

Research Paper
Clinical Pathology

Amniotic membrane as a biological dressing for 5-fluoruracil-induced oral mucositis in rats

G. de M. G. Lima, M. C. Severo,
G. de F. Santana-Melo,
M. A. Carvalho,
M. das G. Vilela-Goulart,
M. A. C. Salgado, M. F. Gomes

Department of Bioscience and Oral
Diagnosis, Universidade Estadual Paulista
"Júlio de Mesquita Filho", São José dos
Campos, São Paulo, Brazil

G. de M. G. Lima, M. C. Severo, G. de F. Santana-Melo, M. A. Carvalho, M. das G. Vilela-Goulart, M. A. C. Salgado, M. F. Gomes: Amniotic membrane as a biological dressing for 5-fluoruracil-induced oral mucositis in rats. *Int. J. Oral Maxillofac. Surg.* 2015; 44: 845–851. © 2015 International Association of Oral and Maxillofacial Surgeons. Published by Elsevier Ltd. All rights reserved.

Abstract. Oral mucositis is a reaction to chemoradiation therapy during cancer treatment. The aim of this study was to investigate the use of amniotic membrane as a biological dressing for oral mucositis lesions in rats. Sixty Wistar rats were divided into three groups ($n = 20$): control, 5-fluoruracil (5-FU), 5-fluoruracil + amniotic membrane (5-FU + AM). Each group was subdivided ($n = 5$) according to the time interval to sacrifice (3, 7, 14, and 21 days). Histology (haematoxylin–eosin staining) and immunocytochemistry (anti-rat antibodies CD4, CD8, VEGF, and PCNA) were evaluated. Immunocytochemistry results were analyzed using one-way ANOVA and Tukey tests. The amniotic membrane (5FU + AM) played an important role in cell proliferation (PCNA 3 days 27.08 ± 4.65 , 7 days 27.90 ± 3.34) and especially in neovascularization (VEGF 3 days 23.00 ± 1.40 , 7 days 26.00 ± 0.95) for all time intervals, when compared to 5-FU (PCNA 3 days 23.12 ± 1.61 , 7 days 37.21 ± 1.20 ; VEGF 3 days 17.05 ± 1.51 , 7 days 8.45 ± 1.35) and control (PCNA 3 days 29.99 ± 0.92 , 7 days 16.33 ± 2.88 ; VEGF 3 days 13.65 ± 0.55 , 7 days 15.70 ± 1.39). It was biocompatible, showing significant differences compared to the other groups in CD4 ($F = 40.72$; $P = 0.001$) and CD8 ($F = 69.99$, $P = 0.001$) staining together, only during the inflammation phase (7 days). Amniotic membrane presented biocompatibility and stimulated cell proliferation and neovascularization, functioning as a promising biological dressing.

Key words: oral mucositis; amniotic membrane; therapy; chemotherapy; wound healing.

Accepted for publication 12 January 2015
Available online 4 February 2015

Oral mucositis (OM) is a common, symptomatic, and regimen-limiting condition related to head and neck chemoradiation protocols.^{1,2} The clinical appearance of OM varies from redness of the intact mucosa to symptomatic ulcers.³ Pathologically, OM results in a thin epithelium and

ulcers that are thought to be caused by inflammation and depletion of the basal layer of epithelial cells, with subsequent bacterial infection.⁴

Studies have suggested that chemotherapy acts on the basal layer of epithelial cells, inducing a loss of self-renewal

capacity.^{3,5} The pathobiology of OM can be divided into five stages: initiation, upregulation and message generation, amplification, ulceration, and healing.^{3,5} According to a model of mucositis, there are two main factors involved in its initiation by radiation or chemotherapy: the

death of clonogenic cells and the generation of reactive oxygen species (ROS) by damaged cells. The initiation phase is a gatekeeper phase, and responding to or preventing it can minimize or prevent regimen-related injury.⁶

According to the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology (MASCC/ISOO), the therapies available for OM involve nutritional support, pain control, oral hygiene, and the management of dry mouth and bleeding.^{2,7}

The amniotic membrane (AM) is the innermost layer of the placenta. It has a thin epithelium and a non-vascular stroma that contain growth factors, cytokines, and other active substances.^{8,9} Although the mechanisms involved in the biological benefits of the AM are not yet completely understood, the AM is known to provide support for cell growth and adhesion.⁹ The AM has been used as a biological dressing in wound repair,^{10–12} vestibuloplasty,¹³ and alveoloplasty¹⁴ with favourable results. However, we could identify no studies investigating the benefits of AM in chemotherapy or radiation-induced mucositis.

The aim of the present study was to evaluate the effect of AM on wound repair of 5-fluorouracil (5-FU)-induced mucositis in rats. We used immunocytochemistry to detect proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), CD4, and CD8 to evaluate cell and vascular proliferation. In this study, we found that the use of AM led to important tissue improvements; it acted as a physical barrier and scaffold during wound repair, and accelerated the healing process.

Materials and methods

Animals

All animal procedures were performed in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA) and Federal Law Number 11794. The experiment was approved by the local ethics committee on research involving animals. All rats received food *ad libitum*.

Prior to the experiment, animals were treated with a single dose of the anthelmintic albendazole (Zentel 150 mg/kg; GlaxoSmithKline Brasil, Rio de Janeiro, RJ, Brazil) and multivitamins (Vita Gold; Tortuga Companhia Zootécnica Agrária, Mairinque, SP, Brazil), 40 drops per litre in drinking water for 15 days.

To obtain AM, 15 pregnant rats (*Rattus norvegicus* var. *albinus*; Wistar) were

maintained in cages (five per cage) for a gestation period of 21 days.

For experimental mucositis, 60 male rats (*Rattus norvegicus* var. *albinus*; Wistar), 90 days old, were divided randomly into three groups: (1) a control group in which an oral ulcer was induced with 50% acetic acid on the buccal fornix on the lower incisors ($n = 20$); (2) a 5-FU group, treated with 5-FU to induce OM + 50% acetic acid to induce an ulcer ($n = 20$); (3) 5-FU + AM group, treated with 5-FU to induce OM + 50% acetic acid to induce an ulcer and then treated with an AM biological dressing ($n = 20$). Each group was subdivided ($n = 5$) according to the time interval to sacrifice after the induction of mucositis (3, 7, 14, and 21 days).

Amniotic membrane harvesting

Fertile female and male rats were maintained in cages overnight. In the morning, female rats were submitted to microscopic evaluation of cervical smears. The female rats that presented smears indicating a mating event (15 rats) were housed in separate cages for 21 days. Hysterectomies were then performed to obtain the AM.

On day 21 of gestation, the females were anaesthetized with a mixture of ketamine (Dopalen 5 mg/kg; Agribands do Brasil Ind. e Com. Ltda, Paulínia, SP, Brazil) and xylazine (Anasedan 10 mg/kg; Agribands do Brasil Ind. e Com. Ltda), and caesarean surgery was performed. After removing the placenta, the AM was separated aseptically from the chorion and washed with a saline solution and phosphate buffer (pH 7.4). The AM was stored in liquid nitrogen immersed in lactated Ringer's solution (Equiplax, Aparecida de Goiânia, GO, Brazil) containing 10 mM HEPES (2.38 g/l) and 2 ml of penicillin (10,000 IU/ml) and streptomycin (10 mg/ml) (Cultlab, Campinas, SP, Brazil) until use. For application as a biological dressing, the AM was washed in phosphate buffer (pH 7.4) and incorporated into an orabase ointment.

Induction of oral mucositis

Rats were treated with three intercalated doses of 5-FU 30 mg/kg/day (Fauldfluor 2.5 g/50 ml; Libbs Farmaceutica Ltda, Embu das Artes, SP, Brazil) via the intramuscular route, to induce OM (days 0, 2, and 4).

After a week, clinical signs of 'chemotherapy' appeared: hair loss, diarrhoea, weight loss, and signals of OM (intact mucosal redness). We then defined the region to be treated. We placed a 9-mm²

filter paper immersed in 50% acetic acid (C₂H₃COOH; 10 µl) on the fornix region for 60 s, in accordance with Fujisawa et al.¹⁵ During treatment with 50% acetic acid, animals were anaesthetized with ketamine 5 mg/kg and xylazine 10 mg/kg (Fig. 1A and B).

Treatment with amniotic membrane

Once the study area was defined, rats from the 5-FU + AM group were anaesthetized (ketamine 5 mg/kg and xylazine 10 mg/kg) and the areas of mucositis (buccal fornix of the mandibular incisors) were treated with AM incorporated into the orabase ointment (Fig. 1C and D); this resulted in a multilayer dressing that was applied only to the area of the ulcer. The inner borders of the lower lip were immediately sutured using an absorbable thread (Vicryl 6-0; Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil). After application of the AM, all rats had their nails cut and were fed with ground food until the end of the experiment. These modifications helped to keep the AM in position until euthanasia.

The clinical aspect of the area of mucositis was evaluated throughout the entire experiment. Details are reported in the Results section together with data on the other clinical aspects as regard the effects of chemotherapy and membrane treatment. We did not measure the ulcer diameter.

Euthanasia

Five animals in each group were euthanized at time intervals of 3, 7, 14, and 21 days after the study area was delimited with 50% acetic acid (ulcer induction). They were anaesthetized with ketamine 10 mg/kg and xylazine 20 mg/kg and decapitated using a guillotine. The entire lower lip was fixed in 4% paraformaldehyde (pH 7.4) for 48 h, washed, and put into 70% ethanol. It was subsequently embedded in Paraplast (Sigma-Aldrich Co. LLC, St. Louis, MO, USA).

Microscopic analysis (histology)

After embedding in Paraplast, 5-µm histological sections were cut, placed on glass slides, and stained with haematoxylin and eosin (HE). The HE-stained section slides were described for their histological aspects.

Immunocytochemistry

For immunocytochemistry, 3-µm slices were placed on silane-coated slides. The

Download English Version:

<https://daneshyari.com/en/article/3132433>

Download Persian Version:

<https://daneshyari.com/article/3132433>

[Daneshyari.com](https://daneshyari.com)