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Note

Observations in simultaneous microencapsulation of 5-fluorouracil and leucovorin for combined pH-dependent release

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Abstract

5-Fluorouracil (5-FU) in combination with leucovorin (LV) is nowadays the standard treatment in colon cancer and would be a candidate to be delivered orally to the colon. Eudragit P-4135F or Eudragit RS100 were used separately to prepare microspheres by an oil/oil emulsification process trapping 5-FU and LV simultaneously. Scanning electron microscopy permitted a structural analysis, process parameters were analyzed and drug loading and release profiles were recorded. Particle size varied between 123 (RS100) and 146 μm (P-4135F). Generally, higher encapsulation rates were found with RS100 (5-FU, 60.3±9.7%; LV, 81.4±8.6%) compared to P-4135F (5-FU, 48.3±2.0%; LV, 55.4±2.7%). Microparticles made from Eudragit RS100 released the incorporated drug combination within 8 h not exhibiting general differences between the kinetics of both drugs. P-4135F was found to maintain the undesired 5-FU release at pH 6.8 lower than 25% within 4 h while at pH 7.4, a nearly immediate release (within 15 min) was observed. Although the release was similar at pH 7.4, at pH 6.8 LV showed a distinct initial drug loss of about 60% and a complete release within 2 h. SEM analyses revealed a substantial presence of LV crystals on the particle surface provoking a distinct burst effect of LV. These observations were concluded to be related to the high lipophilicity of P-4135F provoking a separation between P-4135F and LV during the preparation process.

Keywords: Colon delivery; Colorectal cancer; Microspheres; 5-Fluorouracil; Leucovorin

1. Introduction

Colon cancer is the second most cause of death after lung cancer by cancer diseases. Many different drugs or drug combinations have been tested for a successful therapy. At present, the standard regimen is an intravenous bolus injection of 5-fluorouracil (5-FU) modulated by leucovorin (LV) [1,2].

Only few approaches for an oral administration of anticancer drugs in the treatment of colon cancer have been described in literature. Recently, enzyme-dependent tablet-based systems have been proposed, which might allow an efficient treatment combined with a reduction of adverse effects [3]. Alternatively, pH-dependent drug release systems have been developed for the 5-FU release in the colon [4]. Possible variations in transit time throughout the colon risking incomplete carrier disintegration and a subsequent therapy failure were thought to be reduced with this latter strategy.

However, all proposed drug delivery strategies did not consider that the optimized drug therapy for colon cancer consists of a drug combination [1,2]. The design of systems delivering an entrapped drug combination has been reported in literature earlier for other therapeutic fields [5,6]. Although an oral drug administration was aimed, these systems focused rather on a sustained than a pulsatile drug release which is proposed here.

Besides a possible local effect after absorption of the drug combination which seems to be of minor importance, a major advantage consists in the colon delivery, namely the avoidance of mucosal metabolism which has been reported to be lowest in the colon on the example of other drugs [7]. Similar findings are described for a P-glycoprotein related efflux slightly reduced in the colonic tissue. Both are reasons

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for a generally lower bioavailability of drugs also being responsible for a lower therapeutic effect.

Most of the commercialized systems for the local drug delivery to the lower intestine after oral administration are based on the change of pH during the gastrointestinal passage. The pH-sensitive approaches such as methacrylate/methacryl acid polymers Eudragit® S and L dissolve in aqueous media at pH 6 and 7, respectively, which may be equivalent to a drug release to the distal ileum.

Recently, an additional polymer has been described for the use in colon delivery. It has been applied for film coating purposes on pellets, tablets and the preparation of microspheres [8,9]. Similarly, the described approach of a combined and pH-sensitive drug release of 5-FU and LV is based on the use of Eudragit P-4135F which was reported to allow more effective drug retention during the gastrointestinal passage and a subsequent delivery to the colon [8,9]. Besides, diarrhea has been observed as one of the major adverse effects of the 5-FU therapy [10] which can turn oral standard formulations insufficient. A size reduction of the carrier system might be required in order to circumvent its early elimination, since size-dependent gastrointestinal retention has been reported in diarrhea with an optimum for particles smaller than around 200 μm [11]. Subsequently, the combined microencapsulation of 5-FU and LV could be an advantageous approach.

2. Materials and methods

2.1. Materials

Eudragit® RS100 and Eudragit® P-4135F were kind gifts from Roehm Pharma Polymers, Tokyo, Japan. 5-FU, LV, and polyvinyl alcohol were purchased from Sigma (Deisenhofen, Germany). All other chemicals were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and were of analytical grade.

2.2. Methods

2.2.1. Preparation of microspheres

The preparation of microspheres was based on an oil/oil emulsification-solvent evaporation method. It was optimized as follows: a total polymer amount of 200 mg was dissolved in 5 ml acetone or equivalent volumes of solvent mixtures of acetone/ethanol. Fifty milligrams of 5-FU crystals (diameter [$D_{50\%}$]: 32.7 ± 3.9 μm) and 20 mg of LV crystals (diameter [$D_{50\%}$]: 17.2 ± 7.1 μm) were suspended by ultrasonication in the polymer solution. This solution was poured into 80 ml of liquid paraffin containing 1% w/w Span 80 and an oil/oil-emulsion was formed by stirring with a three-blade propeller at 600 rpm. The emulsion was stirred under vacuum until solvents were removed. Microspheres were collected by filtration and washing steps were performed with *n*-hexane followed by lyophilization.

2.2.2. Scanning electron microscopy and particle size analysis

The external and internal morphology of microspheres was analyzed by scanning electron microscopy (SEM). The microspheres were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (JEOL JSM-T330A scanning microscope, Tokyo, Japan) at 10 kV.

All microsphere batches were analyzed for their size distribution using a LDSA 2400A particle size analyzer (Tohnichi Computer Co. Ltd, Japan).

2.2.3. Determination of drug content and in vitro drug release

The drug loading efficiency in the microparticles was determined by high performance liquid chromatography (HPLC) based on an extraction method described elsewhere in detail [4]. The isocratic HPLC method was developed to our requirements allowing the simultaneous detection of both drugs. The system setup was as follows: RP-18 column (LiChrospher® 100); eluent:acetonitrile:acetate buffer pH 4.4 (6:94); flow rate 0.8 ml/min. Both, LV and 5-FU were detected by UV absorbance at 283 nm, samples of 50 μl were injected into the column.

The in vitro drug release was analyzed by the use of a paddle apparatus (USP XXIII). Drug-loaded microparticles (20 mg) were suspended in 500 ml phosphate buffer systems of different pH. The dissolution medium was kept under stirring at 100 rpm. All the experiments were carried out at 37 °C for 4–8 h. Aliquots of the dissolution medium (300 μl) were withdrawn at predetermined time intervals. Drug concentrations were directly analyzed by the HPLC method described before.

3. Results and discussion

The entrapment of 5-FU and LV in combination by using the newly described pH-sensitive polymer Eudragit P-4135F was intended to deliver the drug towards the distal sections of the intestine. The application of an oil/oil emulsification appeared reasonable due to the hydrophilic properties of both drugs aiming for increased encapsulation rates based on their lower solubility in the external oil phase. Moreover, a recent report on the microencapsulation of 5-FU into Eudragit P-4135F by an oil/water emulsification showed generally encapsulation rates below 40% [4] demanding for alternative preparation methods increasing the drug load.

Particle size of microspheres prepared with Eudragit P-4135F varied around 146 μm (Table 1). Generally, the encapsulation rates were higher compared to those found in