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Monitoring structural features, biocompatibility and biological efficacy of gamma-irradiated methotrexate-loaded spray-dried microparticles

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ABSTRACT

In this study, biodegradable and biocompatible gamma irradiated poly-(DL-lactide-*co*-glycolide) (PLGA) spray-dried microparticles were prepared aiming to improve the efficacy of methotrexate (MTX). The experimental design included three formulations of microparticles containing distinct drug amount (9%, 18%, and 27% w/w) and three distinct gamma irradiation dose (15 kGy, 25 kGy, and 30 kGy). The physicochemical and drug release properties of the microparticles supported their biocompatibility and biological efficacy studies in different cell lines. The irradiation induced slight changes in the spherical shape of the microparticles and the formation of free radicals was dependent on the drug loading. However, the amorphous character, particle size, drug loading, and drug release rate of the microparticles were preserved. The drug release data from all microparticles formulation were evaluated by using four drug kinetic models and by comparison of their similarity factor (f_2). The gamma irradiation did not induce changes in the biocompatibility of PLGA microparticles and in the biological activity of the MTX-loaded microparticles. Finally, the spray-dried MTX-loaded PLGA microparticles enhanced the efficacy of the drug in the human cervical cancer cells (SiHa cell line). This study demonstrated the feasibility of the gamma irradiated spray dried PLGA microparticles for prolonged release of MTX, supporting a promising antitumor-drug delivery system for parenteral (subcutaneous) or pulmonary use.

1. Introduction

Methotrexate (MTX) is a chemotherapeutic and immunosuppressant agent used for autoimmune diseases such as rheumatoid arthritis and psoriasis, childhood acute lymphoblastic leukemia, choriocarcinoa non-Hodgkin's lymphoma, and certain types of cancer, such as breast, skin, head, neck, and lung cancer [1]. The use of drug delivery systems (DDS) can avoid the potential side effects of MTX, such as nausea, vomiting, intestinal mucositis, diarrhea, stomatitis, myelosuppression, hepatotoxicity, pulmonary fibrosis, and renal insufficiency [2]. Polymeric microparticles have demonstrated interesting properties such as biocompatibility, biodegradability and feasibility for adjustable drug doses to be used by parenteral or pulmonary routes. Among the polymers used for this purpose, the poly-(DL-lactic-*co*-glycolic acid) (PLGA) is a copolymer well established for drug delivery, approved by the Food and Drug Administration (FDA) and the European Medicine Agency. Its excellent biocompatibility and tunable structural features as polymeric carriers have been demonstrated for a plethora of drugs and diseases [3–7].

In recent years, many studies have reported the encapsulation of MTX in polymeric nanoparticles and microparticles to be used in cancer treatment. The majority of these studies have reported important approaches concerning the physicochemical characterization and the cytotoxicity evaluation of MTX-loaded nanoparticles in cancer cell lines [8–12]. Some approaches addressing particle engineering of functionalized or decorated MTX nanoparticles with specific ligands. Moreover, the drug targeting performance, mainly for breast cancer have been evaluated [13–17]. However, few studies have reported structural and feasible features of MTX-loaded microparticles intended to pulmonary or parenteral subcutaneous routes. Recently, interesting intra-cavity cylindrical moldable scaffolds, structured as PLGA/PEG microparticles containing MTX were tested for brain tumors [18]. Nevertheless, the authors have not explored physicochemical properties, the slow drug

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release and in vivo biocompatibility. Any sterilization procedure was purposed for the studied polymeric device.

Drug delivery systems intended for pulmonary or parenteral routes requires an intensive control of their physicochemical properties in order to evaluate their biological activity. Indeed, small-sized particles between 1 and 10 μm assure a feasible, precise, and accurate administration of the drug by pulmonary and parenteral routes. In addition, some administration routes did not tolerate a considerable volume of particles, needing a careful design and composition of the DDS, with an ideal drug/polymer ratio. The amount of drug deposition in the lungs is affected by the size, the density, the shape, and the surface roughness of the particles [19]. In addition, the sterility of the DDS for parenteral routes is well controlled by the government regulatory agencies. The contamination of aerosols by microorganisms increase the risk of infection in patients. The risk of transmission of an infection is dependent upon duration of exposure of drugs with pathogens and the procedures taken by respiratory therapists to avoid pathogen exposure. Proper practices of medication handling, device cleaning, and sterilization can greatly reduce this risk [20]. However, the adequate sterilization for drug delivery systems remains a challenge to be efficiently overcome.

Different sterilization methods are recommended for pharmaceuticals, but the PLGA is highly sensitive to moisture and to high temperatures commonly used in conventional methods involving dry heat or autoclaving. The microparticles are too large to be sterile-filtered and the ethylene oxide sterilization is not appropriable due to the undesirable residues [21]. Hence, the sterilization by gamma irradiation is an effective and accessible method for biodegradable drug delivery systems, mainly those prepared with aliphatic polyesters [21,22].

The gamma irradiation assured enough high energy for a powerful penetration in the materials, with consequent inactivation of microorganisms by damage of their nucleic acids. A minimum absorbed dose of 25 kGy should be considered for sterilizing procedure for pharmaceuticals with a low initial bioburden and non-radioresistant spores [23]. The current accepted sterility assurance level (SAL) is limited to 10^{-6} microorganisms, which represents no more than one viable microorganism in one million parts of the final product [24]. However, this radiation can induce simultaneous chain scission and crosslinking of polymeric chains, affecting the drug release properties and consequently the biological efficacy [25].

The effect of the gamma irradiation on the polymeric microparticles is very controversial and seems to be affected not only by the composition, preparing procedure, presence of air, surfactants, and additives, but also by the type of polymer (structure, molecular weight, as well as the composition of blends), and, finally, the irradiation conditions [25]. Some studies have demonstrated the damage of gamma irradiation on the drug-loaded polymeric microparticles [26,27]. In contrast, the protective effect of PLGA microspheres against the harmful degradation effect of this sterilization procedure for proteins [28].

The purpose of this study was to prepare effective and gamma irradiated MTX-loaded PLGA microparticles intended for the parenteral (subcutaneous) or pulmonary routes. The possible adverse effects of gamma irradiation on their physicochemical properties were carefully monitored. The experimental finds were correlated with the drug release kinetics, the biocompatibility, and the biological activity. The gamma irradiation dose of 25 kGy is officially recommended for sterilization of pharmaceuticals [23]. Therefore, the three microparticles formulations were exposed to gamma irradiation using 25 kGy. Alternatively, two other irradiation doses (15 kGy and 30 kGy) were also tested. The spray drying technique was chosen for preparing the microparticles because it is a single-step method that allows the solvent evaporation, producing spherical and narrow sized microparticles. The parameters such as the rate of spraying, the feed rate of the drug/polymer dispersion, the nozzle size, and the inlet and outlet temperatures have been previously studied in our laboratory [29–32].

2. Materials and methods

2.1. Materials

MTX (2S)-2-[[4-[(2,4-diaminopteridin-6-yl)methylmethylamino] benzoyl] amino] was purchased from DEG (São Paulo, Brazil). Poly-(DL-lactide-*co*-glycolic acid) (PLGA) 50:50, from Birmingham Polymers Inc. (inherent viscosity 0.63 dL g^{-1} at 30 °C), was purchased from LACTEL Absorbable Polymers® (Birmingham, USA). Leibowitz (L-15) medium (Invitrogen, New York, USA), DMEM medium, penicillin/streptomycin solution, sodium pyruvate, and 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum was purchased from Invitrogen (São Paulo, Brazil). All other reagents were of analytical grade.

2.2. Preparation of spray-dried MTX-loaded PLGA microparticles

Microparticles were prepared by using the mini spray-dryer Buchi-191 (Flawil, Switzerland) equipped with a 0.7 mm nozzle. An aspirator efficiency of about 90%, air flow of 600 l h^{-1}, spray feed rate of 3 mL·min^{-1} and inlet air temperature of 80 °C were selected. The free drug-loaded PLGA microparticles were prepared by spray drying 100 mL of 0.5% (w/v) PLGA solution in acetone.

The MTX-loaded microparticles containing 9%, 18%, or 27% of drug were prepared preserving the solute concentration of 0.5% (w/v). Different amounts of drug (0.045 g, 0.09 g, or 0.135 g) dissolved in 10 mL of 0.1 M acetic acid solution were mixed with 90.0 mL PLGA solutions containing 0.455 g, 0.410 g, and 0.375 g, respectively. These solutions were spray dried under the same conditions as above for free drug-loaded PLGA microparticles. After preparation, the dried microparticles were collected and stored under vacuum at 25 °C.

2.3. Gamma irradiation

The samples, hermetically closed in glass vials with rubber cap and sealed with aluminum, were exposed to gamma irradiation in a Gamma cell 220 Excel (Ottawa, Canada) with a Cobalt-60 source. The same formulation of microparticles containing 18% of MTX was exposed to different gamma irradiation doses (15 kGy, 25 kGy, and 30 kGy), while the distinct formulations (microparticles containing 9, 18, or 27% w/w of MTX) were exposed to the same procedure using a standard dose of 25 kGy.

2.4. Morphology and particle size measurements

The aspect of the powder was observed in a TM 3000 Microscope Hitachi (Tokyo, Japan) scanning electron microscopy (SEM) with magnification of 2500 times. The particles were dried and mounted on metal subs using double-sided adhesive carbon tape and analyzed by SEM at a voltage of 15.0 kV. The shape and surface of microparticles were further assessed in a SEM SSX550, Shimadzu (Tokyo, Japan) with magnification of 6000 and 15,000 times respectively. The particles were coated with a thin layer of gold in a Sputter Coater, and analyzed with SEM at a voltage of 15.0 kV.

The particle size distribution was determined using dynamic light scattering (DLS) in a Nanotrac NPA252 (Montgomeryville, USA) with Flex software 10.4.3. The microparticles were dispersed in 0.1% polysorbate 80 solution. The corresponding diameters of 10, 50, and 90% of the cumulative particle distribution were determined in triplicate. The index span was calculated by the equation: SPAN = (D90 − D10) / D50.

2.5. X-ray diffraction analysis

The X-ray diffraction (XRD) analysis was performed using a Rigaku diffractometer (Tokyo, Japan) set for an angle range of 5–45°, step size