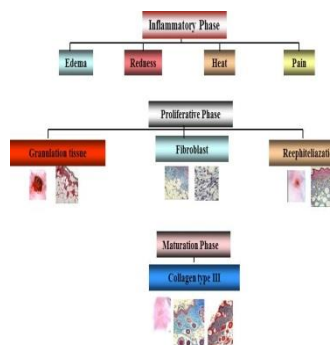


Healing Activity Induced by Glucose/Mannose Lectins in Mice

P-CM29

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Skin Lesions - Healing Process

This work aimed the use of glucose/mannose lectins (Cramoll, EmaL and Con A) in the treatment of cutaneous wounds in mice. Surgical wounds were treated daily with the lectins and parameters such as edema, hyperemia, scab, granulation and scar tissues as well as contraction of wounds were analyzed. The lectin wounds showed higher edema and arrival of more polymorphonuclear cells at the site of lesions when compared with control group (0.15 M NaCl). Granulation tissue and collagen fiber deposition were observed with higher intensity in all lectin treated wounds promoting excellent closing and repair of lesions in less time than other groups. Results showed that Cramoll was more effective in the repair of experimental lesions in mice; however, the glucose/mannose lectins can be used as future cicatricial compounds.

Introduction

The healing process aims to recover anatomical and physiological integrity of tissue. The repair of a wound is a complex sequence of biochemical and cellular events in response to tissue lesion being divided in phases: hemostasis, inflammatory, proliferation or granulation and remodeling of the extracellular matrix (ECM) or maturation [1]. It is important to emphasize those significant variations in the nature, composition and duration of these phases in different wounds, depending on the location of tissue, degree of contamination and bacterial infection, sanguine irrigation and extension of injury to the tissue. The infection compromises reepithelialization and increases collagen deposition. Since prehistory, plants and their by-products were used to treat wounds and these biomaterials can be applied in the rehabilitation of injuries. Biomaterials are defined as any molecule that has the capacity to interact with the biological system without inducing an adverse response in the host. Among them are lectins, which constitute a heterogeneous group of proteins, structurally distinct, with two or more specific binding sites to mono or oligosaccharides. These molecules, purified mainly from different species of plants, have attracted great interest. Lectins have various biological activities, in particular, the interactions of plant lectins with human pathogenic bacteria and their immunomodulatory activity, lymphoproliferation, CD4-mediated signal transduction and functional activation of monocytes and macrophage-like cells [9]. Cramoll is a lectin extracted from seeds of *Cratylia mollis*

Mart., a plant native to Northeastern Brazil. Cramoll is from the same Leguminosae family and Diocleinae subtribe as Concanavalin A (Con A; a lectin from *Canavalia ensiformis* seeds) and like Con A, Cramoll is glucose/mannose specific. Several studies have demonstrated the immunomodulatory profile of this lectin, production of IFN- γ and nitric oxide, mitogenic activity on human lymphocytes and antitumor activity. EmaL is a lectin obtained from *Eugenia malaccensis* L. seeds with antibacterial activity; it is also specific for glucose/mannose. Thus, in order to evaluate the effects of glucose/mannose lectins Cramoll, Con A and EmaL in topical applications, this study investigated *in vivo* clinical and histopathological aspects of cutaneous wounds experimentally performed in healthy and immunocompromised mice, to find an alternative biomaterial for repairing skin lesions.

Methods

C. mollis seed extract (10% w/v prepared in 0.15M NaCl) was fractionated using ammonium sulphate (40–60% w/v) following by 0.3 M D-glucose bio-selectively elution with affinity chromatography in Sephadex G-75 [2]. EmaL was purified from a 10% (w/v) *E. malaccensis* seed extract in 0.15 M NaCl aqueous solution (crude extract, CE). CE was precipitated by 0–80% ammonium sulphate fractionation (F0-80) and the precipitate was further purified by 0.3 M glucose bio-specifically elution with affinity chromatography in Sephadex G-50 column. Con A from *C. ensiformis* (Jack bean) Type IV was purchased from Sigma Chemical Co., USA.

Females of albino Swiss mice (n = 30/group), 12 weeks old (age 35–45 days, weight 25 ± 2 g), were used from the Bioterium of LIKA/UFPE. All mice were treated and killed in accordance with the Ethical Committee of UFRPE for Experiments with Laboratory Animals (Ministry of Health, Brazil, 012/02). Each animal was maintained in an individual cage, under controlled environmental conditions (12-h light/dark cycle, temperature 23±2 °C and humidity 55±10%) with water and commercial food ad libitum (Labina®, Agribands of Brazil). Animals were divided into four groups treated daily with 100 µL (100 µg/mL) of Cramoll (Cramoll group – CraG), EmaL (Emal group – EG) and Con A (Con A group – ConG) solutions and 100 µL of NaCl 0.15 M (Control group – CG1 and CG2), and were anesthetized for the surgical procedure. Each animal was placed in a prone position and prepared for aseptic surgery using 2% chlorhexidine digluconate. A standard wound was performed on the dorsal thoracic region. The clinical characteristics of the experimental wounds were 24 h after surgery, taking into consideration the following aspects: edema, hyperemia, secretion, bleeding, crust formation, scab, granulation, epithelialization and scar tissues. On a daily basis, wound areas were measured using a pachometer and were calculated as follows: $A = \pi R \times r$, where A, R and r are mean area, large ray and small ray, respectively. The calculation of the contraction degree was expressed in percentage using the equations proposed by Ramsey [3], $100 \times (W_0 - W_i)/W_0 = M \pm SE$ (W_0 = initial area of the wound; W_i = area of the wound on the day of the biopsy; M = average; SE = stand error). At each time of biopsy, on the 2nd, 7th and 12th days after surgery, animals were drawn from the experimental groups, subjected to subcutaneous anesthesia and euthanized by cervical disruption. Immediately after the withdrawal of the skin, the samples were placed on a filter paper and settled in formaldehyde 4% (v/v) prepared in PBS 0.01 M pH 7.2 and submitted to the histopathology procedures. All results were expressed as mean values of groups ± standard deviation and were analyzed considering the value of $p < 0.05$ as statistically significant.

Results

Edema and hyperemia are characteristic signs of the pathophysiological point of view of inflammation and are directly related to local vasodilatation determined by vasoactive mediators, resulting in leakage of fluids into the extravascular space [17]. Figure 1 shows the degree of hyperemia and edema of the wounds treated with CraG and ConG in relation to CG1. Edema

was observed in the margins and/or the wound bed in all animals of groups, persisting after the second day except to CraG. The reduction of inflammatory signs were observed in CraG and ConG compared to CG1.

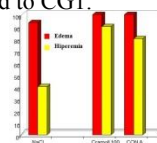


Figure 1. Comparison of measured inflammatory signs (edema and hyperemia) within two days after the wound was promoted.

The evolution of granulation tissue occurred exponentially especially in wounds treated by the CraG and ConG and more slowly in the CG1 (Figure 2A).

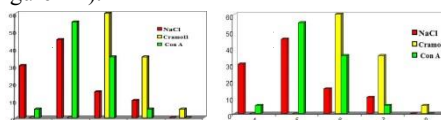


Figure 2. Frequency of granulation tissue (A) and scar tissue (B).

The formation of exuberant granulation, which exceeds the height of the surrounding intact skin tissue was observed in 20% of the CG1; 5% of CraG and 20% in group ConG. In the fourth and fifth day, there was an initial formation of scar tissue in CraG and ConG. On the seventh day the wounds had its edges with formation of scar tissue which was observed in small numbers in wounds of CG1 (Figure 2B). At day 12, cutaneous flaps, got an average of 100% contraction in CraG and ConG and 92.12% in CG1 (Figure 3); noting that in this group, at the 12th day there was still granulation tissue and crusting.

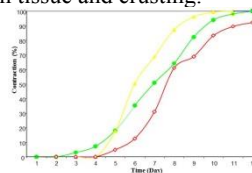


Figure 3. Average wound contraction during the period of 12 days. —◇— NaCl, —□— Cramoll and —●— Con A.

To EG it was observed during the inflammatory period the presence of edema, however, this clinical signal remained until the sixth day after surgery in the EG although in CG2 remained until the eighth day. On the sixth day no hyperemia was observed in EG, while 11.11% of the animals of the CG2 were still presenting this flogistic signal. In the first day it was observed the first crust only

in the group treated with EG (33.3%). Between the 3rd to 5th and the 7th to 9th days 100% of EG presented the first crust, situation that did not occur in CG2. The second crust was identified macroscopically after the seventh day in EG and CG2 (Figure 4A and B). The presence of granulation tissue was observed between the fifth and seventh days in EG and CG2 (Figure 4C and D) and the peak in EG was obtained on the seventh day.

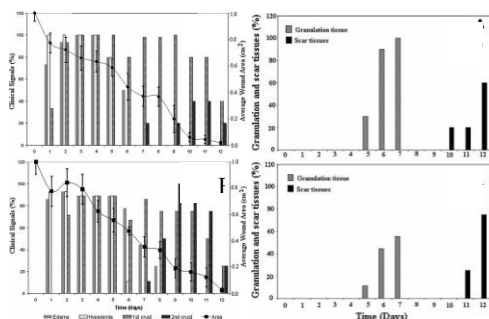


Figure 4. Variation with time of: clinical signals after the topic treatment with Emal (A) and 0.15 M NaCl (B); granulation and scar tissue after the topic treatment with EmaL (C) and NaCl 0.15 M (D).

The microscopic evaluation of the healing process was followed by the crust presence, infiltrated inflammatory cells, angiogenesis, granulation tissue and reepithelialization of lesion. On the seventh day it was observed that there was a greater regression of granulation tissue, collagen deposition largest and most advanced reepithelialization in CraG compared to ConG and CG1 (Figura 5A, B and C). In the twelfth day, it was observed in CraG and ConG wound closure with complete re-epithelialization of the skin, which hyperplasia was associated with the regression of blood vessels in the dermis and better deposition and organization of collagen fibers, and formation of annex sprouts well developed in wounds of CraG. Partial reepithelialization of the wound with missing closing, was observed in CG1. In CraG and ConG during inflammatory and fibroplasia phase, it was observed the presence of different cell types such as neutrophils, eosinophils, lymphocytes, plasma

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cells and mast cells (Figure 5D, E and F). On the seventh day, the transition areas of the injuries of EG were characterized for presenting a more extensive reepithelialization towards the center of injury supported by a fibrovascular granulation tissue compared to CG2 (Figure 6A and B). During the 12th day after surgery, reepithelialization was complete or partial in EG and CG2. With respect to the EG the transition areas were well organized, however, difficult to visualize due to the progression of the phenomenon. Moreover, the granulation tissue was fibrous (rich in collagen), presenting some small vessels (Figure 6C). With respect to CG2, only the presence of granulation tissue with fibrovascular and vascular characteristics was observed in the animals (Figure 6D).

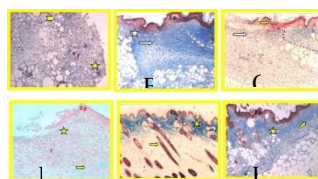


Figure 5. Histopathological views of the cutaneous wounds. Seventh day after surgery: NaCl (A), Cramoll (B) and Con A (C); 12th day after surgery: NaCl (D), Cramoll (E) and Con A (F).

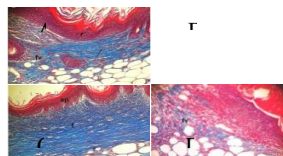


Figure 6. Histopathological views of the cutaneous wounds; 7th day after surgery: EmaL (A) and 0.15 M NaCl (B); 12th day after surgery: EmaL (C) and 0.15 M NaCl (D).

Conclusion

The results demonstrated that the lectins could be used in the process of cutaneous wounds repair. Future research on the applications of lectins obtained from medicinal plants, in biological systems, can be of great importance for therapeutic treatments.