Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

FORMULATION AND EVALUATION OF 5-FLUOROURACIL LOADED CHITOSAN AND EUDRAGIT® NANOPARTICLES FOR CANCER THERAPY

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Received: 17 September 2010; Revised: 30 December 2010; Accepted: 4 January 2011; Available online: 11 January 2011

ABSTRACT

The goal of the present investigation was to formulate and evaluate chitosan and Eudragit® nanoparticles of 5-Fluorouracil for cancer therapy. Nanoparticles of 5- Fluorouracil were prepared using chitosan , Eudragit® S 100, liquid paraffin and Tween -20 using Emulsion droplet coalescence method. The concentration of the polymers Chitosan and Eudragit® S 100 were selected based on the results on preliminary screening. The nanoparticles prepared were evaluated for morphology, loading efficiency, invitro release and invitro anticancer activities. The particle shape and morphology of the prepared 5-Fluorouracil nanoparticles were determined by SEM analysis. The amount of 5-Fluorouracil entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non entrapped drug remaining in the aqueous supernatant. A franz diffusion cell was used to monitor 5-Fluorouracil release from the nanoparticles. The *in-vitro* anticancer activity of formulated nanoparticles was carried out in Dalton-Lymphoma ascites (DLA) cells bearing mice. The formulations CF1, CF2, EF2 and EF3 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours was 74.49, 74.06, 77.68 and 75.51% respectively. However about 15% initial burst release was found at 1 hour in all formulations. EF2 released 77.68% of 5- Fluorouracil in 12 hours with a burst drug release nearly 14.92% of drug within the initial 1 hour. Formulations 4 out of 9 showed good drug release from the polymer, the percentage cumulative drug release after 12 hours were in the range of 74-78 %. Among the four formulations EF 2 (1% Chitosan & 1.5 % Eudragit® S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. *In-vitro* anticancer study revealed that the formulated nanoparticles were found to have good cidal activity on cancer cells in sustained manner.

Keywords: Emulsion droplet coalescence, nanoparticles, chitosan, Eudragit® S 100, 5- Fluorouracil.

INTRODUCTION

The substances with size ranges from 1 to 1000 nm are called nanoparticles. These materials are mainly used in oncology for early detection of malignancy and precise localisation of cancer therapeutics without or with minimal adverse effect to the somatic tissues. These carriers are used to protect drugs, vaccines, nutrients and cosmetics. Nanoparticles exerts its site specific drug delivery by avoiding the reticuloendothelial system, utilising enhanced permeability and retention effect and tumour specific targeting. The formation of nano particles and physicochemical parameters such as pH, monomer concentration, ionic strength as well as surface charge, particle size and molecular weight are important for drug delivery. Further, these nanoparticles have the capability to reverse multi drug resistance, a major problem in chemotherapy.[1]

5-Fluorouracil (initially 7-12 mg/kg iv for 4 days), a cell-cycle-phase-specific anti neoplastic agent, is indicated in colon, rectal, breast, ovarian, cervical, gastric, oesophageal, bladder, liver, and pancreatic cancer. Fluorouracil exerts its cytotoxic activity by acting as an anti metabolite, competing for the enzyme that is important in the synthesis of thymidine, an essential substrate for DNA synthesis. The hydrophillicity of 5-Fluorouracil allowed it to complex with dendrimers after simply incubating the polymer with the drug.[2]

The limitation in conventional cancer treatment can be alleviated by targeted drug delivery, which is a vehicle that will preferentially carry the drug to the target site in the body and thereby reduce the amount of drug in the rest of the body that can cause undesired side effect. These would increase the range in which a drug is both safe and effective. The distinct capability of nanoparticles to provide access to virtually all cell types may be utilised for the delivery of therapeutic agents to wide array of cellular types and targets.[3]

MATERIALS AND METHODS

5-Fluorouracil was a gift sample from Sun Pharmaceuticals, Pondicherry, India. Chitosan was procured from CIFT, Cochin, India. Eudragit® S 100 was obtained from SD Fine Chemical Maharashtra, India. Liquid paraffin, tween 20, sodium chloride was obtained from Spectrum Chemicals and Reagents, Cochin, India.

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Sodium hydroxide, Sodium dihydrogen phosphate and disodium hydrogen phosphate was procured from Burgoyne Uribiges & Co, Mumbai, India.

Preparation of drug loaded nanoparticles[4, 5]

Method: emulsion-droplet coalescence method

Chitosan was dissolved in 1% acetic acid and 50 mg of 5-Fluorouracil in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 5%v/v tween 20. This mixture was stirred using a homogeniser 3 minutes to form water in oil (w/o) emulsion. Similarly, another w/o emulsion consisting of Eudragit® S 100 in 3M sodium hydroxide solution was prepared.[6]

Then these two emulsions were mixed and stirred using homogenizer. As a result of coalescence of the droplets, chitosan in the system was solidified to produce nanoparticles. Eudragit® S 100 producing second coating over chitosan nanoparticles. The resultant 5-Fluorouracil nanoparticles were centrifugated at 3000 rpm was 60 mts (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator.[7] (Table **1**)

Table 1. Formulation of 5- Fluorouracil nanoparticles

Formulation	Conc.of Chitosan	Conc.of Edragit® S 100	Amount of drug (5-Fluorouracil)	Conc.of Tween 20
CF 1	0.5 %	1 %	50 mg	0.5 %
CF 2	1 %	1 %	50 mg	0.5 %
CF 3	1.5 %	1 %	50 mg	0.5 %
CF 4	2 %	1 %	50 mg	0.5 %
CF 5	2.5 %	1 %	50 mg	0.5 %
EF 1	1 %	0.5 %	50 mg	0.5 %
EF 2	1 %	1.5 %	50 mg	0.5 %
EF 3	1 %	2 %	50 mg	0.5 %
EF 4	1 %	2.5 %	50 mg	0.5 %

Evaluation of Nanoparticles

Detection of shape and morphology

The particle shape and morphology of the prepared 5-Fluorouracil nanoparticles were determined by SEM analysis. The nanoparticles were viewed using a Jeol-5610 L V (Tokyo, Japan) for morphological examination. Powder samples of dried nanoparticles were mounted onto aluminium stubs using double side adhesive tape and then sputter coated with a thin layer of gold at 10 Torr for vaccum before examination. The specimens were scanned with an electron beam of 1.2 kv acceleration potential and images were corrected in secondary electron mode.[8]

Determination of loading/entrapment efficiency[9-11]

The amount of 5-Fluorouracil entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non entrapped drug remaining in the aqueous supernatant. The latter was determined following the separation of drug loaded nanoparticles from the aqueous medium by centrifugation at 5000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation.[12] The amount of free 5-Fluorouracil in the supernatant was determined by UV-Visible spectrophotometer (UV1 v7.07 Thermo Scientific, Germany.

***In-vitro* release study**

A franz diffusion cell was used to monitor 5-Fluorouracil release from the nanoparticles.[13] The receptor phase was phosphate buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrance (Hi Media, Mumbai, India) with molecular weight cut off 12,000 to 14000 Daltons was used to separate receptor and donor phases. The latter consisted of a 2ml suspension of nanoparticles containing 10 mg of 5-Fluorouracil, mixed for 5 seconds to aid re-suspension, in a 1% w/v Tween 80 solution in PBS. Samples (1ml) from the receptor phase were taken at time intervals and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of 5-Fluorouracil into the receptor phase was evaluated spectrophotometrically.[14]

Evaluation of *in-vitro* anticancer activity[15-17]

The invitro anticancer activity of formulated nanoparticles was carried out in Dalton-Lymphoma ascites (DLA) cells bearing mice. The cells were aspirated from the peritoneal cavity of tumour bearing mice. The cells were washed three times with phosphate buffer saline. The viability of the cells were checked using trypan blue (cell viability should above 98%) and different dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were made.[18]

The number of cells in the 10^{-3} dilutions was counted using a haemocytometer and the numbers of cells were adjusted to 1×10^{-6} cells/ml. The experiment was set up by incubating different formulations of nanoparticles (100µg/ml) with 1×10^{6} cells/ml. The final volume of the assay mixture was made up to 1 ml using phosphate buffered saline and was incubated at 37°C for 3 hrs. 0.1 ml of trypan blue solution was added after incubation period and the number of dead cells was counted using a haemocytometer. The percentage cytotoxicity was calculated.[19]

RESULTS AND DISCUSSION

In total nine formulations of 5-Fluorouracil loaded nanoparticles were prepared and evaluated for various parameters such as particle size, morphology, drug entrapment efficiency, *in-vitro* release and *in-vitro* anticancer activity.

Preliminary screening for encapsulation efficiency

The nanoparticle drug delivery system is prone for the delivery of drugs to the targeted site. In early stages of formula optimization studies, the w/o emulsion formation was the problem. It was overcome by replacing surfactant; the surfactant selected was tween 20, which had high encapsulation efficiency.

Preparation of nanoparticles

Nanoparticles were prepared by emulsion droplet coalescence method. It is a laboratory method proved for the preparation of nanoparticles. The concentration of the polymers Chitosan and Eudragit® S 100 were selected based on the results on preliminary screening. The surfactant used for the preparation was tween 20. The time taken to complete preparation was around 2 hours.

DSC studies

As DSC is useful tool to monitor the effect of additives on the thermal behaviour of materials, these techniques were used to derive qualitative information about the physicochemical status of drug in particles.[20] The peak for 5-Fluorouracil pure sample was obtained in 152.04°C. The peak in physical mixture and nanoparticles were 154.68°C and 157.51°C respectively.

Entrapment efficiency and loading capacity

The data of drug entrapment efficiency and drug loading capacity for drug loaded nanoparticles were as shown in

the table **2**. The formulation EF1 1 showed around 35% of drug loading.

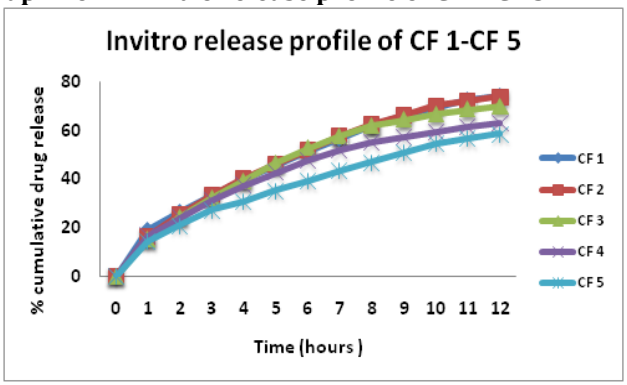
Table 2. Summary of evaluation of nanoparticles

Parameter	CF1	CF2	CF3	CF4	CF5	EF1	EF2	EF3	EF4
pH	7.1	7.5	7.7	7.6	7.8	7.0	7.2	7.4	7.5
Practical yield	130	175	130	265	295	115	225	265	315
Efficiency of particle recovery (mg)	86.67	87.50	86.66	88.33	84.28	76.66	90.00	88.35	90.00
Unencapsulated drug(mg out of 50 mg)	8.5	10.9	11.8	13.0	14.35	14.5	12.3	9.8	14.35
Entrapment efficiency(%)	83.00	78.20	76.40	74.00	71.30	71.00	75.40	78.80	71.30
Loading capacity(%)	31.92	22.34	29.38	13.96	12.08	35.73	30.16	14.86	11.37

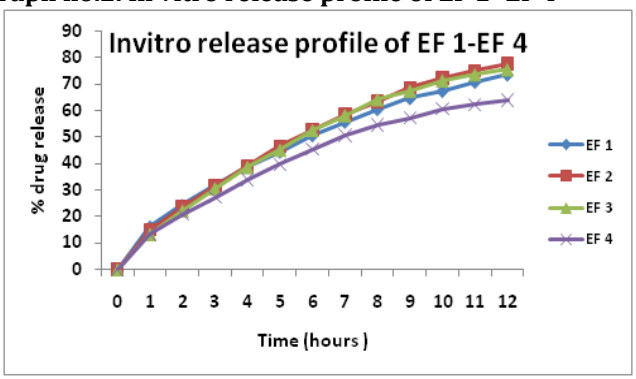
In-vitro diffusion study

The drug release profile from the nanoparticles were as shown in the graphs (Graph **1** & **2**). The formulations CF1, CF2, EF2 and EF3 showed good drug release from the polymer.[15] The percentage cumulative drug release after 12 hours was 74.49, 74.06, 77.68 and75.51% respectively. However about 15% initial burst release was found at 1 hour in all formulations. EF2 released 77.68% of 5-Fluorouracil in 12 hours with a burst drug release nearly 14.92% of drug within the initial 1 hour.

Graph no. 1.In vitro release profile of CF 1-CF 5



Graph no.2. In vitro release profile of EF 1- EF 4



In-vitro anti cancer studies

The *in-vitro* anti cancer study reported that the formulated nanoparticles were found to have cidal activity on cancer cells in sustained manner. Whereas the standard used (10µg/ml of 5 Fluorouracil) gave 100% activity within 2 hours itself. High dead cells are found in the formulation CF1. The percentage cytotoxicity was found to be 75 %.

CONCLUSION

On preliminary screening different formulations were developed with various ratios of polymers and different surfactants. It revealed that formulations with the polymer concentration (1.0-2.5%) and surfactant (tween-20) had better drug release and entrapment efficiency. So the formulations were designed with that polymer concentration and surfactant.

Nine formulations were evaluated and among them CF1, CF2, EF2 and EF3 were found to have good results. Among the four formulations EF2 (1% Chitosan & 1.5% Eudragit® S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. The work on formulation development of 5- Fluorouracil nanoparticle was very much advantageous than the existing dosage forms as the drug is targeting to the cancerous cells, hence better action.

ACKNOWLEDGMENT

The authors are highly obliged to Sun Pharmaceuticals, Pondicherry, India for providing gift sample of 5-Fluorouracil. Authors also thank CIFT, Cochin, India, SD Fine Chemicals, Maharashtra, India for providing generous quantities of required materials and Mother Theresa Post Graduate & Research Institute of Health Sciences, Pondicherry, India for providing best lab facilities necessary for completion of research project.

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