King Saud University

The Saudi Dental Journal

ORIGINAL ARTICLE

Effect of multidrug solution for the treatment of chemotherapy-induced oral mucositis *in vivo*

Rebecca Rhuanny Tolentino Limeira [a], Isabella Lima Arrais Ribeiro [a,b],
Paulo Rogério Ferreti Bonan [c], Danielle da Nóbrega Alves [d],
Elba dos Santos Ferreira [e], Tereza Karla Vieira Lopes da Costa [f],
Cassiano Francisco Weege Nonaka [g], Ana Cláudia Dantas de Medeiros [h],
Frederico Barbosa de Sousa [e,i], Ana Maria Gondim Valença [j],
Ricardo Dias de Castro [k,*]

[a] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[b] *Department of Social Medicine, Medical School of Ribeirão Preto of University of São Paulo, Medical School of Ribeirão Preto of University of São Paulo, Brazil*
[c] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[d] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[e] *Department of Pharmaceutical Sciences, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[f] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[g] *Department of Dentistry, State University of Paraíba, Campus I, Campina Grande, PB, Brazil*
[h] *Department of Pharmaceutical Sciences, State University of Paraíba, Campus I, Campina Grande, PB, Brazil*
[i] *Department of Morphology, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[j] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*
[k] *Department of Clinical and Social Dentistry, Federal University of Paraíba, Campus I, João Pessoa, PB, Brazil*

* Corresponding author at: Health Sciences Center, Federal University of Paraíba, Campus I, 58051-970, João Pessoa, PB, Brazil.
  E-mail addresses: rebecca.rhuanny@hotmail.com (R.R. Tolentino Limeira), isabella_arrais@yahoo.com (I. Lima Arrais Ribeiro), pbonan@yahoo.com (P.R. Ferreti Bonan), dnobregaalves@msn.com (D. da Nóbrega Alves), elbaferreira99@gmail.com (E. dos Santos Ferreira), tereza.vieira.91@gmail.com (T.K. Vieira Lopes da Costa), cfwnonaka@gmail.com (C.F. Weege Nonaka), anaclaudiamedeiros.uepb@gmail.com (A.C. Dantas de Medeiros), fredericosousa@hotmail.com (F. Barbosa de Sousa), anamvalenca@gmail.com (A.M. Gondim Valença), rcastro@ccs.ufpb.br (R. Dias de Castro).

ELSEVIER | **Production and hosting by Elsevier**

Abstract *Objective:* Evaluate the effect of a multidrug solution, adopted by a referral hospital for cancer to control and treat chemotherapy-induced oral mucositis in rats.

Methods: Oral mucositis (OM) was induced by 5-Fluorouracil (5-FU), and the animals were treated with saline (n = 8, G1), 0.12% chlorhexidine (n = 8, G2); and multidrug solution (n = 8, G3). The animals were submitted to clinical and histological analysis of the lesion using mucosal fragments. The animals' food consumption during treatment was also evaluated.

Results: Clinical improvement ($p < 0.05$) was observed in the groups treated with the multidrug solution and 0.12% chlorhexidine digluconate. In G2 and G3, there was a prevalence of reepithelialization covering < 50% of the lesion. Evaluation of the inflammatory infiltrate indicated that the G1 treatment permitted an intense inflammatory response in all animals, yet this evaluation parameter was moderate in groups G2 and G3. The G3 group ($p < 0.05$) presented higher food consumption than the other groups.

Conclusions: The multidrug solution improved the clinical and histological parameters of the chemotherapy-induced oral mucositis, as well as promoted an increase in food intake.
© 2023 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In patients undergoing chemotherapy and/or radiotherapy, oral mucositis (OM) is an important acute adverse effect in the oral cavity, and is considered the most common cause of morbidity and mortality (Zhang et al., 2016). Oral lesions can lead to a considerable decrease in the quality of life. OM causes difficulty in feeding, pain, burning when swallowing, and poor coordination of the speech muscles. In addition, the injury can represent a gateway for opportunistic infections. (Bolouri et al., 2015).

When caused by chemotherapy, OM presents a prevalence of 40%, and when caused by combination radio-chemotherapy the prevalence can reach 100% (Panahi et al., 2010). Direct damage to the mucous membrane due to the production of reactive oxygen species, and secondary infections in the oral region due to immunological depression associated with myelosuppression can both contribute to the appearance of OM (Bian et al., 2015).

From a clinical point of view, OM is characterized by erythematous lesion with the presence of edema or ulceration, and can be accompanied by changes ranging from mild to severe burning sensations (Yoshino et al., 2013). Symptoms such as eating and sleeping disorders, communication difficulties, and acute pain are also associated with the disease and reduce the quality of life of affected individuals (Rodríguez-Caballero et al., 2012). OM can cause loss of consciousness and forced cessation of treatment (Vieira et al., 2012).

The treatments for OM include oral hygiene (Rodríguez-Caballero et al., 2012), mouthwashes with antimicrobial agents (Vieira et al., 2012), the use of anti-inflammatory drugs (Lalla et al., 2014), topical and systemic analgesics (Nicolatou-Galitis et al., 2013), topical antioxidants (Moura et al.,2016), protective agents, mucosa lining treatments with B (Lalla et al., 2014) complex vitamins and cryotherapy (Nicolatou-Galitis et al., 2013). Previous study has demonstrated that the use of low-level laser therapy and antimicrobial based mouthwash solutions are effective, especially 0.12% chlorhexidine digluconate (Moura et al., 2016).

The scarcity of consistent information concerning adoption of validated protocols for treatment of chemotherapy induced OM drives reference hospitals treating cancer to establish their own protocols for treatment and prevention of the disease. One of these therapeutic proposals includes the use of a multidrug solution for daily mouthwash: (nystatin, dexamethasone, diphenhydramine, morphine, lidocaine, B vitamins and saline) (Costa et al., 2018; Ribeiro et al., 2015). So, this study aimed to evaluate this therapeutic proposal for chemotherapy-induced OM in rats, using clinical and histopathological evaluation parameters, food intake, and the pharmaceutical compatibility of the solution components.

2. Materials and methods

2.1. Animals

Twenty-four male Wistar rats, aged between 90 and 100 days, and an average weight of 300 g, were obtained from the Vivarium of the Centro Universitário de João Pessoa, Paraíba, Brazil. The sample size definition was conducted in accordance with ANOVA testing (One-way), and for sample calculation, the highest sample proportion for the treatment was estimated using values obtained from a previous study determining the clinical efficacy of laser therapy, a proven alternative method for the treatment of oral mucositis (Migliorati et al., 2013). The sample design adopted a confidence level of 95% (one-tailed alpha error = 5%), study power of 80% ($1 - \beta$), and an effect magnitude (g of hedge) of 1.3, resulting in a composition for each experimental of 8 animals per group.

2.2. Ethical considerations

The research project was previously approved by the Ethics Committee on the Use of Animals at the Federal University of Paraíba on May 25, 2018, under no. 6464080318.

2.3. Substances used and drug preparation

– Chemotherapy. 5-FU (Fauldfluor® 2.5 g/50 mL, LIBBS Farmacêutica Ltda., São Paulo – SP, Brazil).
– Oral lesion inducer – topical application. 20% acetic acid (C_2H_3COOH, 99.7%, NEON Farmacêutica Ltda., São Paulo – SP, Brazil) administered dose of 100uL. – Reference drug. Chlorhexidine digluconate 0.12% (Periogard®, Reymer, Goiânia – GO, Brazil).
– Multidrug solution. The drugs used to prepare the experimental multidrugs solution are described in Table 1.

2.4. Methodological design

The investigation is characterized as an *in vivo*, controlled, randomized, and double-blind study. The animals were randomly divided into 3 groups:

Group 1 (G1): Negative control, composed of 8 animals that underwent the OM induction protocol and received 0.9% saline.

Group 2 (G2): Positive control, composed of 8 animals that underwent the OM induction protocol and received treatment with 0.12% chlorhexidine digluconate.

Group 3 (G3): Experimental group, composed of 8 animals that underwent the OM induction protocol and received treatment with the multidrug solution.

2.5. Animal experimentation

The rats received an intraperitoneal injection of 5-FU (30 mg/kg/day) on the 1st, 2nd, and 3rd days of the experiment. Subsequently, on the 4th day of the experiment, to form ulcers in the buccal fornix region of the lower incisor, a 9 mm^2 filter paper soaked with 20% acetic acid (100 μL), was applied for 60 s to each animal, according to the recommended technique (Fujisawa et al., 2003).

After OM induction, treatment began on the 5th day, and continued until the 8th day of the experiment. The solutions for groups 1, 2, and 3 were administered every 4 h, 100 μL applied with the aid of a sterile cotton swab for 60 s, with a 12-hour non-use interval (8:00 PM–08: 00 AM), for 4 days.

2.6. Feed/nourishment

All animals were fed in a controlled manner throughout the experiment, with 100 g/day/cage of standard Presence® pellet food at the beginning of each day. They were weighed each following day (24 h period) using residual ration, to calculate food intake, this since the principal signs and symptoms of oral mucositis are pain and difficulty in food intake (Schirmer et al., 2012).

2.7. Clinical evaluation

The OM injuries were evaluated for days 5, 6, 7, and 8 using photographic records from a digital camera (Canon EOS T5i) with good resolution (12.3 MP), and a thirty-five – eighty millimeter (35–80 mm) objective with autofocus. Clinical evaluation of the OM severity was performed by observing the photographs, having been previously mixed, coded, and blind analyzed by a single examiner using the parameters described previously (Sonis et al., 2000). This analysis was performed twice by the same examiner, with an interval of one month, for correct calibration (: 0.87).

2.8. Histological evaluation

Upon finishing the experiments, all animals were euthanized. The specimens obtained from an excisional biopsy of the buccal fornix of the lower incisors were fixed in 10% buffered formaldehyde and included in paraffin. The anatomical specimens were sequentially stained in hematoxylin and eosin (HE), and under light microscopy (Leica DM500, Leica Microsystem Vertrieb GmbH, Wetzlar, DE), a previously trained examiner, with a degree in stomatopathology, performed histomorphological analysis of the specimens. The degree of reepithelization of the wounds was assessed according to criteria proposed previously described (Meireles et al., 2008), and the intensity of the inflammatory infiltrate in the tissues was analyzed based on criteria proposed by the previous study (Isana et al., 2013). The histological examination was performed as a blind test.

Table 1 Multidrugs solution components used to treatment of oral mucositis.

Components	Molecular Formula	Quantity	Action
Nystatin (Micostatin®, Bristol-Myers Squibb Farmacêutica S.A. – São Paulo – SP, Brasil)	$C_{47}H_{75}NO_{17}$	20 mL	Antifungal
Dexamethasone (Decadron®, Aché Laboratórios Farmacêuticos S.A. Guarulhos – SP, Brasil)	$C_{22}H_{29}FO_5$	1 ampule of 1 mL	Anti-inflammatory
Diphenhydramine (Difenidrin®, Cristália Produtos Químicos Farmacêuticos Ltda. – Butantã – São Paulo-SP, Brasil)	$C_{17}H_{21}NO$	1 ampule of 1 mL	Anti-histamine
Morphine (Dimorf®, Cristália Produtos Químicos Farmacêuticos Ltda. – Butantã – São Paulo-SP, Brasil)	$C_{17}H_{19}NO_3$	1 ampule of 1 mL	Analgesic
Lidocaine 2% (Xylocaína®, Hipolabor Farmacêutica Ltda. Borges /Sabará – MG, Brasil)	$C_{14}H_{22}N_2O$	10 mL	Anesthetic (local)
B complex vitamins (Complexo B Medquímica®, Medquímica Indústria Farmacêutica S.A. – Juiz De Fora – MG, Brasil)	$C_6H_6ON_2$	1 ampule of 1 mL	Tissue repair adjuvant
Physiological Saline Solution 0.9% (Cloreto de Sódio 0.9%, Pro Soro Dauf Indústria Farmacêutica S.A, Brasil)	NaCl	250 mL	Vehicle

Source: Protocol for the treatment of oral mucositis used by a referral hospital for cancer treatment (Oliveira et al., 2011).

2.9. Differential Thermal Analysis (DTA)

Differential thermal analysis was performed using a Shimadzu simultaneous thermal analyzer, the derived thermogravimetry model (DTG), and the following parameters were established: closed aluminum crucibles, mass of 2.0 mg (± 0.1) for each sample, and nitrogen atmosphere with gas flow controlled at 50 mL/min. Thermoanalytical curves were performed in the temperature range from 25 to 450 °C, with a heating rate of 10 °C/min. The data were analyzed using Shimadzu's TASYS software. Compatibilities between the active pharmaceutical ingredients (API) were carried out, evaluating the thermal behavior of the drugs alone and in binary mixtures a 1:1 ratio. The compatibility assessments were carried out based on the methods described by (Wesolowski and Rojek, 2013).

2.10. Statistical analysis

The data obtained from the weighing of individual feed rations were recorded in Microsoft Excel and analyzed using descriptive and inferential statistics (normality test of Shapiro-Wilk), where values related to the ration weights presented a normal distribution for all evaluation days in all experimental groups, using paired T-test and one-way ANOVA with duplicate measurements The scores obtained from the reepithelization and inflammatory infiltrate assessments were subjected to statistical analysis using the Kruskal-Wallis test with the Student-Newman-Keuls post-test. It was adopted a significance level of 5%.

3. Results

With administration of 5-FU and topical application of acetic acid (20%), the appearance of induced OM was observed with the presence of ulcers. These ulcers covered from 50% of the application site to complete mucosal ulceration, which confirmed our validation of the proposed protocol for experimental lesion induction described in previous study (Shimamura et al., 2018).

For all groups, the OM induction protocol caused mortality (12.5% of the sample). During the experiment it was observed that some animals were weakened, with clinical signs of alopecia, an infection associated with mucositis and decreased food consumption.

According to the proposed scale (Sonis et al., 2000), all groups started the treatment protocol (5th day) with similar OM degrees, varying between scores of 4 and 5, which respectively indicate either formation of ulcers covering 50% of the region, or complete mucosal ulceration. When comparing all groups at each experimental time, a difference on days 7 and 8 was observed between the animals that used saline solution (G1) and the animals of the other groups, with significant clinical improvement ($p < 0.05$) in the groups who used 0.12% chlorhexidine digluconate solution (G2) and the multidrug experimental solution (G3). These data are presented in Table 2.

Histological analyses were performed for each group separately on the last day of the experiment. No reepithelialization process was observed for the entire saline treated group. In the groups treated with 0.12% chlorhexidine digluconate and with the multicomponent solution, there was evidence of reepithe-

Table 2 Clinical parameter defined in degrees of lesion severity, and assessed during the treatment of chemotherapy-induced oral mucositis in Wistar rats with different therapeutic protocols. Values expressed as medians.

Groups	Days			
	5°	6°	7°	8°
	Degree of severity			
Saline	4	4	5	5
0.12% chlorhexidine digluconate	4	5	3*	2*
Multicomponent solution	5	5	4*	3*

* Significant difference observed when values were compared with values within the same group at the beginning of treatment, and compared at the same time period with values of the control group (saline). $p < 0.05$, Kruskal-Wallis test.

lization (< 50% of the lesion), with no significant difference between the experimental groups ($p = 0.2654$, Kruskal-Wallis). The histological analysis data referring the reepithelialization process are shown in Table 3.

Regarding the reepithelialization process, for all analyzed cases, an area of epithelial lining discontinuity was found, covered by eosinophilic material with a fibroid aspect. Within this fibroid material, varying amounts of colonies of microorganisms were observed. These findings can be seen in Fig. 1A.

Evaluation of the inflammatory infiltrate indicated that the treatment with saline permitted a predominance in intense inflammatory response. However, as shown in Table 3, the majority of animals treated with 0.12% chlorhexidine digluconate, and all animals submitted to the multicomponent solution presented histological sections characterized by moderate inflammatory infiltrate. Statistical analysis indicated that as compared to the saline solution group, a significant improvement in inflammatory response occurred only for the animals treated with the multidrug solution, (Kruskal-Wallis test and Student-Newman-Kewls post-test, $p = 0.0237$).

In evaluation of the inflammatory infiltrate, for all groups the connective tissue underlying the epithelial tissue was characterized with an exuberant granulation reaction, with fibroblasts displaying bulky nuclei and newly formed blood vessels, some of which exhibited inconspicuous lumen. The elements described were arranged in the middle of thin and elongated collagen fibers, being predominantly loose and permeated by mononuclear inflammatory infiltrate consisting of lymphocytes and macrophages. In superficial regions of the connective tissue, without epithelial tissue covering, areas of neutrophilic inflammatory infiltrate were also observed. These findings are presented in Fig. 1B.

The consumption curves are similar for the 0.12% chlorhexidine digluconate and multidrug solution groups, yet all groups presented a decrease on the 5th day, with a variation in consumption until the last day of the experiment, but presenting a decreasing food intake trend for all animals.

Regarding food intake, we observed that shortly following the chemotherapy an increase in feeding occurred. However, at the last ration weighing, all groups presented a significant decrease in feeding ($p < 0.05$, ANOVA one-way test with measurements duplicated). When comparing the groups by food consumption, it was noted for being generally similar in all groups in the differing experimental times, and presenting sta-

Table 3 Degrees of reepithelization and presence of inflammatory infiltrate in lesions of oral mucositis induced by chemotherapy, in Wistar rats, treated with saline (G1), 0.12% chlorhexidine digluconate (G2), or multicomponent solution (G3).

Groups	Degree of reepithelization	n (%)	Degree of inflammatory infiltrate	n (%)
1	Absent	4 (57.1%)	Moderate	2 (28.6%)
	Reepithelization that covers < 50% of the wound	3 (42.9%)	Intense	5 (71.4%)
2	Absent	2 (28.6%)	Moderate	5 (71.4%)
	Reepithelization that covers < 50% of the wound	4 (57.1%)	Intense	2 (28.6%)
	Reepithelization that covers > 50% of the wound	1 (14.3%)		
3	Absent	1 (14.3%)	Moderate	7 (100.0%)
	Reepithelization that covers < 50% of the wound	6 (85.7%)		
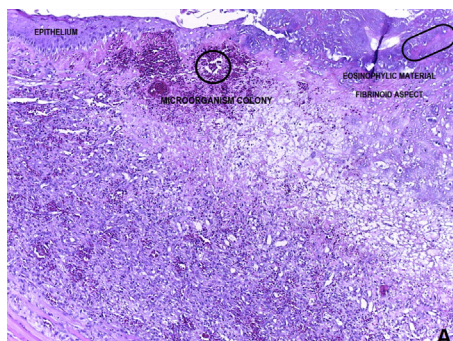
Fig. 1A H&E image of the histological section of the animals' oral mucosa, after treatment, detailing the reepithelialization.
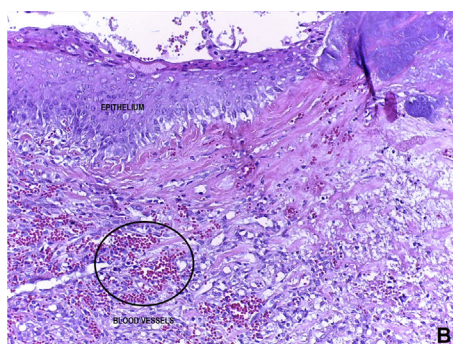
Fig. 1B H&E image of the histological section of the animals' oral mucosa, after treatment, detailing the inflammatory response.

tistical difference ($p < 0.05$) on days 4 and 5 alone, but with progressively decreasing consumption. These results are shown in Table 4.

Table 5 presents the endothermic and exothermic events observed in the binary mixtures analyzed. In the multicomponent solution proposed for OM treatment, five drugs were used with a vitamin complex: diphenhydramine, dexamethasone, nystatin, lidocaine, morphine, and a vitamin B complex. It was observed that all of the analyzed binary mixtures presented potential incompatibilities.

4. Discussion

This is the first experimental study using an animal model to evaluate the effect of a multidrug solution, proposed by a ref-

Table 4 Comparison of average food (grams) consumption per day between the experimental groups.

DAY	GROUP	Average ± SD (g/day)	Significance (P-value)
DAY 02	G1	63.00 ± 1.68	0.379
	G2	66.00 ± 1.87	
	G3	66.25 ± 3.30	
DAY 03	G1	48.50 ± 2.66	0.413
	G2	53.50 ± 3.20	
	G3	51.50 ± 1.44	
DAY 04	G1	59.00 ± 4.02[a]	0.003
	G2	58.75 ± 2.72[a]	
	G3	85.50 ± 6.02[b]	
DAY 05	G1	27.50 ± 0.64[A]	0.002
	G2	30.00 ± 0.91[A]	
	G3	35.50 ± 1.65[B]	
DAY 06	G1	36.75 ± 8.60	0.271
	G2	22.00 ± 2.97	
	G3	27.50 ± 5.20	
DAY 07	G1	10.50 ± 7.00	0.212
	G2	20.00 ± 1.58	
	G3	20.75 ± 2.21	
DAY 08	G1	5.75 ± 3.61	0.089
	G2	15.25 ± 3.96	
	G3	18.50 ± 3.50	

Legend: SD = Standard Deviation; One-way ANOVA with repeated means test; Significance = 5%; Different letters indicate differences between groups.

erence cancer treatment hospital. The results collaborate for a better understanding of drug protocols potentially aimed at treatment of chemotherapy-induced OM.

In this study, the chemotherapeutic 5-Fluoruracil (5-FU), a pyrimidine analogue, was used for the chemotherapy protocol. This medication is widely prescribed for the treatment of breast, head, and neck cancer (Van Kuilenburg and Maring, 2013). It acts, in general, by inhibiting cell division by blocking both DNA synthesis (enzymatic inhibition) and to a lesser extent, RNA (Shimamura et al., 2018). Currently, 5-FU is used in combination with other drugs, such as Busulfan, and Methotrexate, to improve the rate of antineoplastic response (Brunton et al., 2019).

This mortality rate may be related to a more severe mucositis, since the animals studied in this experiment did not present cancer in development, and the only systemic changes caused were due to the intraperitoneal infusion of the antineoplastic drug and topical induction with acetic acid in the oral mucosa.

Table 5 Thermal analysis of the active pharmaceutical ingredients in the multidrug solution.

Active Pharmaceuticals	Event		Incompatibility
	Peak Temperature (°C)	Enthalpy (J/g)	
Diphenhydramine	170.83	−271.48	−
Dexamethasone	228.85	−111.00	−
Nystatin	159.09	−6.39	−
*Multivitamin	129.68	−2,550.00	−
Morphine	−	−	−
Lidocaine	80.17	−275.85	−
Dexamethasone + Nystatin	168.46	−6.30	INC
Dexamethasone + Lidocaine	84.10	−143.44	INC
Dexamethasone + Diphenhydramine	147.10	−121.00	INC
Nystatin + Lidocaine	85.14	−151.00	INC
Nystatin + Diphenhydramine	146.42	−143.00	INC
Lidocaine + Diphenhydramine	84.77	−6.60	INC
Morphine + Dexamethasone	106.40	−881.60	INC
Morphine + Diphenhydramine	129.42	−4,980.00	INC
Morphine + Lidocaine	129.13	−3,500.00	INC
Morphine + Nystatin	162.03	−76.66	INC
Morphine + Multivitamin	103.12	−369.43	INC
Multivitamin + Diphenhydramine	129.18	−9.35	INC
Multivitamin + Dexamethasone	107.83	−26,570.00	INC
Multivitamin + Lidocaine	79.28	−3.56	INC
Multivitamin + Nystatin	109.91	−1.89	INC

Legend: INC – incompatible.
* Multivitamin = Vitamin B complex.

Some studies indicate that chemotherapy acts on rapidly proliferating cells in the basal layer of the epithelium, causing the loss of the tissue's ability to renew itself. Mucous ulcerations, which are associated with OM, are a consequence of these events (Sonis, 2004) and it is therefore suggested that these processes were facilitated by trauma and the action of pathogenic oral microorganisms.

The animals started presenting a complete clinical picture of mucosa ulceration on approximately the 4th day after chemotherapy application. Following the 3rd day of treatment (day 7 of the experiment), a significant clinical improvement was observed for the groups treated with 0.12% chlorhexidine digluconate and the multidrug solution. Previous studies (Costa et al., 2018; Ribeiro et al., 2015) had indicated that complete remission of lesions/healing occurs 5–14 days after the beginning of the protocol. However, the animals had been treated for 4 days. Thus, a gradual remission of symptoms would be possible if the treatment time was increased.

In the multicomponent solution, nystatin, which is insoluble in water was used as an antimicrobial drug. In preparing the test solution, saline was used, which presents water as one of its components. It remains possible that the insolubility of nystatin in the vehicle used to prepare the multicomponent solution prevented its pharmacological effect, and thus contributed to the appearance of colonies of microorganisms (Brunton et al., 2019).

Regarding food intake, these findings corroborate those described in the literature, which point out that of the principal signs and symptoms of oral mucositis, feeding difficulty stands out (Fujisawa et al., 2003; Rodríguez-Caballero et al., 2012).

When comparing the groups by food consumption, this analysis is important because according to (Sacono et al., 2008), the measure of average feed intake reflects the evolution of clinical severity, though it is possible that the improvement in OM observed during the treatment time was not sufficient to improve the animals' food intake.

The binary tested associations presented incompatibility for all of the active pharmaceutical ingredients; the sum of the results of the thermogravimetry curves for the mixtures did not correspond to the individual curve values (Oliveira et al., 2011). However, clinical improvement and reduction of the inflammatory process can be seen from the biological tests, a fact that stimulates our understanding of the pharmacological effects promoted by the API in the multicomponent solution.

Diphenhydramine is among the principal first-generation antihistamines, and presents significant sedation and cholinergic blockage (Wyngaarden and Seevers, 1951) that is, the presence of this drug in the multicomponent solution, administered topically, may be justified by its potential to minimize possible adverse reactions promoted by other components of the solution.

Considering that OM is an inflammatory process, the use of dexamethasone, (a long-acting corticosteroid that plays a role in all phases of the inflammatory process), contributes to the treatment of the mucosal ulcerations, preventing the progression of the inflammatory response and destruction of tissue (Wyngaarden and Seevers, 1951). In this sense, its presence is justified in the multicomponent solution, since it acts directly on the damaged tissue.

Nystatin is an important component of the solution, it is effective in treating fungal infections, such as candidiasis. In view of facing OM, the use of the proposed solution containing nystatin is recommended to both prevent and treat clinical evolution. (Ribeiro et al., 2015). However, during preparation of the multicomponent solution, saline is used, and given the

physicochemical properties of nystatin, solubilization does not occur in this vehicle, which may compromise its pharmacological activity. Thus, one might choose another antifungal that is soluble in this vehicle.

The use of vitamin complexes brings a degree of relief in OM symptoms, a fact that may be associated with the anti-inflammatory action of the multicomponent solution (Peres et al., 2013; Ribeiro et al., 2015), since B complex vitamins have an adjuvant action in tissue repair.

Due to the repercussion of OM symptoms on the patients' quality of life, the use of analgesics and local anesthetics is mentioned by some authors (Brunton et al., 2019; Van Kuilenburg and Maring, 2013; Wesolowski and Rojek, 2013). In severe cases, centrally acting analgesics such as morphine, may be prescribed (Meireles et al., 2008). Local anesthetics with formulations of lidocaine are also reported (Sacono et al., 2008).

Morphine is an opioid analgesic that has important effects on the central nervous system and gastrointestinal system. Since it presents acute toxicity and is also considered a drug of abuse, causing dependence and tolerance (Ribeiro Júnior et al., 2010), its use must be minimized and its presence in the multicomponent solution should be reconsidered.

Lidocaine temporarily relieves pain associated with minor trauma by blocking both initiation and conduction of the nervous impulse, it also reduces neuronal membrane sodium ion permeability. Solutions containing lidocaine can be used alone or in combination, for topical anesthesia, and thus chosen for palliative activity against the pain caused by oral mucositis (Kirk et al., 2017).

The present study demonstrated that chlorhexidine digluconate did not completely eliminate oral mucositis lesions, but does decrease their frequency and severity, likely by minimizing secondary infections. Chlorhexidine digluconate is the drug of reference for control and treatment of superficial oral infections and acts to disorganize the microbial cell membrane, by inhibiting its specific enzymes. However, its side effects, such as changes in tooth color, increases in supragingival calcified deposits, and taste changes contraindicate prolonged use (Logan et al., 2007).

5. Conclusion

The proposed multidrug solution is effective in improving clinical and histological parameters related to the severity of the OM inflammatory process, as was induced by chemotherapy in Wistar rats. The solution also promoted an increase in the amount of food ingested when compared to the untreated animals. Differential thermal analysis between the active pharmaceutical ingredients presents in the solution in binary combinations, indicated incompatibilities.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rebecca Rhuanny Tolentino Limeira, Isabella Lima Arrais Ribeiro, Paulo Rogério Ferreti Bonan, Danielle da Nóbrega Alves, Elba dos Santos Ferreira, Tereza Karla Vieira Lopes da Costa, Cassiano Francisco Weege Nonaka, Ana Cláudia Dantas de Medeiros, Frederico Barbosa de

Sousa, Ana Maria Gondim Valença and Ricardo Dias de Castro. The first draft of the manuscript was written by Rebecca Rhuanny Tolentino Limeira and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bian, L. et al, 2015. The role of Smad7 in oral mucositis. Protein. Cell 6, 160–169.

Bolouri, A.J. et al, 2015. Preventing and therapeutic effect of propolis in radiotherapy induced mucositis of head and neck cancers: a triple-blind, randomized, placebo-controlled trial. Iran. J. Cancer Prevent. 8 (5).

Brunton, L.L., Chabner, B.A., Knollmann, B.C., 2019. Goodman & Gilman: Las bases farmacológicas de la terapéutica. [s.l.] McGraw Hill.

Costa, R.C. et al, 2018. Associação terapêutica no manejo da mucosite oral quimioinduzida em pacientes pediátricos. Revista Família, Ciclos de Vida e Saúde no Contexto Social 6 (2), 256–263.

de Oliveira, M.A., Yoshida, M.I., de Lima Gomes, E.C., 2011. Análise térmica aplicada a fármacos e formulações farmacêuticas na indústria farmacêutica. Química Nova 34, 1224–1230.

Fujisawa, K., Miyamoto, Y., Nagayama, M., 2003. Basic fibroblast growth factor and epidermal growth factor reverse impaired ulcer healing of the rabbit oral mucosa. J. Oral Pathol. Med. 32 (6), 358–366.

Isana, C.L.S. et al, 2013. Effect of the maltodextrin-induced chemical reticulation on the physical properties and healing potential of collagen-based membranes containing Brazilian red propolis extract. Int. J. Med. Med. Sci. 5 (12), 514–524.

Kirk, L.M. et al, 2017. Beyond-use dating of lidocaine alone and in two "magic mouthwash" preparations. Am. J. Health Syst. Pharm. 74 (9), e202–e210.

Lalla, R.V. et al, 2014. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer 120 (10), 1453–1461.

Logan, R.M. et al, 2007. The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. Cancer Treat. Rev. 33 (5), 448–460.

Meireles, G.C.S. et al, 2008. Effectiveness of laser photobiomodulation at 660 or 780 nanometers on the repair of third-degree burns in diabetic rats. Photomed. Laser Surg. 26 (1), 47–54.

Migliorati, C. et al, 2013. Systematic review of laser and other light therapy for the management of oral mucositis in cancer patients. Support. Care Cancer 21 (1), 333–341.

Moura, L.B. et al., 2016. Estadiamento TNM Para O Tratamento De Câncer Bucal. [s.d.].

Nicolatou-galitis, O. et al, 2013. Systematic review of anti-inflammatory agents for the management of oral mucositis in cancer patients. Support. Care Cancer 21 (11), 3179–3189.

Panahi, Y. et al, 2010. Allopurinol mouth rinse for prophylaxis of fluorouracil-induced mucositis. Eur. J. Cancer Care 19 (3), 308–312.

Peres, P. et al, 2013. Pediatric dentistry applied to childhood cancer-clinical manifestations and protocol service. J. Manag. Prim. Health Care 4 (3), 191–199.

Ribeiro Júnior, O., Borba, A.M., Guimarães Júnior, J., 2010. Prevention and treatment of oral mucositis: the fundamental role of dentist: review. Rev. Clín. Pesq. Odontol. 6 (1), 57–62.

Ribeiro, I.L.A., Valença, A.M.G., Bonan, P.R.F., 2015. Protocolo de tratamento da mucosite oral grave durante o tratamento quimioterápico em paciente pediátrico. RGO-Revista Gaúcha de Odontologia 63, 467–471.

Rodríguez-caballero, A. et al, 2012. Cancer treatment-induced oral mucositis: a critical review. Int. J. Oral Maxillofac. Surg. 41 (2), 225–238.

Sacono, N.T. et al, 2008. Light-emitting diode therapy in chemotherapy-induced mucositis. Lasers Surg. Med.: Off. J. Am. Soc. Laser Med. Surg. 40 (9), 625–633.

Schirmer, E.M., Ferrari, A., Trindade, L.C.T., 2012. Oral mucositis evolution after nutritional intervention in cancer patients under palliative care. Revista Dor 13, 141–146.

Shimamura, Y. et al, 2018. A mouse model for oral mucositis induced by cancer chemotherapy. Anticancer Res. 38 (1), 307–312.

Sonis, S.T. et al, 2000. Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. Oral Oncol. 36 (4), 373–381.

Sonis, S.T., 2004. The pathobiology of mucositis. Nat. Rev. Cancer 4 (4), 277–284.

van Kuilenburg, A.B.P., Maring, J.G., 2013. Evaluation of 5-fluorouracil pharmacokinetic models and therapeutic drug monitoring in cancer patients. Pharmacogenomics 14 (7), 799–811.

Vieira, D.L. et al, 2012. Tratamento odontológico em pacientes oncológicos. Oral Sci., 37–42

Wesolowski, M., Rojek, B., 2013. Thermogravimetric detection of incompatibilities between atenolol and excipients using multivariate techniques. J. Therm. Anal. Calorim. 113 (1), 169–177.

Wyngaarden, J.B., Seevers, M.H., 1951. The toxic effects of antihistaminic drugs. J. Am. Med. Assoc. 145 (5), 277–282.

Yoshino, F. et al, 2013. Alteration of the redox state with reactive oxygen species for 5-fluorouracil-induced oral mucositis in hamsters. PLoS One 8 (12), e82834.

Zhang, L. et al, 2016. Dendritic cell vaccine and cytokine-induced killer cell therapy for the treatment of advanced non-small cell lung cancer. Oncol. Lett. 11 (4), 2605–2610.