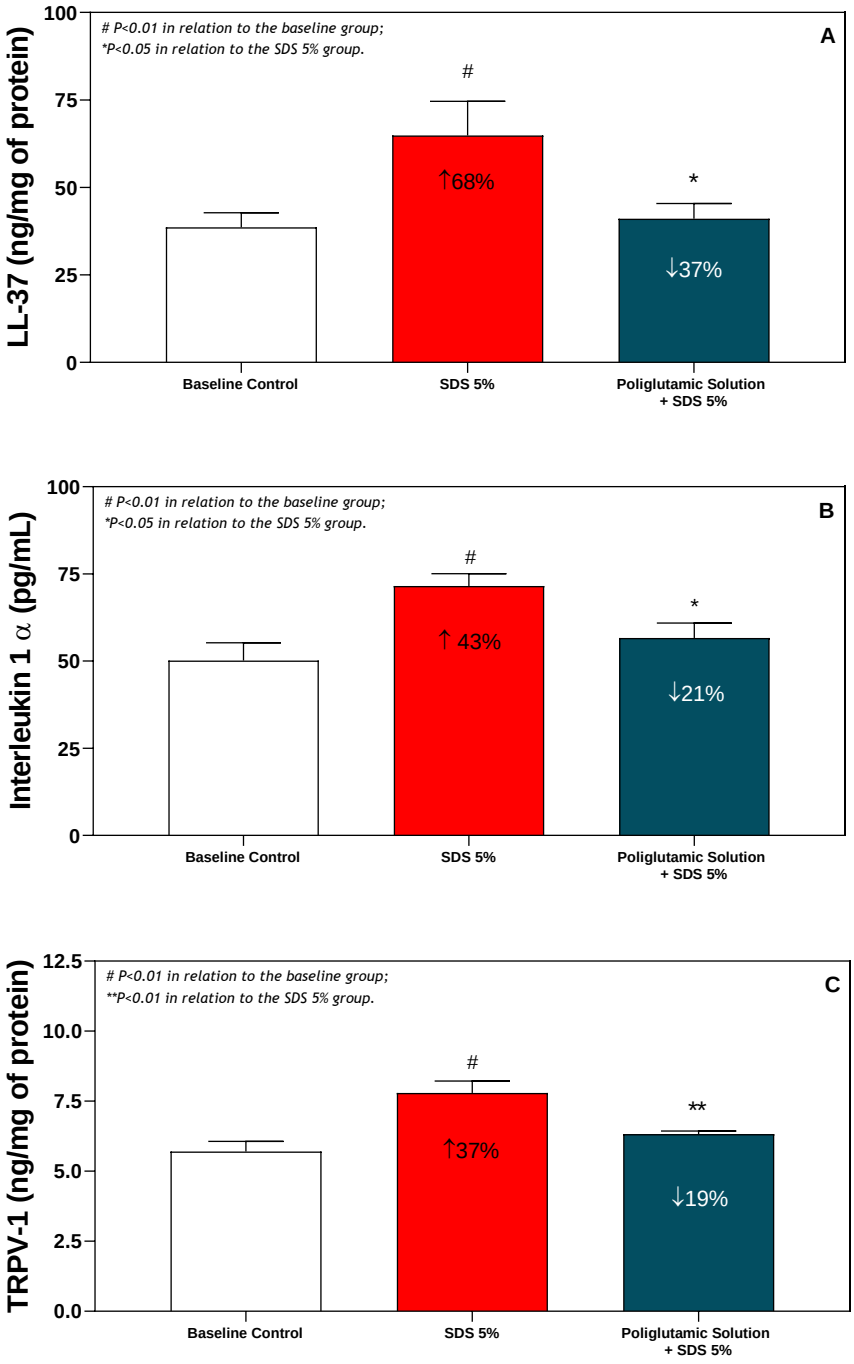

Figure 1. Quantification of LL-37, IL-1α, and TRPV-1 synthesis in *ex vivo* sensitized human skin culture treated with POLYGLUTAMIC SOLUTION. *Ex vivo* sensitized human skin fragments (SDS 5%) were treated for 4 days with the product, for subsequent quantification of LL-37 (A), IL-1α (B), and TRPV-1 (C) synthesis. # P<0.01 in relation to the baseline group; *P<0.05 and **P<0.01 in relation to the SDS 5% group.

Additionally, we observed that exposure to UV radiation increased VEGF synthesis by 36% (*P<0.001*), when compared to the basal control group. *Ex vivo* skin treatment with the polyglutamic solution reduced VEGF synthesis by 15% (*P<0.01*) when compared to the control group (exposed to UV radiation without treatment). These results are presented in figure 2.

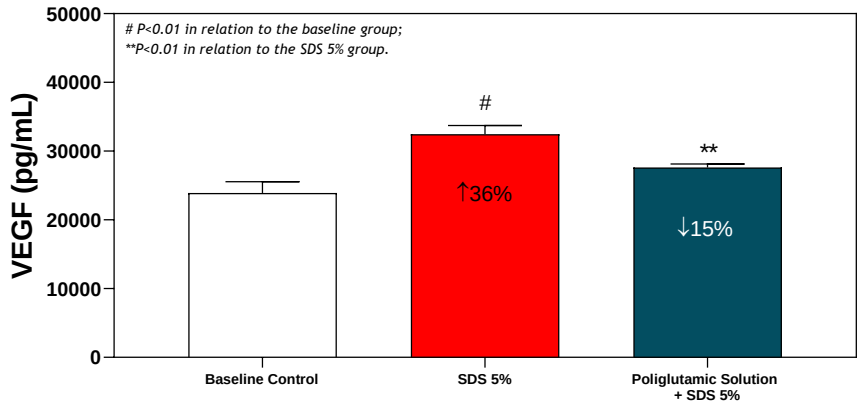

Figure 2. Quantification of VEGF synthesis in *ex vivo* human skin culture subjected to UV radiation and treated with POLIGLUTAMIC SOLUTION. Fragments of *ex vivo* human skin exposed to two doses of 10J/cm^2 of UV-A were treated for 4 days with the product for subsequent quantification of VEGF synthesis. # P<0.01 in relation to the baseline group; **P<0.01 in relation to the SDS 5% group.

Discussion.

Rosacea can be initiated or aggravated by a variety of endogenous and exogenous trigger factors, including heat, noxious cold, ultraviolet (UV) irradiation, and food and beverages. Activation pathways to some of the rosacea triggers have been delineated recently and might point to future therapeutic targets.

Transcriptomic analysis and immunohistochemical findings indicate that the transient receptor potential family—in particular, members of the ankyrin subfamily (TRPA1) and the vanilloid subfamily (TRPV1 and TRPV4)—might convey cellular responses to several of the rosacea-specific trigger factors [14-15]. TRPV1 and TRPA1 are well-described targets for various pungent compounds such as capsaicin (TRPV1) [16] and could render rosacea stimuli such as heat (TRPV1) [17], possibly cold temperatures (TRPA1) [18], UVB irradiation (TRPV4) [19], and toxins

and cosmetics ingredients [20] into clinical rosacea manifestations. In particular, neuronally expressed TRP channels could be responsible for the activation of the cutaneous vasculature leading to flushing, one hallmark feature of rosacea, and erythema by a neurovascular mechanism involving neurogenic inflammation mediators (see Figure 1) [21-23].

Although the mechanisms involved in the pathophysiology of rosacea have not yet been completely elucidated, the relationship between an exacerbated neurogenic inflammatory response and tissue hypervascularization with clinical manifestations of discomfort in patients with rosacea is well described and established. In our study, we first confirmed the relevance of the ex vivo human skin model for evaluating skin disorders and, subsequently, we demonstrated the effectiveness of the polyglutamic solution in regulating the main biological parameters described in the literature for modulating the skin response in patients with rosacea.

Conclusion.

Our results together highlight the modulatory role of the polyglutamic solution in the main biological parameters that are deregulated in rosacea, reducing the exacerbation of the inflammatory response, hypervascularization and skin sensitivity. The adherence to skin care advice and consequent application of adequate non-irritating skin care can significantly prevent events of rosacea aggravation and improve the patient’s quality of life. Skin care consists primarily of avoidance of trigger factors, usage of sunscreen and application of dermocosmetics specific for this condition.

Conflict of Interest Statement.

NONE.

References.

1. Holmes AD, Spoendlin J, Chien AL, Baldwin H, Chang ALS. Evidence-based update on rosacea comorbidities and their common physiologic pathways. J AmAcad Dermatol. 2018;78(1):156–66.
2. Weinstock L. Crohn's disease and rosacea: effect of rifaximin and adalimumab. Am J Gastroenterol. 2011;106:S449.
3. Two AM, Wu W, Gallo RL, Hata TR. Rosacea: part I. Introduction, categorization, histology, pathogenesis, and risk factors. J Am Acad Dermatol. 2015;72(5):749–58 (quiz 759–760).
4. O'Reilly N, Menezes N, Kavanagh K. Positive correlation between serum immunoreactivity to Demodex-associated Bacillus proteins and erythematotelangiectatic rosacea. Br J Dermatol. 2012;167(5):1032–6.
5. Wong F, Rayner-Hartley E, Byrne MF. Extraintestinal manifestations of Helicobacter pylori: a concise review. World J Gastroenterol. 2014;20(34): 11950–61.
6. Addor FAS. Skin barrier in rosacea. An Bras Dermatol [Internet]. 2016Jan;91(An. Bras. Dermatol., 2016 91(1)).
7. Wang G. Human antimicrobial peptides and proteins. Pharmaceuticals (Basel). 2014;7:545-94.
8. Yamasaki K, Schauber J, Coda A, Lin H, Dorschner RA, Schechter NM, et al. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J. 2006;20:2068-80.
9. Reinholz M, Ruzicka T, Schauber J. Cathelicidin LL-37: An Antimicrobial Peptide with a Role in Inflammatory Skin Disease. Ann Dermatol. 2012;24:126-35.
10. Steinhoff M, Buddenkotte J, Aubert J, Sulk M, Novak P, Schwab VD, et al. Clinical,cellular, and molecular aspects in the pathophysiology of rosacea. J Investig Dermatol Symp Proc. 2011;15:2-11.

11. Voegeli R, Rawlings AV, Doppler S, Schreier T. Increased basal transepidermal water loss leads to elevation of some but not all stratum corneum serine proteases. Int J Cosmet Sci. 2008;30:435-42.
12. Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. Physiol Rev. 2007;87:165-217.
13. Sulk M, Seeliger S, Aubert J, Schwab VD, Cevikbas F, Rivier M, et al. Distribution and expression of non-neuronal transient receptor potential (TRPV) ion channels in rosacea. J Invest Dermatol. 2012;132:1253-62.
14. Steinhoff M, Buddenkotte J, Aubert J, et al.: Clinical, cellular, and molecular aspects in the pathophysiology of rosacea. J Investig Dermatol Symp Proc. 2011; 15(1): 2–11.
15. Sulk M, Seeliger S, Aubert J, et al.: Distribution and expression of nonneuronal transient receptor potential (TRPV) ion channels in rosacea. J Invest Dermatol. 2012; 132(4): 1253–62.
16. Everaerts W, Gees M, Alpizar YA, et al.: The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. Curr Biol. 2011; 21(4):316–21.
17. Caterina MJ, Schumacher MA, Tominaga M, et al.: The capsaicin receptor: a heatactivated ion channel in the pain pathway. Nature. 1997; 389(6653): 816–24.
18. Story GM, Peier AM, Reeve AJ, et al.: ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell. 2003; 112(6): 819–29.
19. Moore C, Cevikbas F, Pasolli HA, et al.: UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin-1 signaling. Proc Natl Acad Sci U S A. 2013; 110(34): E3225–34.
20. Mihara S, Shibamoto T: The role of flavor and fragrance chemicals in TRPA1 (transient receptor potential cation channel, member A1) activity associated with allergies. Allergy Asthma Clin Immunol. 2015; 11(1): 11.

21. Drummond PD, Su D: Endothelial and axon reflex vasodilatation to acetylcholine in rosacea-affected skin. Arch Dermatol Res. 2012; 304(2): 133–7.

22. Steinhoff M, Ständer S, Seeliger S, et al.: Modern aspects of cutaneous neurogenic inflammation. Arch Dermatol. 2003; 139(11): 1479–88.

23. Aubdool AA, Brain SD: Neurovascular aspects of skin neurogenic inflammation. J Investig Dermatol Symp Proc. 2011; 15(1): 33–9.