Analysis of the Anti-Inflammatory Potential of Pure and Microemulsified Bullfrog Oil in Acute Lung Injury

André Luiz Silva Davim[1] Tereza Neuma de Castro Dantas[2] Márcia Rodrigues Pereira[2]

[1] Natural Products and Inflammation Laboratory, Department of Morphology, Centro Universitário do Rio Grande do Norte - UNI-RN, Natal, RN, Brazil
[2] Tensoactive Technology Laboratory, Institute of Chemistry, Universidade Federal do Rio Grande do Norte – UFRN, Natal, RN, Brazil

Address for correspondence André Luiz Silva Davim, PhD, Laboratório de Produtos Naturais e Inflamação, Departmento de Morfologia, Centro Universitário do Rio Grande do Norte - UNI-RN, Rua Prefeita Eliane Barro, 2000, Tirol, Natal, Rio Grande do Norte 59014-545, Brasil (e-mail: anatomia@unirn.edu.br).

J Morphol Sci 2018;35:102–105.

Abstract

Infectious diseases account for more than a third of all hospital admissions, and are highly prevalent in intensive care units. Currently, sepsis is one of the diseases with the highest morbidity and mortality rates worldwide, with death rates reaching up to 60% among intensive care patients, according to statistics from low-income countries. The prominence of multi-resistant microorganisms is rising, while the possibilities of development of new target drugs are being exhausted. Thus, the objective of the present study was to evaluate the anti-inflammatory potential of bullfrog oil in its pure state and in a microemulsion system in an experimental model of sepsis. Mice were separated into three groups and treated with bullfrog oil in its pure state, in a microemulsion, and with saline solution, and subsequently submitted to induction of sepsis. Bronchoalveolar lavages were performed for cell counts, as well as analyses of lung tissue samples. When the washings were analyzed, no statistically significant difference was observed in cell migration between the experimental groups, but a difference was observed between these groups and the saline solution group. When the lung tissue samples were analyzed, intense tissue wear was observed in the bullfrog oil groups, with the presence of cellular infiltrate and rupture of respiratory bronchioles and alveoli. However, in the microemulsion group, no major tissue wear was observed, and the pulmonary parenchyma was more preserved. Thus, we concluded that bullfrog oil in pure form and in a microemulsion system are good modulators of the inflammatory response, with the microemulsion system being more efficient in protecting lung tissue.

Keywords
- sepsis
- bullfrog oil
- microemulsion system
- acute lung injury

Introduction

Sepsis morbidity and mortality in intensive care units worldwide are more common nowadays, with no significant reduction in the last decades.[1,2] Especially in Brazil, the incidence of patients with sepsis is becoming more frequent, but it is still difficult to precisely determine the numbers due to lack of notification, and due to the cases in which it is associated with other severe diseases.[3,4] As a consequence of the septic picture, the development of acute pulmonary injury due to increased microvascular permeability is observed in many patients. The lungs, being heavily perfused organs, become possible targets for the occurrence of injuries, due to the excessive migration of immune system cells.[5] Despite the pharmacological, technological and surgical advances, mortality due to sepsis and/or associated diseases remains high throughout the world; thus, the search for affordable and inexpensive therapeutic alternatives becomes relevant in an attempt to contain the progression of this disease.

received
January 24, 2017
accepted
April 28, 2018
published online
September 3, 2018

DOI https://doi.org/10.1055/s-0038-1669933.
ISSN 2177-0298.

Natural products of diverse origins, be them vegetable or animal, have been playing an important role in different industry sectors, such as the pharmaceutical industry, because some of these substances act in a beneficial way on the human immune system. Many hypotheses are based on the performance of these compounds in the modulation of the inflammatory response, thus preventing the occurrence of tissue injuries. Among the natural products with possible therapeutic potential are polyunsaturated fatty acids, which show efficacy in their proposed effect, but present serious restrictions due to the induction of hepatotoxicity, because their metabolism is hepatic.[6,7] In an attempt to attenuate or even eliminate the deleterious effects of the direct administration of polyunsaturated fatty acids, the use of microemulsion systems is a possible alternative as a vehicle for drug release, since the release of fatty acids occurs gradually through this system, thus avoiding hepatic overload and, consequently, the toxicity of this organ.[8]

Bullfrog oil, which is rich in polyunsaturated fatty acids, is a potential modulator of the inflammatory response, and has been used by several populations in Brazil for the treatment of numerous inflammatory diseases, such as bronchitis, asthma, lichen sclerosis, furunculosis, sebaceous cyst, and to heal the skin and mucous membranes.[9] The goal of the present study was to evaluate the anti-inflammatory potential of bullfrog oil in its pure state and in a microemulsion system in acute pulmonary injury, in an experimental sepsis model.

Materials and Methods

All experimental procedures are in accordance with the Guide for the Care and Use of Laboratory Animals (US) and the Brazilian College of Animal Testing (Colégio Brasileiro de Experimentação Animal, COBEA, in the Portuguese acronym). The present study was approved by the Ethics Committee on the Use of Animals of Universidade Federal do Rio Grande do Norte, Brazil, under protocol no. 052/2014.

Materials

Obtaining the Microemulsion System (MeS)

The method of preparation was based on volumetric titration with analytical weighing of the v/v ratios to obtain the respective mass ratios. In order to construct the diagram, various proportions were weighed in the active material (surfactant) and aqueous components, followed by the titration of the oily component, describing the phase transitions by visual observation, after shaking and centrifugation of the sample. The phase equilibrium characterization was performed based on the Winsor classification. The microemulsion system was of the water-in-oil (W/O) type, containing 60% oil phase (bullfrog oil), 35% surfactant (soy lecithin) and 5% aqueous phase.

Experimental Model

A Swiss strain of *Mus musculus* mice was used to carry out the experiments; they were male, with a mean age of 35 ± 5 days, and had a mean weight of 30 g ± 5 g. The animals were kept at ideal temperature conditions (22°C ± 3°C) and humidity (50% ± 20%), with controlled luminosity in a rate of 12 hours of light/dark periods, and with the ingestion of water and feed (Labina, Purina, St. Louis, Missouri, US) ad libitum.

The mice were randomly selected, packed in polypropylene cages, and divided into three groups: 1) the saline solution group (the controls, to which saline solution was administered); 2) the pure oil group (one of the experimental groups, to which pure bullfrog oil was administered); and 3) the microemulsion group (the other experimental group, to which the bullfrog oil was administered in a microemulsion system). All animals used in this study came from the laboratory of Centro Universitário do Rio Grande do Norte (UNI-RN).

A total volume of 100 µl/day was administered to all groups through the gavage technique during the period of 30 consecutive days. On the thirtieth day of administration, each group was surgically treated using the cecal ligation puncture technique for the induction of sub-lethal sepsis.

Methods

Sepsis Induction

Sepsis was induced as described by Baker et al (1983),[10] with some modifications. For this purpose, an incision of ~ 1.0 cm was made in the longitudinal axis in the abdomen of the animals, reaching the abdominal cavity and exposing the cecum. The appendix was punctured by crossing it with a 21-gauge (G) needle so that there was extravasation of feces into the abdominal cavity and, consequently, induction of sepsis by the lipopolysaccharide (LPS) of the cell wall of bacteria. Then, the skin and peritoneum were sutured, and the animals were exposed to light for temperature control.

Bronchoalveolar Washing

For the analysis of the migration of cells to the lungs of the animals, an opening was made in the trachea, and a cannula was introduced with 1.0 ml of sterile saline, and 1 volume was injected until maximum lung insufflation. The aspirates were then centrifuged for subsequent cell count in the Neubauer chamber.

Histopathological Analysis of the Samples

Pulmonary and hepatic tissue samples were removed and placed in a 10% formalin solution. All samples were prepared and subsequently stained using the hematoxylin-eosin (HE) technique. At the end of the process, the slides were photographed with a digital camera, and their images were captured and processed using the Universal Desktop Ruler software, version 3.6, for a detailed visualization of the tissues.

Statistical Analysis

For the statistical analyses of the cell migrations, the SigmaStat (Systat Software, San Jose, California, US) software, version 3.10 was used, and the analysis of variance (ANOVA) for non-parametric data (Kruskal-Wallis test) was performed in it. When statistically significant differences were found, the Student-Newman-Keuls post-hoc test was used for the multiple comparisons of means.

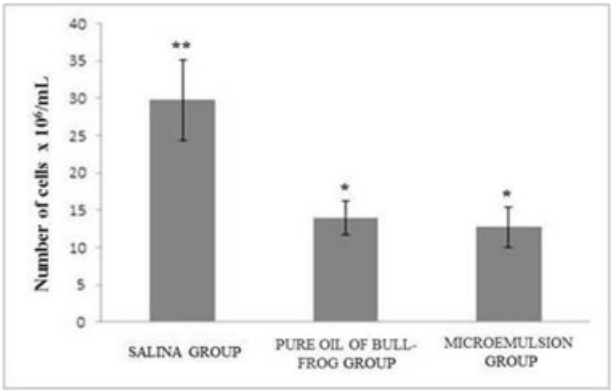

Fig. 1 Evaluation of cell migration to the lungs after induction of sepsis. $^{**}p \leq 0.01$; $^{*}p > 0.05$.

Results

Evaluation of Migration of Leukocytes to the Lungs through Bronchoalveolar Wash in Septic Mice

A total of 100 µL/day of saline solution and of pure and microemulsified bullfrog oil were administered orally for 30 days through the gavage technique. After this period, sepsis was induced in the animals, bronchoalveolar lavages (BAL) were performed after 8 hours, and the number of cells was determined from the Neubauer chamber count (►**Fig. 1**).

We observed that in the saline group there was an intense migration of neutrophils into the lung tissue. When cell migration was analyzed in the groups treated with the two kinds of bullfrog oil, a statistically significant reduction ($p \leq 0.01$) of cell migration was observed when compared with the saline group. When comparing the pure oil and microemulsion groups, no statistically significant difference was observed ($p > 0.05$).

Histopathological Analysis of Pulmonary Tissue of Septic Mice

After eight hours of sepsis, the animals in the pure oil and microemulsion groups were euthanized for subsequent removal of lung tissue samples and histopathological analysis. In all of the analyzed samples of animals treated with the bullfrog oil pure, an exacerbated leukocyte migration to the lungs was observed, with the presence of cellular infiltrate and rupture of the alveolar walls (►**Fig. 2**). The establishment of a scenario of acute lung injury (ALI) (►**Fig. 2**) was observed in the histological analysis of this group.

In all of the analyzed samples of animals treated with the microemulsion, a greater integrity of the lung tissue was observed, with reduced leukocyte migration and consequent reduction in cellular infiltrate and tissue damage (►**Fig. 3**). When compared with the group treated with pure bullfrog oil, less wear of the lung parenchyma was observed in the samples of the animals treated with the microemulsion (►**Fig. 3**).

Discussion

The ethnopharmacological bioprospection for drugs based on natural sources has become quite common since the isolation of pharmacologically active compounds, increasing the therapeutic arsenal against various diseases.[11] In this context, natural oils have been widely used in folk medicine for the treatment of many diseases due to their high content of secondary metabolites and fatty acids. Numerous biological functions are attributed to bullfrog oil, largely due to the presence of polyunsaturated fatty acids, which, in particular, play an important role in the structure of the cell membrane, in the metabolic processes and in the production of inflammatory mediators. They interfere in several stages of the inflammatory response, such as vascular contraction, chemotaxis, adhesion, diapedesis, activation and cell death.[12] All of these events are important for the activation and modulation of the inflammatory response, since a minimal response causes damages to the ability of the organism to fight microorganisms, and an exacerbated response causes damages to the intensely vascularized tissues.

Oils are the main biological constituents of microemulsion systems, which are pharmaceutical formulations obtained by the dispersion of two immiscible phases (usually water and oil), in which the hydrophilic or lipophilic parts of the dispersing phase determine the type of emulsion (oil in water [O/W] or W/O).[9] There are scarce reports in the literature of studies analyzing the therapeutic potential of bullfrog oil. In a study by Amaral-Machado, the researchers observed that bullfrog oil in a nanostructured system was a potential candidate for the development of drugs, as it is free of toxicity and quite effective in antineoplastic therapy. These data corroborate the findings

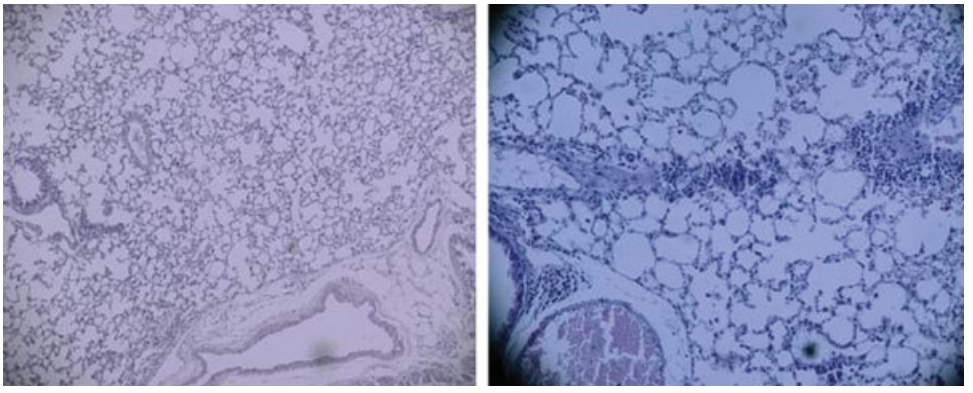

Fig. 2 Histological section of animal lung tissue from the pure oil group (10x magnification).

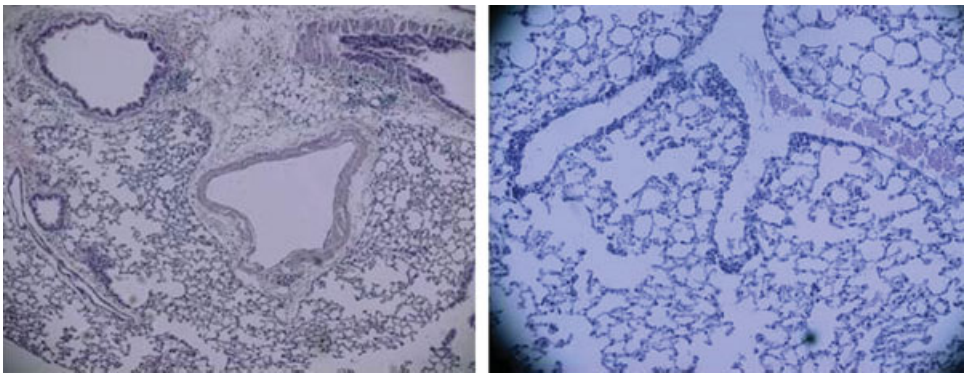

Fig. 3 Histological section of animal lung tissue from the microemulsion group (20x magnification).

of the present study, evidencing bullfrog oil in pure state and in micro and nanostructured systems as a product of animal origin with an important and promising therapeutic potential.

Conclusion

The results obtained in the present study allow us to conclude that bullfrog oil in its pure form and in a microemulsion system presents good therapeutic potential, since it modulated the inflammatory response, acting effectively against the progression of inflammation, as well as reducing the excess of cell migration to the target organs of the septic scenario. Thus, the microemulsion system used in this study is a possible alternative for a new drug delivery system (NDDS).

Acknowledgments

The researchers would like to thank the Centro Universitário do Rio Grande do Norte and Universidade Federal do Rio Grande do Norte for the financial support to carry out this research. Furthermore, they would like to thank Dr. Carmen Elizabeth Rexach and Dr. Jennifer McDonald, both professors at Mt. San Antonio College, US, for their support in the preparation of the histological slides.

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