WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 8, Issue 4, 1584-1592 Research Article ISSN 2278 – 4357

BIO-MIMETIC VESICLES ENCAPSULATING IRINOTECAN HYDROCHLORIDE ALONG WITH HERBAL BIO POTENTIATORS FOR BIOAVAILABILITY ENHANCEMENT: PREPARATION, CHARACTERISATION AND EVALUATION

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Article Received on
18 Feb. 2019,

Revised on 11 March 2019,
Accepted on 02 April 2019

DOI: 10.20959/wjpps20194-13550

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ABSTRACT

The present study was aimed to prepare sustained release Biomimetic vesicles of irinotecan Hydrochloride alone (R1 to R8) and along with bioenhancers (BP1 to BP7) by thin film hydration technique using span 40 as surfactant, cholesterol as membrane stabilizing agent, Genistein and Quercetin as Drug bioavailability enhancers and dicetyl phosphate (DCP) as charge inducing agent. All the formulations of Vesicles were characterized on the basis of physical appearance and entrapment efficiency. The *in-vitro* release studies of optimized formulation of Irinotecan Hydrochloride alone and along with efficiency improvers were performed and compared with pure drug released. The entrapment efficiency of irinotecan hydrochloride in optimized formulation of Biomimetic Vesicles containing Irinotecan along with Efficiency improvers was found to 59.9 % and entrapment efficiency of Genistein and Quercetin was found to be 30.30% and 60.1%respectively. In vitro drug release of optimized formulations of Irinotecan Hydrochloride without and with bio potentiators was found to be 99.77% and 60.11% at the end of 12 h respectively. Results concluded that Vesicles of Irinotecan containing bioenhancers followed sustain release pattern.

KEYWORDS: Irinotecan Hydrochloride, Thin Film Hydration Technique, Entrapment efficiency, *Invitro* study, Genistein, Quercetin.

INTRODUCTION

The success of cancer chemotherapy depends on many factors such as active agent, dosage form, patient condition and chemotherapy regimen.Usage of nanotechnology to develop better dosage forms in cancer treatment has brought new hopes for cure.Irinotecan Hydrochloride is effective anti cancer agent but its availability is unfortunately hampered due to its low bioavailability. This is because of low aqueous solubility, low permeability and high affinity for efflux pump cytochrome P-450 and P-Glycoprotein.

there are several approaches to increase the bioavailability of irinotecan hydrochloride such as using P-gp inhibitors and to stop Irinotecan elimination.However this type of approach leads to serious side effects as depression of immune system.Another approach drug carrier systems are extensively studied to enhance the bioavailability and reduce the side effects.[123] As antitumor agents have high potential to induce side - effects and toxicity, localization of the drug to the tumor site would certainly optimize the therapy. The concept of targeted drug delivery is designed for attempting to concentrate drug in the tissues of interest and thereby reducing the relative concentration of medication in the remaining tissues.[4] Certain Vesicles or cellular carriers like erythrocytes and lymphocytes may be used to ferry the drug to the required site. Ideally, such Vesicles should be targeted the pathological area to provide the maximum therapeutic efficacy. Biomimetic Vesicles have been suggested to besafe vesicular systems with higher chemical stability. These potential carriers have received attention for their potential as drug delivery vehicles due to advantages like higher flexibility, better bioavailability, increased efficacy, and therapeutic index.[5,6] Bioavailability of drug encapsulated in vesicles can be enhanced by encapsulating the drug along with bio potentiators of herbal origin. The co-administration of bioenhancers like Genistein and Quercetin with Irinotecan Hydrochloride inhibiting the P-glycoprotein and cytochrome p-450 enzymes enhances the efficacy of drug, makes drug more effective against cancer and transporter inhibitors like Genistein increases the intracellular drug accumulation and restores the chemosensitivity.[7,8] Irinotecan HCL is an antineoplastic drug of topoisomerase I inhibitor. It is semisynthetic derivative of camptothecin, an alkaloid extract from plants such camptotheca acuminata or is chemically synthesized.It acts by inhibiting topoisomerase I. Irinotecan HCL is a standard chemotherapeutic agent which exhibits a dose dependent toxicity. The most common problem encountered with Irinotecan is the development of resistance to tumors. Relatively small increase in drug resistance in cancer cells is thus sufficient to render the drug ineffective. Hence there is a need to improve its acceptability by

minimizing the intensity of side effects and thus increasing the therapeutic efficacy of the drug. The aim of the present study was to utilize the principles of Vesicular Drug carriers to formulate a sustained release system for Irinotecan alone and along with Efficiency Improvers (a mixture of Genistein and Quercetin) by thin film hydration technique such that an increased entrapment with prolong the release of drug from Carriers and also provided better stability to the formulation.

MATERIALS AND METHODS

Materials: Irinotecan, cholesterol, dicetylphosphate (DCP) were purchased from Sigma Aldich, Bangalore. Span 40(surfactant) was obtained from Lobachemie Pvt. Ltd. (Mumbai, India). Methanol, hydrochloric acid and chloroform were obtained from Merck India Ltd, (Mumbai, India). Potassium dihydrogen phosphate were obtained from HiMedia Laboratories Pvt, Ltd. (Mumbai, India). Genistein extract and Quercetin extracts were obtained from Green Grover's Pvt Ltd. (Bangalore, India). Sodium chloride, sodium hydroxide and disodium hydrogen phosphate were obtained from CDH Laboratory Ltd. (Delhi, India). All chemicals used were of analytical grade.

Methods

1. Preparation of Irinotecan Loaded Biomimetic Vesicles alone and along with Herbal efficiency improvers: Biomimetic vesicles of Irinotecan HCL alone and along with bioavailability potentiators were prepared by thin film hydration technique using rotary flash evaporator as described method of Bangham, reported by Juliano and Daoud[9] . Accurately weighed quantity of cholesterol, span 40 and DCP were dissolved in minimum quantity (about 3 ml) of a mixture of chloroform: methanol (2:1) in a 250 ml round bottom flask[10,11]. Round bottom flask was then attached to a rotary evaporator. The organic solvent mixture was evaporated in a rotary flash evaporator under a vacuum of 25 inches of Hg at 60 ± 2ºC and the flask rotated at 100 rpm until a very thin, smooth and dry film of surfactant was formed on the inner surface of the flask, The dry lipid film was slowly hydrated with 5 ml phosphate buffer saline (PBS) of pH 7.4 containing 10 mg Irinotecan drug alone and with 10 ml PBS pH 7.4 containing 10 mg Irinotecan drug and accurate quantity of bioenhancers at a temperature of 60 ± 2 ºC for a period of 1h. It formed homogenous suspension of multilamellar vesicles (MLVs). TheMLVs suspension was sonicated to form small unilamellar vesicles (SUVs) of niosomes by using probe sonicator in a ultrasonic bath. The final Vesicular suspension was further hydrated at 4ºC for overnight to stabilize the

formulation. The amount of span 60, cholesterol and bioenhancers to be loaded was selected on the basis of entrapment efficiency of the vesicles. The compositions of different formulation of niosomes are given in Table 1a and 1b.

2. Characterization of drug loaded vesicles alone and along with Bio Potentiators

2.1. Entrapment efficiency

Encapsulation efficiency of vesicles was estimated after disruption of vesicles in methanol and NaOH. For determination of entrapment efficiency, non entrapped drug in the Vesicular formulation was separated using centrifugation at 10,000 rpm for 1 h at 4 ºC. The supernatant contains non entrapped Irinotecan HCL was removed and the remaining pellet in the centrifuge tube resuspended in 0.1 N sodium hydroxide (as Irinotecan is highly soluble in 0.1 N NaOH) and vortexed thoroughly for 3 min. After vortexing 1 ml of the suspension was taken in a micropipette and transferred to a test tube. To this added 5 ml of methanol and was further vortexed for 2 min. The absorbance of resulting solution was measured using a shimadzu UV Spectrophotometer (1700) at 341 nm after suitable 10 dilution with methanol.

2.2. *In Vitro* release study

In vitro release of Irinotecan HCL from vesicles was investigated by dialysis method.A volume of 1ml Vesicular dispersion (encapsulation efficiency: 59.9%) was put in a dialysis bag (MWCO 12,000 Da,Sigma-Aldrich, USA.). The dialysis bag was suspended in 50 ml phosphate buffer pH 7.4 and maintained at 37±0.2°C. The medium was stirred continuously at 100 rpm during the release study. At predetermined time intervals of 15 min,30 min, 45 min, 60 min, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h, 5 ml, aliquots were sampled and replaced with 5 ml fresh phosphate buffer pH 7.4. The concentration of Irinotecan was determined by the UV spectrophotometer (Shimadzu UV 11,12 1700) at 341 nm.

RESULT AND DISCUSSION

Vesicular systems of Irinotecan HCL without and with bio potentiators such as Genistein and Quercetin were prepared by thin film hydration method by using span 40 and cholesterol as a surfactant. They are optimized on the basis of observation and maximum percentage drug entrapment (PDE).Entrapment efficiency The optimization of Biomimetic Vesicles of Irinotecan HCL without bioenhancers depends on the basis of entrapment efficiency. The entrapment efficiency was varied as concentration of surfactant varied. The amount of span 40 was increased by keeping drug and cholesterol concentration constant. As the amount of span 60 increased, the PDE of drug was also increased upto the formulation R6 (1:15 ratio of

drug and span60) and further increasing the amount of span 60 did not change encapsulation efficiency. The higher entrapment of span 40 may be due to their high phase transition temperature and hydrophobic in nature. The amount of cholesterol was increased by keeping drug and surfactant ratio constant. The ratio 1:15:2 gave highest encapsulation efficiency due to stabilizing effect of cholesterol. The cholesterol in Biomimetic vesicular systems greatly affects the membrane properties of the bilayers by reducing the rotational freedom of hydrocarbon chains. Cholesterol also eliminates the gel to liquid phase transition of the vesicle bilayers and induces permanent transition of the gel-state bilayer to an ordered liquid crystalline state. Both these mechanisms make the bilayers more stable leading to increase in entrapment efficiency. Further increase in cholesterol concentration, decreased the fluidity of the bilayers by filling empty spaces among the surfactant molecules and results of the membrane become more rigid and ultimately decreased the encapsulation efficiency (Table 2a). Optimization of Vesicles of Irinotecan with bio potentiators was done on the basis of entrapment efficiency. The formulation in which bio potentiators were added (10mg of genistein and 15 mg of Quercetin) produced a uniform dispersion with lower drug entrapment. Further increase in Genistein concentration (50mg) by keeping Quercetin concentration constant (15mg) produced a uniform suspension with an acceptable PDE. Further increase in concentration of Quercetin (>15mg)reduce the entrapment efficiency. So the formulation BP 2 containing genistein (10mg) and Quercetin (15 mg) as a bioenhancers was found to be an optimized formulation which gave highest drug entrapment (55.1±0.49 %) (Table 2b).

In Vitro Release Study: The invitro release study revealed that the release of the drug was sustained on encapsulation in Vesicles. The free drug released approximately 98.89% of the drug within 60 min whereas the same percentage of drug release from Vesicles of irinotecan HCL was occurring at the end of 11 h. Release of Irinotecan from Vesicles was biphasic with an initial faster release for 3 h followed by a period of slow release. Thus, the study revealed that initially there was a high rate of drug release, which may be due to the release of the adsorbed drug from the lipophilic region of Vesicle carriers which help to achieve the optimum loading dose. The drug diffuses slowly after 3 h due to the presence of cholesterol in the formulation which affects the fluidity by making it more rigid. As the amount cholesterol increased, they filled the pores of vesicular bilayers and abolished the gel-liquid phase transition of the niosomal systems. This confirms that addition of cholesterol acted as a membrane stabilizing agent that decreased the permeability and helped to sustain the release.

The maximum release of drug from Vesicles containing Irinotecan along with bioenhancers was 60.97% at the end of 12 h. The reason for slower release of the drug from Irinotecan HCL encapsulated complex may be the interaction of complex with the lipid/surfactant bilayers and biopoentiators.These results indicate that the release of Irinotecan HCL followed a sustain release pattern (Figure: 1).

Table. 1a: Composition of different formulation of Vesicles of Irinotecan HCL without bioenhancers.

Batch Name	Irinotecan HCL (mg)	Span 40 (mg)	Cholesterol (mg)	DCP (µmol)	Organic Solvent (ml)	Hydration volume (ml)
R1	10	50	10	7	3	4
R2	10	75	10	7	3	4
R3	10	100	10	7	3	4
R4	10	125	10	7	3	4
R5	10	150	10	7	3	4
R6	10	175	10	7	3	4
R7	10	150	20	7	3	4
R8	10	150	30	7	3	4

Table. 1b: Composition of different formulation of Vesicles of Irinotecan HCL along with EfficiencyImprovers.

Batch Name	Irinotecan HCL (mg)	Span 40 (mg)	Cholesterol (mg)	Genistein (mg)	Quercetin (mg)	DCP (µmol)	Organic Solvent (ml)	Hydration volume (ml)
BP1	10	150	20	5	10	7	3	10
BP2	**10**	**150**	**20**	**10**	**15**	**7**	**3**	**10**
BP3	10	150	20	20	15	7	3	10
BP4	10	150	20	30	15	7	3	10
BP5	10	150	20	40	15	7	3	10
BP6	10	150	20	50	20	7	3	10
BP7	10	150	20	50	30	7	3	10

Table. 2a. Optimization Of Vesicles of Irinotecan without bioenhancers.

Batch name	Observation	%Drug entrapped
R1	Flaking	20.17±1.667
R2	Flaking	26.89±1.025
R3	Flaking	37.49±1.351
R4	Flaking	43.35±0.920
R5	Uniform dispersion	50.73±0.714
R6	Non-uniform dispersion	23.35±0.840
R7	**Uniform dispersion,without flaking**	**56.9±1.33**
R8	Uniform dispersion,lower PDE	50.85±0.818

*Data are expressed as Mean±SD. SD = Standard Deviation

Table. 2b: Optimization of vesicles of Irinotecan with bioenhancers.

Batch Name	Observation	%Drug entrapped	%Genistein entrapped	% Piperine entrapped
BP1	Flaking	18.90±0.21	27.66±0.66	10.05±0.36
BP2	**Uniform dispersion**	**55.1±0.49**	**30.30±0.67**	**60.11±0.96**
BP3	Flaking	25.89±0.44	32.78±0.12	24.30±0.64
BP4	Uniform dispersion	27.67±0.62	35.94±0.51	32.10±0.26
BP5	Uniform dispersion	32.24±0.51	39.01±0.21	39.00±0.82
BP6	Non Uniform Dispersion	36.09±0.51	36.02±0.51	33.89±0.51
BP7	Non-uniform Dispersion	49.89±0.53	33.87±0.54	29.00±0.38

Table: 2c *In vitro* release of pure drug Irinotecan.

Time (min)	Free methotrexate (percent Drug release)*
10	20.21±0.6
20	34.80±1.9
30	56.39±0.3
40	79.19±1.6
50	99.77±0.9

*Data are expressed as Mean ±SD. SD = Standard Deviation (n=3).

Table 2d: *In vitro* release profile of Vesicular formulation of Irinotecan HCL alone and along with Bioenhancers.

Time (min)	Biomimetic Vesicles containing Irinotecan*	Biomimetic Vesicles containing Irinotecan along with bioenhancers*
0	0	0
1	27.71±1.19	10.49±1.00
2	33.79±1.27	20.57±0.15
3	55.56±0.07	29.46±0.11
4	55.65±0.24	34.63±0.59
5	67.01±0.77	46.14±0.72
6	69.92±0.06	48.62±0.32
7	72.68±0.57	52.67±0.21
8	74.81±1.67	54.68±0.01
9	88.4±0.40	54.71±0.23
10	93.57±0.16	59.7±0.12
11	98.55±0.22	60.37±0.49
12	99.77±0.10	60.11±0.66

*Data are expressed as Mean ±SD. SD = Standard Deviation (n=3).

CONCLUSION

The use of various pharmaceutical nanocarriers has become one of the most important areas of nanomedicine. Vesicles of drug alone and along with bioenhancers such as Genistein and Quercetin were prepared by thin film hydration method by using surfactant span 40 and

cholesterol that were optimized on the basis of entrapment efficiency. The in vitro study revealed that the release pattern of the drug was sustained in Carriers and it was further significantly sustained by addition of bioenhancers. Further *in vivo* and stability studies can be performed to see the pharmacological activity as well as the stability of the formulation because the stability of Vesicular systems is a great issue and a major challenge in commercializing the formulations.

ACKNOWLDEGEMENTS

The authors are expressing their sincere thanks to Malik Deenar College of Pharmacy for performing the research work.

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