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Research Article

Antitumor efficacy of Folic Acid conjugated Polymeric Nanoparticles of SN-38 after oral delivery

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ABSTRACT

The objective of the present research was to develop folic acid conjugated polymeric nanoparticles (FCsPNP) and to investigate its therapeutic effectiveness in xenograft Colon tumor models after oral delivery. Chitosan coated PLGA nanoparticles (CsPNP) were prepared by polyelectrolyte complexation method and it was further conjugated with Folic acid. Optimized formulation was investigated for particle size, zeta potential, polydispersity index (Pdl), % entrapment drug loading and *in vitro* release. The morphology was observed by SEM and TEM images. Tumor regression studies were conducted on Balb/c mice implanted with Colo-26 cells. FCsPNP were successfully prepared and optimized. *In vitro* parameters viz. Particle size, Zeta potential, Pdl were found to be optimum. The *in vitro* % release is directly correlated with the nature of polymers and folate conjugation. *In vivo* tumor regression studies found the formulations to be less toxic than Irinotecan hydrochloride (IHCl). CsPNP and FCsPNP were successfully prepared and evaluated for antitumor efficacy after oral delivery. FCsPNP were more effective in the colon tumor treatment and found to be less toxic than IHCl thus making it a potential drug delivery candidate for future anticancer therapy.

Keywords: Nanoparticles, Xenograft colon tumor, Chitosan, Irinotecan hydrochloride**Article Info:** Received 09 June 2019; Review Completed 19 July 2019; Accepted 20 July 2019; Available online 15 August 2019

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INTRODUCTION

Colorectal cancer is third widely diagnosed cancer in the whole world¹. Effective chemotherapy is still the need of hour as in spite of so many researches the side effects and toxicity of chemotherapeutic agents are still prevailing in the cancer patients. Multifunctional nanoparticles have reached many milestones in the field of anticancer research^{2,3}. Polymeric nanoparticles have shown excellent therapeutic efficacy in colorectal cancer treatment^{3,4}. Because of the numerous advantages of nanoparticles they are mainly used for controlled delivery of drugs and imaging agents and have attracted remarkable interests in recent years^{5,6}. Drugs are protected from being degraded during *in vivo* by these nanoparticles. They provide targeted and controlled drug release and are capable of accumulating in colon tumor by active and passive targeting mechanisms. However, after intravenous administration nanoparticles are often eliminated rapidly by macrophages present in the reticulum endothelial system (RES) which are located mainly in the liver and spleen and which leads to short circulation half-life

after intravenous administration^{7,8}. Oral chemotherapy is not only convenient but can also greatly improve the quality of life of the patients. This is especially important for the patients with advanced or metastatic cancer. Oral chemotherapy can eventually start a new concept of chemotherapy: 'chemotherapy at home'⁹⁻¹¹. In the present research polymeric nanoparticles are developed by polyelectrolyte complexation method using a cationic polymer (Chitosan) and an anionic polymer (PLGA). Chitosan is a biodegradable and versatile polymer which is soluble in acidic media and is used for colonic delivery. These are hydrophilic, natural and positively charged polymer which interacts with negatively charged polymers, polyanions and macromolecules. Polyelectrolyte complexation is a method for the formation of nanoparticles and it has a potential for oral controlled release^{12,13}. These nanoparticles are better than other synthetic nanoparticles as they are more stable, have simple preparation methods, low toxicity and can be given by versatile routes of administration¹²⁻¹⁴. PLGA (Poly(lactide-co-glycolide)) is biodegradable polymer widely used for the formation of polymeric nanoparticles as they

can provide a solution to adjuvant therapy and may provide a better efficacy and fewer side effects. It is a frequently-used drug delivery carrier for nanoparticulate systems¹⁵⁻¹⁷. Biodegradable polymer can be used as carriers for drug and for the preparation of biodegradable NPs. Other advantages of biodegradable NPs include lesser frequency of administration, less systemic side effects, sustained drug release and reduced dosage^{7, 15, 18}. PLGA is approved for human use by the USFDA as implantable devices, surgical sutures and drug delivery systems. Chitosan and PLGA are applied as Pgp (Para glyco protein) inhibitors and are also used as drug carriers^{19,20}. SN-38 (7-ethyl-10-hydroxycamptothecin) is 1000 fold more cytotoxic than CPT-11. It has anticancer activity against colon cancer, lung cancer, cervical cancer and pancreatic cancer. It is the active metabolite of Irinotecan (CPT-11), which is a topoisomerase I inhibitor and is available commercially as Camptosar®²¹⁻²³. It has poor aqueous solubility and poor oral bioavailability (8%)²⁴. It is formed in the liver and tumors from CPT-11 but its metabolic conversion rate is less than 10% of the original volume of CPT-11²⁵. For increasing the hydrophilicity, bioavailability and for providing sustained action various approaches like, Liposomes, Polymeric micelles, Nanoparticles and Polymer drug conjugates were prepared but most of these formulations were given by intravenous administration although oral administration is chosen by 89% patient³. Folic acid is widely used as a targeting ligand to deliver the polymeric nanoparticles (NPs) into cells primarily via receptor-mediated endocytosis^{26,27}. Folate-receptor-targeted SN-38 NPs was produced earlier using PLGA NPs covalently bound to Poly (ethylene glycol)-folate, which demonstrated more cytotoxicity on HT-29 cells as compared to control NPs²⁸⁻³⁰. These nanoparticles were given via the intravenous route and showed that the bioavailability of SN-38 was significantly increased^{24,31}. However, oral delivery of nanoparticles is more practical as compared with the intravenous route because of greater convenience, cheaper cost, better patient compliance and further simplicity for long-standing treatment of chronic diseases^{9,32-34}. The aim of present study is to determine the potential application of Folate conjugated chitosan coated PLGA NPs for improving the oral absorption of SN-38 and to show its therapeutic effectiveness against solid colon tumor.

MATERIAL AND METHODS

Material

SN-38 was obtained from Avra Labs (Hyderabad, India). PLGA (50:50) (Resomer®) was obtained from Evonik Industries (Mumbai, India). All other solvents and chemicals are of analytical grade purchased from Hi Media Labs (Mumbai, India).

Methods

Preparation of Chitosan coated PLGA nanoparticles (CsPNP)

CsPNP was prepared by polyelectrolyte complexation method as given by Prasad et al²². Briefly, Pluronic F 68 was added to chitosan and magnetically stirred at room temperature³⁵. This dispersion is added to SN-38 dispersed in PLGA solution with a syringe and was stirred overnight. Then it was centrifuged, sonicated, sediment was washed and lyophilized. The nanoparticles thus formed was preserved at 2-4°C till further use.

Preparation of folic acid (FA) conjugated CsPNP (FCsPNP)

5mg of FA and 4 ml of NPs suspension (5mg/ml, distilled water used as solvent) were co-mixed in the presence of 10

mg of (Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) EDC and 19 mg of (N-hydroxy Succinamide) NHS and stirred at room temperature under dark condition for 2 hr and a yellow FA-NPs suspension was obtained^{36,37}. Then it was centrifuged, sediment washed and lyophilized for 48 hrs.

In Vitro Characterization

Particle size, polydispersity index (Pdl) and zeta potential

The mean particle size and PDI were determined by particle size analyzer (SALD 2201, Shimadzu) and zeta potential was determined by Zetasizer (Nano ZS 90, Malvern Instruments).

In vitro release in simulated gastrointestinal fluids

The *in vitro* release studies were conducted by a dialysis membrane method with modification²². The membrane was soaked in double-distilled water for about 12 h, prior using the membrane. SN-38 containing CsPNP were put inside 100 ml of Simulated gastric fluid (SGF), Phosphate buffer saline (PBS) (pH 7.4), Simulated intestinal Fluid (SIF) and in Simulated Colonic Fluid (SCF) for 48 hrs after enclosing in a dialysis bag (MWCO 8 kDa, Hi Media, Mumbai). The tests were conducted at 37°C ± 1°C in a constant temperature shower mixer at 100 rpm. The amount of SN-38 released was measured by HPLC, C-18 reverse phase (Young lin instrument, # 899-6, Korea) at a wavelength of 265 nm²¹. The Cumulative % release in SGF, SIF, SCF and PBS is shown in Fig. 2.

Electron microscopic images

Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) images evaluate the size and shape of optimized batch of nanoparticles. TEM images were taken by FEI Philips Morgagni 268D (2, 80,000x). TEM images are shown in Fig. 3. The shape and surface morphology of blank CsPNP and drug loaded CsPNP were investigated using scanning electron microscopy (SEM). The samples photomicrographs were taken with a SEM (Jeol JSM-1600, Tokyo, Japan). The SEM images of blank CsPNP and SN-38 loaded CsPNP and FCsPNP are given in Fig. 4.

Entrapment efficiency and drug loading

CsPNP and FCsPNP (0.25%w/v) was dispersed in dichloromethane. To the above solution acetonitrile (5ml) was added and centrifuged for 20 minutes. After dilutions the supernatant was analyzed for SN-38 at a wavelength of 265 nm with HPLC²¹.

$$\text{Entrapment efficiency\%} = \frac{\text{Wt of SN 38 calculated}}{\text{Wt of SN 38 added}} \times 100$$

$$\text{Drug loading efficiency} = \frac{\text{Wt of SN 38 calculated}}{\text{Wt of nanoparticles}} \times 100$$

In Vivo Characterization

Tumor regression studies on male Balb/c mice

All *in vivo* studies were performed in accord with the study protocol as approved by the Institutional Ethical Committee, SLT Institute of Pharmaceutical Sciences, 994/a/GO/06/CPCSEA, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India. The studies were conducted on male Balb/c mice 6-7 week old obtained from ACTREC, Tata Memorial Centre, Navi Mumbai, India. Four groups (n=6) were taken. The animals were housed 15 days before the experiment and fed the usual commercial diet and water *ad*

libitum. All mice were pre weighed before experiment. Tumor was induced in the mice by subcutaneously injecting Colon-26 cells (1×10^7 cells) as per the protocol of Morton *et al* ³⁸. Treatment started when the implanted tumour reaches nearly 100mm³ in volume as measured by Vernier callipers as per the following formula,

$$\text{Tumor volume} = \frac{A \times (B)^2}{2}$$

Where A and B are length and width of the tumor, respectively. 1st group was control group to which Normal saline solution (0.9%w/v) was given. 2nd, 3rd and 4th group were given FCsPNP, CsPNP and Irinotecan hydrochloride in solution form respectively, for 30 days orally at a dose of 80 mg/Kg. Tumor volume was measured every 3-5th day. Body weight, clinical signs and mortality were observed during these 30 days. Animals which showed toxicity by clinical signs and body weight loss of 25% or more were considered as moribund and euthanized immediately.

Statistical Analysis

Statistical analysis was performed with Graph Pad prism 7.01. The mean data from different formulations were compared by one way ANOVA. Differences were considered significant when $P < 0.05$.

Results

Optimization of CsPNP and FCsPNP and *in vitro* characterization

The formulation having 1.43% (w/v) polymer concentration, 0.21% (w/v) surfactant concentration, and 7.25min sonication time is chosen as optimized formulation and prepared accordingly²². Particle size, entrapment efficiency, polydispersity index (PDI), zeta potential (ZP) and drug loading of CsPNP and FCsPNP are given in Table 1. Ultrasonication resulted in smaller nanoparticles. The magnetic stirring speed was optimized at 1200 rpm for 5hrs. 0.25 % (w/v) of cationic polymer (chitosan) was kept fixed. FCsPNP were prepared by a method as given by Yang *et al* with modifications⁴⁰. Folic acid was added in the suspension with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride in the ratio of 1:3 after optimization of EDC concentration. Conjugation efficiency was determined and found to be 73.3 ± 2.1 % which show that folic acid is conjugated accurately with CsPNP. The schematic diagram of Folic acid conjugation with the polymers is shown in Fig.1.

Electron microscopy

TEM helps in the evaluation of size and shape of the optimized batch of nanoparticles. TEM images of the CsPNPs showed spherical shape of nanoparticles, non aggregation with narrow size distribution and without clumping of nanoparticles. Blank CsPNP were found spherical with nanocapsular structure and SN-38 loaded CsPNP were found having structures of nanospheres with matrix inside. The nanoparticles were in the size range of 90-200 nm as observed in the electron microscopic images. It shows that drug loaded nanoparticles were having a coating of chitosan with a matrix of SN-38 and PLGA inside. The particles were clearly visible with spherical structures in nanometre range. Surface morphology of blank CsPNP and drug loaded CsPNP were visualized by SEM images. It depicted rough surface of CsPNP. Rough surface is due to chitosan in the outermost layer of nanoparticles. It depicted the formation of nanoparticles in the range of 200-400 nm and with a rough surface. TEM images of FCsPNP were taken at a magnification of 40,000X and it was in the range of 70-180 nm. The images (Fig. 3) clearly showed bilayer structure, one

of chitosan and other of folic acid. SEM images (Fig. 4) were taken at magnification of 20,000X and it depicted spherical FCsPNP with somewhat smoother surface as compared to CsPNP, which reveals that the rough surface due to chitosan was covered with smooth layer of Folic acid.

In vitro drug release

Drug release studies in simulated gastrointestinal fluids are done to simulate the human body metabolic activity in gastrointestinal fluid and colonic fluid. The release study of SN-38 from CsPNP was conducted for 96 hrs in simulated gastrointestinal fluids. There was negligible release of SN-38 in SGF. In SIF (pH 6.8) it released nearly 62.9% drug and in SCF the release was 72.5% in 24 hrs and 84.7% in 96 hours. In PBS (pH 7.4) CsPNP released nearly 31.1% drug in 72 hrs. However, pure SN-38 released only 7.4 % drug in 72 hrs. The drug release for FCsPNP was conducted in Phosphate buffer saline (pH 7.4) for 72 hrs by dialysis membrane method. It is represented graphically in Fig. 2. It was found to release 20.1% in 24 hrs and 22.1% in 72 hrs.

In vivo characterization

Tumour regression studies

The tumor regression studies were conducted on xenograft colon tumor models of Balb/c mice transplanted with Colon 26 cells. The photographs of colon tumor cells transplanted mice on 1st day and last day is shown in Fig.6. The percentages of survival of different groups are shown in Fig. 5(A) and data shown in Table 2. It showed that plain aqueous solution of Irinotecan hydrochloride was more toxic as compared to other treated and untreated groups and the survival rate reached 67% on 28th day. Models treated with FCsPNP showed 100 % survival. CsPNP significantly showed better survival rate (83 %) on 28th day as compared to the positive control group. Tumor volume data and graph is given in Table 3 and depicted in Fig. 5(B) respectively. There was maximal control of tumor growth in the group which was treated with FCsPNP (Tumor volume reached 2.53 cm³) as compared to control groups (positive and negative) and CsPNP group. The animal weights taken at different time points are shown graphically in Fig. 5(C) and the data is given in Table 4. A weight loss ≥ 4 gm is considered to be toxic. No animal showed a weight loss of ≥ 4 gm and but there are significant differences in the animal weights of CsPNP and FCsPNP when compared with the positive and negative control groups ($P < 0.05$). There is increase in the weight of control group due to uncontrolled tumor growth. In the treated groups 4th group treated with IHCl had significantly more weight loss as compared to 2nd and 3rd group.

Graph of T/C values from RTV data of different groups with respect to days

Relative tumor volume (RTV) was calculated as follows,

$$\text{RTV} = \frac{\text{Tumor volume on day of measurement}}{\text{Tumor Volume on day 1}}$$

It shows that FCsPNP significantly have maximum control on tumor growth and minimum on treatment with IHCl solution ($P < 0.05$). The data of T/C values is depicted graphically in Fig. 5 (D) and given in Table 5. Antitumor activity is seen when T/C values ≤ 0.4 . The data shows that FCsPNP is showing antitumor activity during the study period. However, CsPNP and IHCl is successful in controlling the tumor growth up to some extent (T/C values is nearly equal to 0.9).

Discussions

Polyelectrolyte complexation is a novel method for the formation of chitosan based nanoparticles⁴¹. This method is economic and involves negligible use of organic solvents. Chitosan is a biodegradable polymer and is mainly used for colonic delivery. These are hydrophilic, natural and positively charged polymer which interacts with negatively charged polymers, polyanions and macromolecules. These nanoparticles are better than other synthetic nanoparticles as they are more stable, have simple preparation methods, low toxicity and can be given by versatile routes of administration. Poly lactic co glycolic acid (PLGA) is biodegradable polymer widely used for the formation of polymeric nanoparticles as they can provide a solution to adjuvant therapy and may provide fewer side effects and better efficacy^{42,43}. Polymeric nanoparticles can incorporate both hydrophilic and lipophilic drug and improves the oral delivery of those drugs having poor chemical, enzymatic or metabolic stability and permeability and releases the drug at a specific site^{44,17}. Stability of nanoparticles is indicated by high zeta potential (positive or negative) as particles which have high charge are dispersed and less aggregated and thus have lesser Pdl^{45,46}. Poly dispersity index symbolises the homogeneity of dispersions. For a homogeneous suspension it should be less than 1⁴⁷. Here it was found to be homogeneous in nature. Positive zeta potential is due to the concentration of chitosan being more than PLGA. Targeting moieties or ligands are very often used to specifically target the receptors of tumor cells and deliver them within cells by receptor mediated endocytosis. Folic acid (FA) is widely preferred in the delivery of anticancer agents due to its non immunogenic nature, small size, low cost and high tumor tissue specificity⁴⁸. Colorectal tumor cells are devoid of folic acid so folic acid linked nanocarriers have comparatively high binding affinity to folate receptors expressed on tumor cells⁴⁰. EDC acts as a catalyst for the coupling reaction between the carboxyl group of folic acid and free amino group of chitosan. The results of drug loading and entrapment efficiency show that conjugation with folic acid does not interfere with drug loading and entrapment efficiency. Zeta potential of FCsPNP is less than that of

CsPNP. It is due to the anionic nature of folic acid which compensates the positive potential of chitosan and decreases the overall zeta potential. Particle size of FCsPNP increased due to the conjugation with folic acid. However, for oral administration the size range of 200-400 nm is required to protect the particles from Pgp (Para-glycoprotein) efflux pump²⁰. As these particles are in the range and are hence prevented from Pgp efflux pump. The pattern of *in vitro* drug release by CsPNP shows sustained release action and there is very less release of SN-38 from FCsPNP in PBS (pH 7.4) due to poor solubility of lactone form of SN-38 in PBS. Guo and his team²⁰ also found that the cumulative release percentages of SN-38 from chitosan nanoparticles in 24 hrs is 27.03% and it had very slow and sustained release in PBS at pH 7.4 during 7 days study. It also shows that CsPNP has more potential for drug release than pure SN-38 in PBS, but lesser in simulated colonic fluid. This pattern was sustained release and favoured the aim to target the drug for colon cancer by oral route. Antitumor efficacy data revealed that no animal showed a weight loss of ≥ 4 gm and there is not any significant change in the animal weight as compared to the initial weight when compared within the groups. When compared in between the groups there was significant difference in the weights of animals of the treated group and positive and negative control groups. It showed that the formulations are not showing any side or toxic effects to the animals. However, IHCl was found to be more toxic than FCsPNP and CsPNP according to the changes in weight of the animals. Relative tumor volume data shows that the control on tumor growth is predominantly shown during the treatment with FCsPNP as compared to CsPNP and IHCl. It is due to the targeting efficacy of folic acid to the folate receptors. Antitumor Activity is seen when T/C values ≤ 0.4 . The results show that FCsPNP is showing antitumor activity during the study period whereas CsPNP is successful in controlling the tumor growth up to some extent (T/C values is nearly equal to 0.5). Earlier studies on SN-38 oral delivery were limited to *in vitro* cytotoxicity studies^{20,49,50}. The *in vivo* studies of the present research shows antitumor efficacy after oral delivery of polymeric nanoparticles of SN-38.

Table 1: *In vitro* evaluation parameters of CsPNP and FCsPNP (n=3, Data represents Mean $\pm$ S.D, P<0.05)

Formulation code	Particle size (nm)	Zeta potential (mV)	Drug entrapment efficiency (%)	Drug loading (%)	Pdl
CsPNP	225.5 $\pm$ 3.75	27.6 $\pm$ 0.173	78.3 $\pm$ 0.44	13.5 $\pm$ 0.4	0.157 $\pm$ 0.0036
FCsPNP	310 $\pm$ 2.5	15.6 $\pm$ 1.8	78.7 $\pm$ 1.6	13.6 $\pm$ 0.6	0.571 $\pm$ 0.03

Table 2: Percent survival of xenograft models at different time points for different groups

Weeks	Days	Control	FCsPNP	CsPNP	IHCl
0.00	1	100	100	100	100
0.71	5	100	100	100	100
1.29	9	100	100	100	100
1.71	12	100	100	100	100
2.14	15	100	100	100	100
2.57	18	100	100	100	100
3.00	21	100	100	100	100
3.57	25	100	100	100	67
4.00	28	83	100	83	67

Table 3: Tumour volume in cm³ of different groups treated with nanoparticles at different time points during 30 days study (n=6, Data represents Mean±S.D, P<0.05)

Week	Days	Control	FCsPNP	CsPNP	IHCl
		Tumour volume			
0.00	1	0.05±0.01	0.05±0.01	0.04±0.01	0.04±0.01
0.71	5	0.33±0.09	0.07±0.02	0.11±0.04	0.15±0.06
1.29	9	1.37±0.61	0.30±0.12	0.81±0.24	1.12±0.32
1.71	12	2.89±0.74	0.49±0.19	1.26±0.47	1.53±0.41
2.14	15	3.60±0.71	0.93±0.46	1.93±0.84	2.49±0.38
2.57	18	5.09±0.87	1.37±0.56	2.38±0.84	3.19±0.51
3.00	21	6.07±1.00	1.75±0.72	3.18±0.75	3.95±0.56
3.57	25	6.91±0.78	2.32±0.89	4.17±0.96	4.37±0.52
4.00	28	7.77±1.55	2.53±0.77	4.38±0.75	4.66±0.41

Table 4: Animal weight in grams at different days during treatment period of 30 days (n=6, Data represents Mean±S.D, P<0.05)

Weeks	Days	Control	FCsPNP	CsPNP	IHCl
0.00	1	22.9±0.5	22±0.8	23.1±0.7	22.0±0.6
0.71	5	23.1±0.4	21.0±0.9	22.8±0.3	19.7±0.7
1.29	9	22.5±0.6	21.1±0.	23.1±0.2	19.5±0.2
1.71	12	22.7±0.4	20.8±0.4	22.9±0.8	19.3±0.6
2.14	15	22.6±0.1	19.1±0.6	22.2±1.0	19.0±0.5
2.57	18	23.0±0.2	19.6±0.9	21.6±0.8	18.8±0.4
3.00	21	23.2±0.5	19.4±1.0	21.4±0.9	18.7±0.3
3.57	25	23.5±0.7	19.6±0.8	21.4±0.7	18.5±0.8
4.14	29	24.2±0.3	20.5±0.3	21.9±0.2	18.6±0.4

Table 5: Data of T/C values of different groups with respect to days

Weeks	Days	FCsPNP	CsPNP	IHCl
	1	1	1	1
0.00	5	0.238572	0.510278754	0.692171
0.71	9	0.273985	0.919423793	1.291801
1.29	12	0.273015	0.907288638	1.095923
1.71	15	0.317109	0.886828463	1.080931
2.14	18	0.330839	0.758162939	1.006423
2.57	21	0.3586	0.833361711	1.021854
3.00	25	0.408758	0.942047971	1.047742
3.57	28	0.385722	0.892929536	0.955581

Figure 1: Schematic diagram of conjugation of folic acid with CHI-PLGA Polyelectrolytic complex

Figure 2: Cumulative % release of SN-38 A) from CsPNP in simulated gastric fluids B) from CsPNP and SN-38 in PBS (pH 7.4) and C) from FCsPNP in PBS (pH 7.4)

Figure 3: TEM images of A) Blank CsPNP B) SN-38 loaded CsPNP and C) FCsPNP

Figure 4: SEM images of A) Blank CsPNP B) SN-38 loaded CsPNP and C) FCsPNP

Figure 5: Graphs of A) % survival of animals at different time points B) Tumor volume in cm^3 of different groups treated with nanoparticles with respect to days C) Average animal weight in grams with respect to days taken at different days during 30 days study and D) T/C values from RTV data of different groups with respect to days (n=6, Data represents Mean $\pm$ S.D)

Figure 6: Photographs of Colon tumor xenograft mice of different groups on 1st day and last day of treatment

CONCLUSIONS

Polymeric nanoparticles and targeted nanoparticles of SN-38 were successfully prepared and evaluated for *in vitro* and *in vivo* characterization. *In vitro* drug release studies in simulated fluids showed sustained action of CsNP and targeted action of FCsNP. The relation between formulation additives and product performance was elucidated by drug release studies. The formulations were found to be less toxic and effective in treatment of colorectal cancer. Thus polymeric nanoparticles prepared by polyelectrolyte complexation method are a viable approach for oral chemotherapy.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

- Hamzehzadeh L *et al.* New Approaches to Use Nanoparticles for Treatment of Colorectal Cancer; a Brief Review New Approaches to Use Nanoparticles for Treatment of Colorectal Cancer; A Brief Review. *Nanomed Res J* 2016; 1(2): 59–68. doi:10.7508/nmrj.2016.02.001.
- Torchilin VP. Multifunctional nanocarriers. *Adv Drug Deliv Rev* 2006; 58(14): 1532–55. doi:10.1016/j.addr.2006.09.009.
- Yu MK *et al.* Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. *Theranostics* 2012; 2(1): 3–44. doi:10.7150/thno.3463.
- Ma Y. Controlled delivery of nanoparticles to the colon for tumour targeting. 2015. doi:10.14264/uql.2015.596.
- Na JH *et al.* Effect of the stability and deformability of self-assembled glycol chitosan nanoparticles on tumor-targeting efficiency. *J Control Release* 2012; 163(1): 2–9. doi:10.1016/j.jconrel.2012.07.028.
- Min KH *et al.* Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. *J Control Release* 2008; 127(3): 208–218. doi:10.1016/j.jconrel.2008.01.013.
- Venkataraman S *et al.* The effects of polymeric nanostructure shape on drug delivery. *Adv Drug Deliv Rev* 2011; 63(14–15): 1228–46. doi:10.1016/j.addr.2011.06.016.
- Van Vlerken LE *et al.* Poly(ethylene glycol)-modified nanocarriers for tumor-targeted and intracellular delivery. *Pharm Res* 2007; 24(8): 1405–1414. doi:10.1007/s11095-007-9284-6.
- Sharma S. Oral Chemotherapeutic Agents for Colorectal Cancer. *Oncologist* 2000; 5(2): 99–107. doi:10.1634/theoncologist.5-2-99.
- Neill VJO, Twelves CJ. Oral cancer treatment: developments in chemotherapy and beyond. 2002: 933–937. doi:10.1038/sj.bjc.6600591.
- Diasio BRB. Current Status of Oral Chemotherapy for Colorectal Cancer. *Oncology* 2001: 1–5.
- Patel MP *et al.* Chitosan Mediated Targeted Drug Delivery System: A Review. 2010; 13(3): 536–557.
- Dash M *et al.* Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci* 2011; 36(8): 981–1014. doi:10.1016/j.progpolymsci.2011.02.001.
- Taetz S *et al.* The influence of chitosan content in cationic chitosan/PLGA nanoparticles on the delivery efficiency of antisense 2'-O-methyl-RNA directed against telomerase in lung cancer cells. *Eur J Pharm Biopharm* 2009; 72(2): 358–69. doi:10.1016/j.ejpb.2008.07.011.
- Chronopoulou L *et al.* Colloids and Surfaces B: Biointerfaces PLGA-based nanoparticles: Effect of chitosan in the aggregate stabilization. A dielectric relaxation spectroscopy study. 2012; 97: 117–123.
- Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol((R))): PLGA nanoparticles containing vitamin E TPGS. *J Control Release* 2003; 86(1): 33–48. doi:10.1016/S0168-3659(02)00320-6.
- Xie X *et al.* PLGA nanoparticles improve the oral bioavailability of curcumin in rats: Characterizations and mechanisms. *J Agric Food Chem* 2011; 59(17): 9280–9289. doi:10.1021/jf202135j.
- Javadzadeh Y *et al.* Preparation and physicochemical characterization of naproxen-PLGA nanoparticles. *Colloids Surf B Biointerfaces* 2010; 81(2): 498–502. doi:10.1016/j.colsurfb.2010.07.047.
- Desai PP *et al.* Overcoming poor oral bioavailability using nanoparticle formulations - Opportunities and limitations. *Drug Discov Today Technol* 2012; 9(2). doi:10.1016/j.ddtec.2011.12.001.
- Guo M *et al.* Mechanisms of chitosan-coated poly(lactic-co-glycolic acid) nanoparticles for improving oral absorption of 7-ethyl-10-hydroxycamptothecin. *Nanotechnology* 2013; 24(24): 245101. doi:10.1088/0957-4484/24/24/245101.
- Prasad S, Dangi JS. Determination of SN-38 contents in nanocapsules by chromatographic and spectrophotometric methods. *World J Pharm Res* 2015; 4(09): 1227–1242.
- Prasad S, Dangi JS. Development and characterization of pH responsive polymeric nanoparticles of SN-38 for colon cancer. *Artif Cells, Nanomedicine Biotechnol* 2016; 44(8): 1824–1834. doi:10.3109/21691401.2015.1105239.
- Prasad S, Dangi JS. Optimization and Characterization of Biodegradable Polymeric. *Int J Pharm Pharm Sci* 2014; 6(SUPPL. 2): 685–689.
- Bala V *et al.* Lipophilic Prodrugs of SN38: Synthesis and in Vitro Characterization toward Oral Chemotherapy. *Mol Pharm* 2016; 13(1): 287–294. doi:10.1021/acs.molpharmaceut.5b00785.
- Hu ZP *et al.* Simultaneous determination of irinotecan (CPT-11) and SN-38 in tissue culture media and cancer cells by high performance liquid chromatography: Application to cellular metabolism and accumulation studies. *J Chromatogr B Anal Technol Biomed Life Sci* 2007; 850(1–2): 575–580. doi:10.1016/j.jchromb.2006.12.056.
- Whittum-hudson J, Panyam J. NIH Public Access. *Mol Pharm* 2013; 9(7): 612–626. doi:10.1021/mp2005388.Folic.
- Song H *et al.* Folic acid-chitosan conjugated nanoparticles for improving tumor-targeted drug delivery. *Biomed Res Int* 2013; 2013: 6. doi:10.1155/2013/723158.
- Ebrahimnejad P *et al.* Preparation and in vitro evaluation of actively targetable nanoparticles for SN-38 delivery against HT-29 cell lines. *Nanomedicine Nanotechnology, Biol Med* 2010; 6: 478–485. doi:10.1016/j.nano.2009.10.003.
- Garcia-Bennett A *et al.* In search of the Holy Grail: Folate-targeted nanoparticles for cancer therapy. *Biochem Pharmacol* 2011; 81(8): 976–984. doi:10.1016/j.bcp.2011.01.023.
- Boddu SH, Vaishya R. Preparation and Characterization of Folate Conjugated Nanoparticles of Doxorubicin using Plga-

- Peg-Fol Polymer. *Med Chem (Los Angeles)* 2012; 02(04): 68–75. doi:10.4172/2161-0444.1000117.
31. Bala V *et al.* Prodrug and nanomedicine approaches for the delivery of the camptothecin analogue SN38. *J Control Release* 2013; 172(1): 48–61. doi:10.1016/j.jconrel.2013.07.022.
 32. Jiao Y *et al.* In vitro and in vivo evaluation of oral heparin-loaded polymeric nanoparticles in rabbits. *Circulation* 2002; 105(2): 230–235. doi:10.1161/hc0202.101988.
 33. des Rieux A *et al.* Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. *J Control Release* 2006; 116(1): 1–27. doi:10.1016/j.jconrel.2006.08.013.
 34. Dinarvand M *et al.* Oral delivery of nanoparticles containing anticancer SN38 and hSET1 antisense for dual therapy of colon cancer. *Int J Biol Macromol* 2015; 78: 112–121. doi:10.1016/j.ijbiomac.2015.03.066.
 35. Hamman JH. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. *Mar Drugs* 2010; 8(4): 1305–1322. doi:10.3390/md8041305.
 36. Xu Q *et al.* Polymeric Carriers for Gene Delivery: Chitosan and Poly(amidoamine) Dendrimers. *Curr Pharm Des* 2010; 16(21): 2350–2368. doi:10.2174/138161210791920469.
 37. Wang Y *et al.* Chitosan-modified PLGA nanoparticles with versatile surface for improved drug delivery. *AAPS PharmSciTech* 2013; 14(2): 585–92. doi:10.1208/s12249-013-9943-3.
 38. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. *Nat Protoc* 2007; 2(2): 247–250. doi:10.1038/nprot.2007.25.
 39. Plengsuriyakarn T *et al.* Anticancer activity of Atractylodes lancea (Thunb.) DC in a hamster model and application of PET-CT for early detection and monitoring progression of cholangiocarcinoma. *Asian Pacific J Cancer Prev* 2015; 16(15): 6279–6284. doi:10.7314/APJCP.2015.16.15.6279.
 40. Yang SJ *et al.* Folic acid-conjugated chitosan nanoparticles enhanced protoporphyrin IX accumulation in colorectal cancer cells. *Bioconjug Chem* 2010; 21(4): 679–689. doi:10.1021/bc9004798.
 41. Hamidi M *et al.* Hydrogel nanoparticles in drug delivery. *Adv Drug Deliv Rev* 2008; 60(15): 1638–49. doi:10.1016/j.addr.2008.08.002.
 42. Chronopoulou L *et al.* Chitosan-coated PLGA nanoparticles: A sustained drug release strategy for cell cultures. *Colloids Surfaces B Biointerfaces* 2013; 103: 310–317.
 43. Picone CSF, Cunha RL. Chitosan-gellan electrostatic complexes: Influence of preparation conditions and surfactant presence. *Carbohydr Polym* 2013; 94(1): 695–703. doi:10.1016/j.carbpol.2013.01.092.
 44. Carie A *et al.* IT-141, a Polymer Micelle Encapsulating SN-38, Induces Tumor Regression in Multiple Colorectal Cancer Models. *J Drug Deliv* 2011; 2011: 1–9. doi:10.1155/2011/869027.
 45. Soppimath KS *et al.* Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release* 2001; 70(1–2): 1–20. doi:10.1016/S0168-3659(00)00339-4.
 46. Nafee N *et al.* Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. *Nanomedicine* 2007; 3(3): 173–83. doi:10.1016/j.nano.2007.03.006.
 47. Saremi S *et al.* Thiolated chitosan nanoparticles for enhancing oral absorption of docetaxel: Preparation, in vitro and ex vivo evaluation. *Int J Nanomedicine* 2011; 6(1): 119–128. doi:10.2147/IJN.S15500.
 48. Stella B *et al.* Design of folic acid-conjugated nanoparticles for drug targeting. *J Pharm Sci* 2000; 89(11): 1452–1464. doi:10.1002/1520-6017(200011)89:11<1452::AID-JPS8>3.0.CO;2-P.
 49. Kolharkar RB *et al.* Potential Oral Delivery of 7-Ethyl-10-Hydroxy-Camptothecin (SN-38) using Poly (amidoamine) Dendrimers. *Pharm Res* 2009; 25(7): 1723–9. doi:10.1007/s11095-008-9572-9.Potential.
 50. Zamboni WC *et al.* Altered irinotecan and SN-38 disposition after intravenous and oral administration of irinotecan in mice bearing human neuroblastoma xenografts. *Clin Cancer Res* 1998; 4(2): 455–462.