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Influence of gamma radiation onto polymeric matrix with papain

Gislaine Zulli [a], Patrícia Santos Lopes [b,*], Maria Valéria Robles Velasco [c], Mara Tânia Silva Alcântara [a], Sizue Ota Rogero [a], Ademar Benévolo Lugao [a], Monica Beatriz Mathor [a]

[a] Nuclear and Energetic Research Institute, IPEN-CNEN/SP, São Paulo, SP 05508-000, Brazil
[b] Pharmacy Department, University of Sorocaba, Sorocaba, SP 18023-000, Brazil
[c] Pharmacy Department, University of São Paulo, São Paulo, SP 05508-900, Brazil

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ABSTRACT

Papain is a proteolytic enzyme that has been widely used as debridement agent for scars and wound healing treatment. However, papain presents low stability, which limits its use to extemporaneous or short shelf-life formulations. The purpose of this study was to entrap papain into a polymeric matrix in order to obtain a drug delivery system that could be used as medical device. Since these systems must be sterile, gamma radiation is an interesting option and presents advantages in relation to conventional agents: no radioactive residues are formed; the product can be sterilized inside the final packaging and has an excellent reliability. The normative reference for the establishment of the sterilizing dose determines 25 kGy as the inactivation dose for viable microorganisms. A silicone dispersion was selected to prepare membranes containing 2% (w/w) papain. Irradiated and non-irradiated membranes were simultaneously assessed in order to verify whether gamma radiation interferes with the drug-releasing profile. Results showed that irradiation does not affect significantly papain release and its activity. Therefore papain shows radioresistance in the irradiation conditions applied. In conclusion, gamma radiation can be easily used as sterilizing agent without affecting the papain release profile and its activity onto the biocompatible device is studied.

1. Introduction

Papain is a proteolytic enzyme isolated from the latex of green papaya leaves and fruits (*Carica papaya* Linné) (O'Neil, 2001). According to Monetta (1987, 1988, 1990), latex has been used in wound healing since a long time by American, African and Caribean Island tribes. The therapeutic value of papain is specially recognized in hard recovery treatments (Mc Grath, 1988; Monetta, 1987, 1988).

However, papain has low stability, which makes its commercialization in a defined pharmaceutical form difficult. An option found to enhance papain stability and to allow the development of an established dosage form was to develop a topical drug delivery system.

Medical devices that intend to contact affected skin must be sterile. Gamma radiation is an interesting option to attend this requirement. Sterilizing agents cause microorganism inactivation by irreversible damage to essential molecules of the cell, such as proteins and DNA (Pinto et al., 2000). According to Phillips (1997), ionizing radiations present advantages in sterilizing medical products compared to conventional agents: no radioactive residues are formed; the product can be sterilized inside the finish packaging and has an excellent reliability. ISO 13409 (1996) is the normative reference for establishment of the sterilizing dose, which determines 25 kGy as the necessary dose for the inactivation of viable microorganisms in heath care products.

2. Experimental

2.1. Preparation of polymeric membranes for in vitro releasing test

A monocomponent silicone dispersion in xylene (MSD – Nusil Technology MED-6605) was used to prepare 2.0% w/w papain (30,000 USP/mg, Merck) and 1.6% w/w L-cysteine hydrochloride monohydrate-containing membranes by a simple mixture. Each membrane was prepared with 5 cm diameter and 0.08 g weight. MSD is a medical-grade polymer, which cures at room temperature after 5 days by an acetoxy-based cure system. Half of the membranes prepared were irradiated with 25 kGy dose of gamma radiation at a 2 kGy/h dose rate in order to evaluate whether radiation can interfere with the release profile of papain. The other part was maintained non-irradiated.

* Corresponding author. Tel.: +55 15 32335853.
E-mail address: patricia.lopes@prof.uniso.br (P.S. Lopes).

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2.2. Release test using franz diffusion cells

Franz diffusion cells of static and vertical flux were used for release test using a cysteine-EDTA phosphate buffer as receptor fluid and maintained at 37 °C in a heated bath. Test was carried out over 30 h and samples were collected at established periods. In each test a papain-containing membrane (irradiated or non-irradiated) and a control membrane, prepared just with the polymer, were assessed together in order to evaluate the polymeric matrix interference on the results. Three replicates of each membrane type were carried out.

2.3. Specific substrate dosage test

Each collected sample from the release test was used to quantify the amount of papain released in each time period. The test is based on the enzymatic reaction of active papain and its specific substrate Z-Phe-Arg 7-amido-4-methylcoumarin hydrochloride (Sigma Aldrich), which forms as product methylcoumarin, a highly fluorescent substance that is sensitive for papain quantification (Pinto et al., 2007). The amount of methylcoumarin is directly proportional to papain release. Therefore, it is possible to evaluate whether gamma radiation inactivates papain released from irradiated membranes.

3. Results

Results obtained from control membranes were not significant (data not shown). The average flux of papain obtained for three replicates of irradiated and non-irradiated membrane is shown in Fig. 1. The graph shows a very similar flux profile for each membrane. It can be observed that a burst effect occurs until 2 h after the beginning of the test. After that the amount of papain released decreases greatly and remains relatively constant until 30 h.

The cumulative amount of papain released over the 30-h test period for each membrane type assessed is shown in Fig. 2. As can be seen, the release profile for both membranes is very similar, which indicates that irradiation does not interfere significantly on the releasing. A constant release profile can be observed up to 12 h after the beginning of the test.

4. Discussion

Papain release profile from irradiated and non-irradiated membranes was determined *in vitro* by the release test in Franz diffusion cells. Analysis of collected samples from control membrane did not show significant results, which indicates that the polymeric matrix does not interfere with the results obtained.

A burst effect occurs up to 2 h after the beginning of the test for both irradiated and non-irradiated papain-containing membranes (Fig. 1). It indicates that the portion of papain released initially was in the surface of the membrane. After that papain is continually released, until the end of the test probably from the interior of the polymeric matrix.

The cumulative amount of papain released was very similar to irradiated and non-irradiated membranes (Fig. 2), which indicates that irradiation does not affect significantly papain release. Furthermore, it is important to note that papain shows a radioresistance, at least for the irradiation conditions applied.

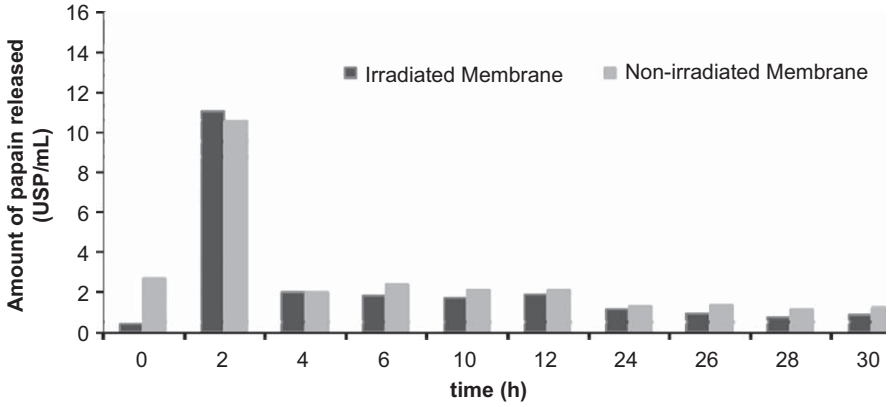

Fig. 1. Flux of papain released from prepared membranes.

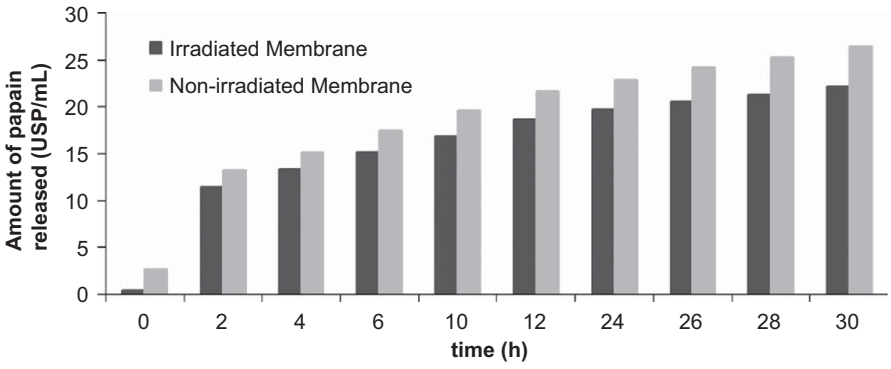

Fig. 2. Cumulative amount of papain released from prepared membranes.

The same result was found for papain dry powder and frozen suspension of papain-hybrid with activated chitosan beads irradiated with 25 kGy (Furuta et al., 2002) as well as for frozen papain solution (Furuta et al., 2000). In addition, a constant flux of papain can be identified up to 12 h after the beginning of the test for both membranes (Fig. 2).

Clinically it can signify that the device could be used for a bidaily application, but *in vivo* tests are necessary to prove that a healing effect occurs with the amount of papain released from the matrix.

5. Conclusions

Regarding the results obtained, it is possible to conclude that the developed device has a huge potential as a drug delivery system of papain. Gamma radiation can be easily used as the sterilizing agent without affecting the papain release profile. *In vivo* tests are necessary to verify whether the amount of papain released is sufficient to present a clinical healing effect.

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