Controlled release of an antitumor agent from radiation-crosslinked EVA matrices

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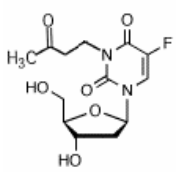
The effect of -radiation doses of 12.5 to 380 kGy on the infrared spectra, gel content, mechanical properties, and the release of oxobutyl-5-Fluoro-2'-deoxyuridine (OfdUrd, an antitumor agent) from poly(ethylene-co-vinyl acetate) (EVA) films was studied. The results showed that the application of radiation doses produced a crosslinking reaction leading to a maximum gel content of about 85% in the case of 150 kGy. The mechanical properties of EVA membranes were affected after the exposition to -radiation. Irradiation dose over 50 kGy caused an increase in the Young's modulus of EVA and at the same time a decrease in the strain percent. Moreover, the network structure formed after irradiation reduced significantly the OFdUrd release from EVA membranes. In this manner, the radiation dose applied to the polymeric matrix modulated the release of OFdUrd avoiding the high concentrations that may cause severe systemic toxicity. The loading of OFdUrd to EVA matrices triggered a slight hyperemia after implantation, while the inflammatory reaction was only observed during the first two days.

Introduction

5-Fluorouracil (5-FU) is a pyrimidine antagonist widely used in the chemoradiotherapy treatment of cancer. In despite the use of 5-Fluorouracil in the cervical cancer treatment their administration is accomplished by severe toxic side effects and delivery problems.[1-3] More recently, drug delivery systems containing a prodrug of 5-FU, (+)-5-Fluoro-2'-deoxyuridine (5-FdUrd) and their derivatives have been reported to be more effective in terms of alleviating some of the collateral effects allieds to the 5-FU administration.[4] Fluorouridine has been found to be a highly effective compound for the treatment of various solid tumors. In order to obtain good radiochemical yield in a minimal reaction time Some clinical studies have suggested that the addition of 2-oxoalkyl side chain to 5-FdUrd at the N(3) position generate derivatives more effective in the cancer treatment due to the higher radiochemical yield of these prodrugs.[5]

Most of the present-day companies provide polymer devices for controlled drug delivery based on poly(ethylene-co-vinylacetate) (EVA). EVA is an inexpensive polymer with biocompatible properties approved for human use by Food and Drug Administration (FDA).[6] In this study, we have investigated the release characteristics of the prodrug oxobutyl-FdUrd (OFdUrd) (Scheme 1) from EVA matrix crosslinked by -irradiation. A combination of radiation-crosslinked EVA and OFdUrd, maintaining therapeutic levels for several days, may be helpful in the treatment of cervical cancer.



Scheme 1: Chemical structure of OFdUrd

Materials and Methods

Prodrug synthesis: N-(3-oxobutyl)-5-fluorouridine (OfdUrd) was prepared by reacting 5-fluorouridine (Sigma) with an appropriate chloroformate prepared from the corresponding alcohol.

Matrix preparation: A weighed amount of the dry OFdUrd powder was dispersed in DMSO. The EVA copolymer (Politeno, Brasil) was dissolved in the drug suspension at 50 ºC. The mixture was poured onto a siliconed glass plate to evaporate the solvent, and the resulting membrane was dried under vacuum. Then, the membrane was melt-pressed to obtain films of uniform thickness (600 m). The OFdUrd content in EVA matrix was analyzed by UV/Vis spectrometry.

Gamma irradiation: The ^{60}Co- -irradiation source was performed using a 22,000 Ci activity ^{60}Co source (Nuclear and Energetic Research Institute, IPEN/CNEN-SP). The samples were -irradiated at a dose rate of 12.15 kGy.s^{-1} in PE bags under nitrogen atmosphere. The doses of gamma radiation ranging from 12.5 kGy to 380 kGy were measured using a Perspex dosimeter.

Determination of gel fraction: EVA samples were extracted with o-xylene in a soxhlet. The remaining insoluble sample was rinsed with methanol and fully dried in a vacuum oven. The gel fraction was calculated gravimetrically.

Stress-strain measurements: Tests were carried out according to ASTM D638 standard recommendations.

Infrared spectroscopy: Attenuated total internal reflectance-FTIR (ATR-FTIR) spectra were obtained.

In vitro drug release studies: The release behavior of loaded films was studied in PBS pH 7.4 at 37 °C. The concentration of the drug was determined by UV/V spectroscopy at 266 nm.

In vivo evaluation: The ethical committee of IPEN-CNEN/USP approved the animal experimentation. Before implantation, films were sterilized by -

radiation (25 kGy). The biocompatibility of drug-loaded films was investigated subcutaneously in the back of Wistar rats and in the cage-implant system, according to the procedure described in the literature. Histopathological observations were made at 3, 7, 14 and 21 days after implantation.

Results and Discussion

Radiation-crosslinking and polymer characterization: The gel content of EVA increased rapidly up to a dose of 50 kGy and doses greater than 100 kGy led to a slow increase of the gel content. Young's modulus (E) also increased rapidly up to a dose of 50 kGy. A clear linear relationship was observed between irradiation dose and E indicating an increased stiffness probably due to the formation of crosslinked EVA chains as confirmed by gel content results. The strength of EVA increased gradually with increase irradiation dose up to 114 kGy, while the strain percent decrease with the radiation treatment. The behavior of EVA matrices after -irradiation is related with the formation of a crosslinked EVA structure as confirmed by gel content analysis. Higher radiation doses produced a brittle film displaying a low strain percent as a consequence of the high crosslinking density and damage of the EVA structure. The spectra of irradiated EVA shows significant variation of absorbance with radiation dose at 3300 and 1598 cm^{-1} due to methylene crosslinking.

Drug release:

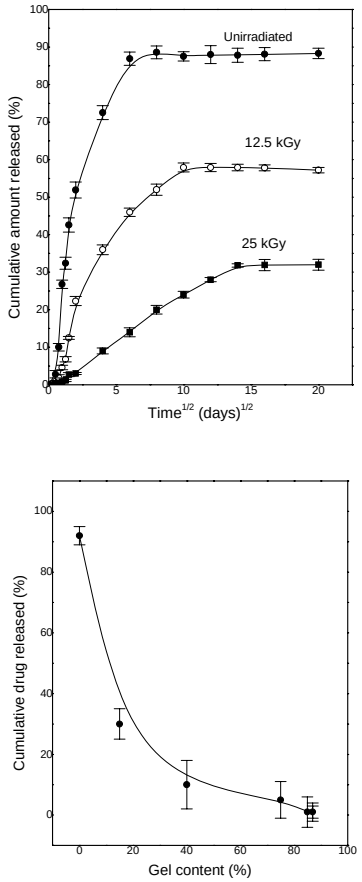


Figure 1: Effect of radiation dose (upper graph) and gel content on the cumulative release of OFdUrd from EVA matrices at 37 ºC

The variation of the drug released from the -irradiated and unirradiated matrices was characterized by three phases (Fig. 1): an initial period of rapid release of OFdUrd (burst effect), due to the release of drug molecules at the matrix surface; a period when release of the OFdUrd drug was approximately linear with respect to $t^{1/2}$; and a final period where release tapered off, due to the increased difficulty of diffusion of drug occluded inside the matrix with decrease of the initial drug concentration in the membranes. As expected, the release rate decreased with increasing gel content.

In vivo analysis:

The concentrations of polymorphonuclear cells and lymphocytes as well as the extra- and intracellular activities of acid and alkaline phosphatases were not significatively different from the control. The loading of OFdUrd to EVA film triggered a slight hyperemia. However, the inflammatory reaction was only present during the first two days. No irreversible changes occurred in the tissues, indicating an overall good biocompatibility.

Conclusions

-Irradiation produced a crosslinked network in EVA films, as showed by the presence of a gel fraction, the content being increased with the irradiation dose. The change in the polymer structure had an important effect in the mechanical properties, evidenced by the observed elastic modulus and the strain percent values. The crosslinked network modulated the release of the 5-FdUrd from the EVA films, avoiding the high concentrations that may cause severe systemic toxicity. Loaded EVA film displayed a low inflammatory reaction after implantation.

References

1. J.A. Houghton; P.J. Houghton, PJ; R.S. Wooten. *Cancer Res.* 1979, *39*, 2406–13.
2. J.D. Schuetz; H.J. Wallance; R.B. Diasio, *Cancer Res.* 1984, *44*, 1358–63.
3. Y. Kimura; H. Okuda. *J. Ethnopharm.* 1999, *68*, 39–45.
4. K. Ulbrich; V. Subr. *Adv. Drug Deliv. Rev.* 2004, *56*, 1023–50.
5. Y. Shibamoto; Y. Tachi; K. Tanabe; H. Hatta; S.I. Nishimoto. *Int. J. Radiat. Oncology Biol. Phys.* 2004, *58(2)*, 397-402.
6. R. Langer; H. Brem; D. Tapper. *J. Biomed. Mater. Res.* 1981, *15*, 167-277.