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ESTIMATION OF PHARMACOKINETIC PARAMETERS AND SAFETY STUDIES OF 5-FLUOROURACIL DRUG LOADED IN SUCRALFATE SUSPENSION

PROCENA FARMAKOKINETSKIH PARAMETARA I BEZBEDNOSNE STUDIJE UDARNE DOZE 5-FLUOROURACILA U SUSPENZIJI SUKRALFATA

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Key words

5-Fluorouracil, Sucralfate, Sodium carboxymethyl cellulose, Xanthangum, Suspension.

Ključne reči

5-fluorouracil, sukralfat, natrijum karboksimetil celuloza, ksantan guma, suspenzija.

Abstract

Objective: The object of the present investigation was estimation of pharmacokinetic parameters and safety studies of 5-Fluorouracil drug loaded sucralfate suspension as drug delivery system. **Methods:** Suspension was prepared by hydration using part of vehicle, carboxymethyl cellulose, sodium carboxymethyl cellulose and xanthan gum used as suspending agent. The effects of different parameters on the drug content and the release of the drug from the suspension and physical stability of suspension were investigated. Pharmacokinetic parameters such as Cmax, Tmax, Half life (T1/2), Elimination rate constant (h-1), AUC0-t , AUC0-inf, AUMC0-t, AUMC0-inf, and MRT (mean residence time) were determined using rat as animal model. **Results:** From the above results, formulations (FS3, FS5, FS6), have shown high viscosity due to increase in concentration of carboxymethyl cellulose and sodium carboxymethyl cellulose. Formulation FS6 containing 2.5% of sodium carboxymethyl cellulose, as a suspending is sufficient to remain particles dispersed in the suspending medium. FS6 formulation appears to be the best of all formulations with highest percentage drug release at the end of 60 min (92.54±0.4). Tmax and elimination rate constant of the 5-Fuorouracil loaded sucralfate suspension was found to be 14 & 0.37696 - 0.40339h-1. MRT of suspension was in the range of 1.998-2.2756 for optimized FS6 formulation.**Conclusion:** Thus, suspension supplies a constant release of drug at specific site, mimics the tumor and improves the patient compliance.

INTRODUCTION

Oral delivery of anticancer drugs offers certain advantages over the current regimen of chemotherapy by injection or infusion. It can provide a continues and prolonged exposure of anticancer drugs to cancer cells at a relatively lower and thus safer concentration. Despite these merits, oral delivery of anticancer drugs has been a challenge since most anticancer drugs have poor bioavailability either due to their poor aqueous solubility, stability or permeability. Therefore, oral administered anticancer drugs have little chance to get in to blood system and thus reach the tumor site (1).

Sucralfate is a non-absorbable, basic aluminum salt of a sulphated disaccharide which has proven effective in the treatment of gastric and duodenal ulcers (2, 3, 4). Sucralfate forms polyvalent bridges to the positively charged proteins present in the mucosa and form pasta like adhesive substances. Thus, protective barrier is formed against further mucosal damage (5). The binding of sucralfate is most effective at low pH but may still occur at higher pH 3 and 5.

Sucralfate suspension has gained increased importance not just for the delivery of anti ulcer drugs for the treatment of ulcers, gastroenteritis but also cytoprotective action used

in the treatment of cancer chemotherapy and radiation induced toxicity [6, 7]. However, there are certain beneficial areas, especially the treatment of upper gastroesophageal disorders (e.g. gastroesophageal reflux, heartburns, dyspepsia, and esophageal cancer) where prolonged drug retention within esophageal region is often desired [8, 9].

5-Flourouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is an antimetabolite of pyrimidine analogue type with a broad spectrum activity against solid tumors such as gastrointestinal tract, pancreas, ovary, brain, breast, etc. Due to its structure, 5-FU interferes with nucleoside metabolism and can incorporate into RNA and DNA, leading to cytotoxicity and cell death [10,11]. Limitations are short biological half-life due to rapid metabolism, incomplete and non uniform oral absorption due to rapid metabolism by dihydropyramidine dehydrogenase and nonselective action against healthy cells.

The aim of the present work is to design 5-Fluorouracil drug loaded in sucralfate suspension, which to avoid first pass metabolism and to improving the desired bioavailability by delivery system at the carcinoma site.

MATERIALS AND METHODS

Materials: 5- Fluorouracil (5Fu) was obtained from Celone Pharmaceuticals Pvt. Ltd., India. Carboxymethyl cellulose, Sodium carboxymethyl cellulose, Xanthangum, Sucralfate was purchased from Yarrow chem. Products (Mumbai), Mohali. india. MethylParaben were purchased from S.D Fine Chemicals Mumbai. Water used in the experiments was deionized filtered (Milli-QAcademic, Millipore). All other chemicals were of analytical grade and used without further purification.

Preparation of 5-Fluorouracil drug loaded Sucralfate suspension:

Sucralfate suspension containing 500 mg/5mL of sucralfate were prepare using (0.1-0.5% xanthangum), (1-5% Carboxymethyl cellulose), (1-5% sodium carboxymethyl cellulose). Mucillages of the gums were prepared by hydration using part of the vehicle. The solid components of the formulation were finely triturated with the aid of mortar and pistle [12]. The suspending agents (xanthangum, carboxymethyl cellulose, sodium carboxymethyl cellulose) were added to 5-Fluorouracil & sucralfate powder dissolved in water and triturated until homogeneous slurry was obtained. Methylparaben was used as the preservative and saccharin (1%) was used as sweetener.

Table 1. Formulation of 5-Fu drug loaded sucralfate suspension

S.No	Ingredients (mg)	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9
1	Sucralfate	500	500	500	500	500	500	500	500	500
2	5-Fluorouracil	20	20	20	20	20	20	20	20	20
3	CMC	5	10	15						
4	Na CMC	-	-	-	5	10	15	-	-	-
5	Xanthan gum	-	-	-	-	-	-	0.5	2	2.5
6	Citric acid	15	15	15	15	15	15	15	15	15
7	Sorbital	35	35	35	35	35	35	35	35	35
8	Methylparaben	5	5	5	5	5	5	5	5	5
9	Water (ml)	5	5	5	5	5	5	5	5	5

Physical stability studies of 5- Fu drug loaded sucralfate suspension

All prepared suspensions were kept at 30° and 45°C and evaluated for their physical and chemical properties including pH, density, sedimentation volume, viscosity.

pH: It is important parameter for the suspension stability. The pH determination study was carried out by using digital pH meter. The pH meter was calibrated and the sample of suspension was taken and pH was measured at room temperature.

Density: Density of the formulated suspension was determined by specific gravity bottle. The values are presented in table 2.

Sedimentation volume: Sedimentation volume of suspension was determined by transferred the prepared suspension in to 50 mL measuring cylinder and make up to final volume. The cylinder was then inverted 10 times to ensure complete mixing and placed in a constant temperature water bath at 25 to 0.1°C. However, no wet ability problems were encountered in the preparation of the suspension. The sedimentation volume can be expressed as the ratio of the final volume of sediment to original volume of the suspension before settling [13].

Viscosity: The viscosity of the prepared formulations was determined at different angular velocities at 25°C using a rotary viscometer (DV-III, Brookfield, USA).The rotation speed was 20 rpm, with spin 18. The average of two readings was used to calculate the viscosity.

***In-vitro* release study:** Dissolution studies was performed in a 6 channel in-vitro dissolution assembly (Electrolab) using a modified official paddle method. 5mL of 5-Fluorouracil drug loaded suspension was introduced in to 900 mL of 0.1 N Hydrochloric acid of pH 1.2 in a 1000 ml dissolution vessel maintained at 37.0 ± 0.1°C. Triplicate studies were performed for all the formulations in simulated gastric fluid. 5mL of aliquots were withdrawn using a pipette with a filter attachment at regular intervals during a period of 1 hr. 5-Fluorouracil released was assayed spectrophotometrically at 254 nm.

Chromatographic system

A gradient High pressure Liquid chromatography (Schemadzu HPLC Class VP series) with two LC 10 -AT VP PUMP variable wave length programmable UV/VIS Detector SPD-10 A VP, CTo -10 AS VP Column oven (Shimadzu) , SCL-10 A VP system controller (Shimadzu) and RP C-18 Column (250 mm x 4.6 mm I.D; particle size 5 µm YMC, Inc, Wilmington, NC 28403, U.S.A) was used. The HPLC system was equipped with the soft ware "Class-VP" series version 5.03 (Shimadzu)

Preparation of mobile phase

The mobile phase used was HPLC grade Methanol and acetonitrile filtered through 0.2m membrane filter and degassed with the helium spurge for 15 minutes before use and pumped from solvent reservoir to the column at a flow rate of 0.8 mL/min which

yielded column back pressure of 200-225 kg/cm^2. The column temperature was maintained at 40°C. The volume of each injection loop was 20 µL.

Establishment of Calibration curve

Preparation of stock solution: Stock solution of 5-FU was prepared in triplicate by dissolving 10 mg 5-FU in 100 mL (Methanol: Acetonitrile 15:85) resulting in a solution containing 100µg/mL . From the prepared stock 1 take 0.5; 1; 1.5; 2; 2.5 and 3mL, and diluted with 2, 4, 6, 8, 10 and 12 µg/mL solution. Absorbance was monitored at 254 nm by photo diode detector at HPLC. A calibration curve was established based on the linear regression. The independent variable is 5-Fu concentration (x) and dependent variable is peak area (x). Fitting a linear regression model gave an equation having the form Y=ax+b where a is the regression coefficient and b is a constant. There is a good linear relationship between x and y for each sample range. The stability, recovery, linearity, accuracy and specificity of the method were evaluated in agreement with the criteria widely accepted. Also, value for day-to-day precision was considerd. Recovery rate of all the samples under study were between 84.6% and 100.5%. The regression equation was Y=78.5 and r=0.9941, n=6.

Study design: The present study was approved by the Institutional Animal Ethical Committee (IAEC) license no: 1035/ac/09/cpcsea. Wistar rats weighing 150-200 g were used. The animals were maintained in a restricted –access room maintained at 25°C. They were housed at a maximum of three rats per cage, and left for stabilization for one week. The rats were fasted for 12 h prior to and during the experiments but were allowed free access to water.

Extraction of procedure and analytical method validation in rat plasma

Rats were assigned randomly in two groups of five rats. Orally suspension was administered to the rats via a polyethylene cannula (diameter 2 mm) with 1mL water under light ether anaesthesia, at a 5-FU dose equivalent to 15mg/kg. This treatment was repeated daily for 7 days [14]. Blood samples (1.0 mL) were collected from the fossa orbitalis vein into heparinized tubes at the following time points: 0, 2, 5, 7, 9, 11, 14, 16, 18, 21 and 24 h for the rats given suspension. The heparinized blood samples were immediately centrifuged at 1000 g for 10 min in a research centrifuge, and the plasma separated and transferred to micro centrifuge tubes for storage at –20°C. Frozen plasma samples were thawed. A 0.2 mL aliquot transferred into a glass tube with a Teflon-lined cap, to which was added 0.2 mL methanol. The mixture was vortexed for 10 min and then centrifuged at 1000 g for 15 min. The supernatant was then dried under a stream of nitrogen and resuspended in 0.1 mL mobile phase, vortexed for 3 min and centrifuged at 1000 g for 5 min; 0.02 mL of the subsequent supernatant was subjected to HPLC for analysis of 5-FU as described below.

Pharmacokinetic anaiysis: Pharmacokinetic parameters were calculated by noncompartment analysis based on the stastistical moment theory using PK1, PK2 excell function such as maximum plasma concentration (Cmax), and time of maximum concentration (Tmax) were obtained directly from the Plasma concentration-time plots. Half life (T1/2), elimination rate constant (h^{-1}), $AUC0_{-t}$, $AUC0_{-inf}$, $AUMC_{0-t}$, $AUMC_{0-inf}$, and mean residence time (MRT) was calculated as AUMC/AUC.

Statistical analysis: Variation in Pharmacokinetic parameters was tested using analysis of variance. Difference in mean PK Parameters of 5-Fu drug loaded Sucralfate suspension using Carboxymethyl cellulose and Sodium carboxymethyl cellulose in 1-2.5% concentration was subjected to t-test to find the statistical significance. In all the cases a value of $P< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

5-Fu drug loaded Sucralfate suspension was prepared with different concentrations of Carboxy methyl cellulose, Sodium carboxy methyl cellulose, xanthan gum as suspending agents.

SNO	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9
Density (gm/cc)	1.23	1.45	1.62	1.42	1.55	1.72	1.02	1.35	1.45
Viscosity (cps)	110	120	130	125	140	150	105	115	125
PH	1.2	2.3	4.2	1.5	2.8	3.2	1.3	2.4	3.2

***Table 2**. Physical stability characters of sucralfate suspension*

Time (days)	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9
0	1	1	1	1	1	1	1	1	1
2	0.85	0.88	0.9	0.94	0.96	0.99	0.84	0.9	0.94
4	0.79	0.82	0.85	0.88	0.84	0.96	0.8	0.84	0.88
6	0.68	0.74	0.79	0.82	0.88	0.92	0.77	0.8	0.82
8	0.58	0.66	0.72	0.72	0.79	0.86	0.66	0.64	0.72
10	0.48	0.54	0.62	0.64	0.7	0.8	0.56	0.58	0.64
14	0.36	0.42	0.48	0.56	0.62	0.7	0.46	0.49	0.56

***Table 3**. Sedimentation volume of sucralfate suspension*

Time	**FS1**	**FS2**	**FS3**	**FS4**	**FS5**	**FS6**	**FS7**	**FS8**	**FS9**
0	0	0	0	0	0	0	0	0	0
5	10.56 ±0.1	13.32 ±0.31	14.51 ±0.21	12.35 ±0.5	15.34 ±0.1	18.21 ±03	7.03 ±0.11	10.31 ±0.2	15.32 ±0.2
10	14.28 ±0.5	17.33 ±0.2	21.32 ±0.34	14.89 ±0.21	19.23 ±0.8	29.9 ±0.5	12.22 ±1.0	15.14 ±0.5	17.56 ±0.25
20	23.04 ±0.2	27.65 ±0.15	32.13 ±0.5	25.34 ±1	30.54 ±1.0	43.58 ±0.8	20.14 ±0.5	28.65 ±1.0	35.87 ±0.5
30	32.49 ±1	35.46 ±0.5	43.84 ±0.61	34.14 ±0.52	42.35 ±0.42	57.24 ±1	35.15 ±0.8	38.48 ±0.8	46.32 ±1.0
45	42.3 ±0.4	48.61 ±1	60.22 ±0.86	50.61 ±0.9	68.12 ±0.5	84.42 ±0.2	45.51 ±0.22	51.36 ±0.3	80.21 ±0.8
60	63.4 ±0.2	66.22 ±0.5	71.35 ±1.0	71.32 ±0.71	80.23 ±1.2	92.54 ±0.4	68.36 ±0.51	74.71 ±1.0	91.22 ±1.2

***Table 4**. In vitro dissolution studies of 5-Fu drug loaded sucralfate suspension*

Pharmacokinetic parameters	FS3	FS5	FS6	FS9
C_{max}	22.8±0.8	23±0.7	22±1.0	24±1.2
T_{max}	14±0.01	14±0.01	14±0.01	14±0.01
Elimination rate Constant(h^{-1})	0.43171±1.2	0.38190±1.3	0.40339±1.22	0.37696±1.32
Half Life (h)	1.605581±1.22	1.81498±1.42	1.718285±0.9	1.8387±1.32
AUC_{0-t}	80.2±3.2	85±3.6	86.7±3.4	87.1±3.66
AUC_{0-inf}	18.875±0.01	18.875±0.01	18.875±0.1	18.875±0.1
$AUMC_{0-t}$	1349.3±8.39	1441.3±11.4	1472.8±8.217	1465.6±7.21
$AUMC_{0-inf}$	40.1456±0.8	39.9527±0.9	39.873±0.86	38.274±1.2
MRT (h)	1.998±0.86	2.05±0.8	2.174±1.2	2.275±1.42

Table 5. *Pharmacokinetic Parameters of sucralfate suspension*

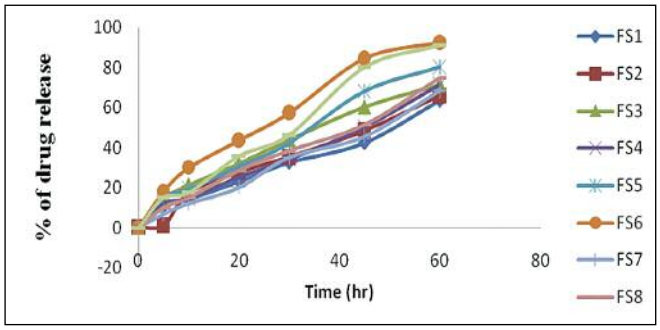

Figure 1 *In vitro dissolution studies of 5-Fu drug loaded Sucralfate suspension*

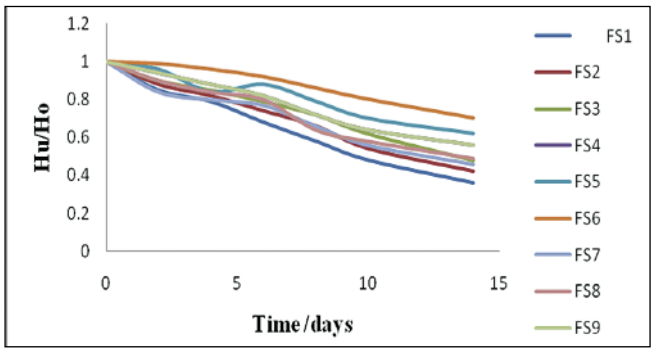

Figure 2 *Sedimentation volume of 5-Fu loaded Sucralfate suspension*

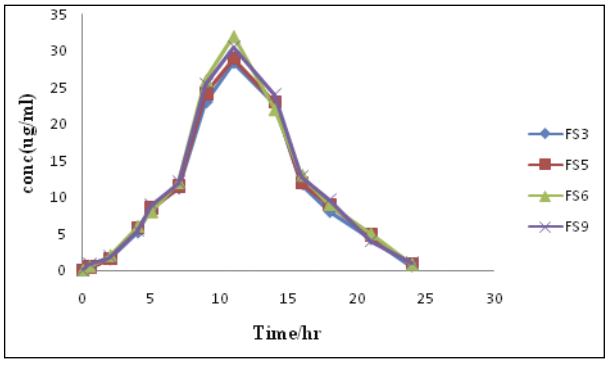

Figure 3 *Pharmacokinetic parameters of 5-Fu loaded sucralfate suspension*

Formulations FS3, FS5, FS6 exhibited high viscosity due to presence of high concentration of Carboxymethyl cellulose, and Sodium carboxymethyl cellulose (Table 2). Formulation FS6 containing 2.5% of Sodium carboxymethyl cellulose as a suspending is sufficient to remain particles dispersed in the suspending medium for sufficient period of time and also sufficient to withdraw suspension from the container. The viscosity of all formulations ranged between 110-150cps.

The pH of formulations FS3, FS5, FS6 was found to be in the range of 2.8-4.2, which is desirable for the stability of the drug (Table 2).

Formulation FS6 has shown maximum value (0.7 closest to 1 as compared to other formulations) of sedimentation volume followed by FS5, FS9, FS4, FS8 respectively (Table 3). Formulations FS6, FS5, remained homogeneous after reconstitution and did not exhibit any signs of caking.

FS6 shows an optimized sedimentation volume and good physical stability characters in fig. 2 where used as a tool for nanoparticles incorporated into sucralfate suspension.

In-vitro drug release performed indicates all the formulations appear to release the drug with an intial lag time observed. FS6 formulation appears to be the best of all formulations with highest percentage drug release at the end of 60 min (92.54±0.4). The observed studies are illustrated in table 4 and percentage of drug release is shown in fig 1 of the graph.

Pharmacokinetic parameters such as C_{max} , T_{max}, Half life ($T_{1/2}$) , Elimination rate constant (h^{-1}), AUC_{0-t}, AUC_{0-inf}, $AUMC_{0-t}$, $AUMC_{0-inf}$, and MRT were determined using PK1 and PK2 excel function for 5-Fu drug loaded sucralfate suspension. All data collected are displayed in table 5.

From the results it is observed that Tmax of the 5-Fu loaded sucralfate suspension was 14 which indicates that no variation was seen between the formulation FS3, FS5, FS6, and FS9. The elimination half life in 5-Fu loaded sucralfate suspension was from 0.37696 - 0.40339h^{-1}.

MRT of 5-Fu loaded sucralfate suspension was found to be in the range of 1.998-2.2756 for optimized FS6 formulation. Thus, suspension gives a constant release of drug at specific site and mimics the tumor. This mechanism was mention earlier which will improve the bioavailability due to cationic and mucoadhesive character.

CONCLUSION:

Stable suspension of 5-Fu drug loaded sucralfate suspension could be formulated at pH 4.2 with 2.5% sodium carboxymethyl cellulose as suspending agent. Oral site specific release of 5-Fu delivery has proved to reduce systemic side effects and to also provide an effective and safe therapy, mimics the tumor and improves the patient compliance.

Sažetak

Cilj: Cilj datog istrazivanja je bila procena farmakokinetičkih parametara i bezbednosne studije udarne doze 5-fluorouracila u suspenziji sukralfata kao način primene leka.
Metode: Suspenzija je pripremljena hidratacijom korišćenjem dela nosača, a karboksimetil celuloza, natrijum karboksimetil celuloza i ksantan guma korišćeni su kao suspenziona sredstva. Ispitivani su efekti različitih parametara na sastav leka i njegovo oslobađanje iz suspenzije kao i fizička stabilnost suspenzije. Farmakokinetički parametri kao što su Cmax, Tmax, poluvreme ($T_{1/2}$), konstanta brzine eliminacije (h^{-1}), AUC_{0-t}, AUC_{0-inf}, $AUMC_{0-t}$, $AUMC_{0-inf}$ i srednje vreme zadržavanja leka, su određeni koristeći pacova kao životinjski model.
Rezultati: Dobijeni rezultati su pokazali da su formulacije (FS3, FS5, FS6) imale visoki viskozitet usled povećanja koncentracije karboksimetil celuloze i natrijum karboksimetil celuloze. Količina od 2,5% natrijum karboksimetil celuloze kao suspenzionog sredstva u formulaciji FS6 je dovoljna da zadrži čestice dispergovane u suspenzionom medijumu. FS6 formulacija se pokazala kao najbolja od svih formulacija jer ima najveći procenat oslobađanja leka na kraju 60. minuta (92,54±0,4). Tmax i konstanta brzine eliminacije udarne doze 5-fluorouracila u suspenziji sukralfata iznosili su 14 i 0,37696 – 0,40339h-1. Srednje vreme zadržavanja optimizovane suspenzije FS6 formulacije, bilo je u rasponu od 1,998 - 2,2756.

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