

Formulation and Evaluation of Irinotecan Suppository for Rectal Administration

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Abstract

Irinotecan suppository was prepared using the moulding method with a homogeneous blend. A sensitive and specific fluorescence method was developed and validated for the determination of irinotecan in plasma using HPLC. The pharmacokinetics of intravenous administered and rectal administered in rabbits was investigated. Following a single intravenous dose of irinotecan (50 mg/kg), the plasma irinotecan concentration demonstrated a bi-exponential decay, with a rapid decline over 15 min. C_{max} , $t_{1/2}$, AUC_{0-30h} and $AUC_{0-\infty}$ were 16.1 ± 2.7 g/ml, 7.6 ± 1.2 h, 71.3 ± 8.8 μ g·h/ml and 82.3 ± 9.5 μ g·h/ml, respectively. Following rectal administration of 100 mg/kg irinotecan, the plasma irinotecan concentration reached a peak of 5.3 ± 2.5 μ g/ml at 4 h. The AUC_{0-30h} and $AUC_{0-\infty}$ were 32.2 ± 6.2 μ g·h/ml and 41.6 ± 7.2 μ g·h/ml, respectively. It representing ~50.6% of the absolute bioavailability.

Key Words: Irinotecan, Oral, Rectal, Pharmacokinetics, HPLC

INTRODUCTION

The anti-neoplastic agent irinotecan hydrochloride (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin; CPT-11) is a semi-synthetic derivative of the natural product camptothecin (Kunimoto *et al.*, 1987; Sawada *et al.*, 1991). This drug has demonstrated good antitumor activity both *in vitro* and *in vivo* against various experimental tumor models, including multidrug-resistant lines (Tsuruo *et al.*, 1988). CPT-11 has shown anticancer activity against a variety of solid tumors in clinical trials, including colorectal cancer, gynecologic cancers, non-small cell and small cell lung cancers, and refractory cervical cancer (Sandler, 2002; Hind *et al.*, 2008; Hirasawa *et al.*, 2013; Takahashi *et al.*, 2013; Takatori *et al.*, 2013). Like camptothecin, CPT-11 acts by inhibiting mammalian DNA topoisomerase I (Kanzawa *et al.*, 2001). Cell death results from stabilization of cleavable complexes formed between topoisomerase I and DNA during DNA replication, transcription, and repair (Koizumi *et al.*, 2004; Barth *et al.*, 2010).

Irinotecan is a camptothecin derivative with low oral bioavailability due to active efflux by intestinal P-glycoprotein receptors. Hence, no oral formulation is marketed for irinotecan till date. Currently in the China market, the branded goods of irinotecan is irinotecan from the American company Pfizer. It is mainly sold in the form of injections, with the major adverse

events of neutropenia and diarrhea which is difficult to tolerate by the long-term high-dose users. As the injections are inconvenient to use, the patients have poor compliance. Foreign literatures pointed that use irinotecan in nanometers from microemulsion formulation for oral administration can significantly improve the oral bioavailability of the drug (Negi *et al.*, 2013). Thus it is shown that administration through blood vessels is not the only route for irinotecan. This research group has developed a new type of irinotecan suppository aiming to increase the safety and compliance by rectally. The present work focuses on the pharmacokinetics of two irinotecan formulations (suppository and injection) in rabbits.

MATERIALS AND METHODS

Materials

Irinotecan (hydrochloride salt) and irinotecan injection were a gift from Hengrui Pharmaceutical Company, Jiangsu, China. Irinotecan suppository were developed in our laboratory. PEG1500, PEG4000 (BASF, Germany). All other chemicals and solvents were of analytical reagent grade. Water was purified and deionized by the Millipore® Simplicity system (USA).

Open Access <http://dx.doi.org/10.4062/biomolther.2013.087>

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Received Oct 24, 2013 Revised Nov 30, 2013 Accepted Dec 3, 2013

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Suppository preparation

All suppositories were prepared using the moulding method. To obtain suspended irinotecan suppositories, PEGs (PEG1500/PEG4000=1:1) were pre-melted at high temperature above the melting points of all constituents (130°C) in an oven and then irinotecan was dispersed under mechanical stirring at 150 rpm. The preparation was left in the oven until stirred to obtain a homogeneous blend.

Measurement of mucoadhesive force

The mucoadhesive force of suppository was measured using a modified balance (Fig. 1) according to previously reported method (Yong *et al.*, 2004). A section of rectal tissues was cut from the fundus of the rabbit (New Zealand White) and instantly secured with mucosal side out onto glass vials using a rubber band and an aluminum cap. The diameter of each exposed mucosal membrane was 7.1 mm. The vials with the rectal tissues were stored at 36.5°C for 10 min before measuring the mucoadhesive force. One vial was connected to the balance and the other vial was placed on a height-adjustable pan. A 0.2 g sample of suppository was then spread between the mucosal membranes on the vials to attach them. The weights on the other side of the balance required to separate the vials' membranes was read. The mucoadhesive force of the suppository per unit area (dyne/cm²) of mucosa was calculated using the following equation $F=0.98 m/\pi r^2$ where m and r represent the balance weight (g) and radius of the vial (i.e., 7.1 mm), respectively.

In vitro release

In vitro release of irinotecan from rectal formulations was monitored by the USP paddle method at a rotating speed of 100 rpm in 500 ml phosphate buffer as a dissolution medium, pH=7.4 at 37 ± 0.5°C. One gram of each formulation containing 50 mg irinotecan was placed into a semipermeable cellulose membrane tube (5×1.5 cm, length×diameter). Both sides of the tube were tied up with a thread to prevent leakage. The semipermeable membrane tube was placed in a dissolution tester (DST-600, Erweka, Germany). Five-milliliter samples were withdrawn at predetermined time over a 4-h period and replaced with the same volume of fresh dissolution medium. The samples were analyzed spectrophotometrically by fluorescence detector which operated at an excitation wavelength of 370 nm and an emission wavelength of 470 nm.

Animals

Twelve adult healthy New-Zealand white rabbits (2.0~2.5 kg) were supplied by Laboratory Animal Center (Hangzhou). Prior to use, all rabbits were maintained under standard laboratory conditions on a 12 h light-dark cycle and were fed standard chow and sterilized tap water. All experimental procedures were carried out in accordance with the guidelines of the Hospital Animal Ethics Committee.

Pharmacokinetic studies

The rabbits were divided randomly into two groups. Group One was the irinotecan intravenous injection group (six rabbits, 50 mg/kg). Group Two was the irinotecan suppository group (six rabbits, 100 mg/kg). Each animal was identified by means of a tattoo on the ventral aspect of one pinna and an individual identification card on the cage. In order to improve the reliability of suppository we tried: 1) The insertion into the

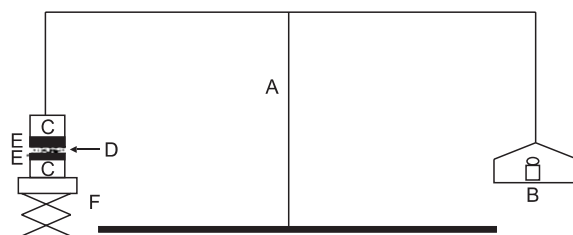


Fig. 1. Bioadhesive force-measuring device: (A) balance, (B) weights, (C) glass vial, (D) suppository, (E) rectal tissue, (F) height-adjustable pan.

animal's rectum was performed using 1 ml syringe for liquid formulations and 2) 1 ml pipette like device with large aperture and piston for dry forms.

Heparinized blood (1 ml) was serially collected from the marginal ear vein before treatment and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 30 h via the central ear vein. The plasma was separated by centrifugation at 12,000 rpm for 10 min and stored frozen until analyzed. A 500 µl volume of plasma was obtained and stored at -20°C until analysis. Pharmacokinetic parameters were calculated from the plasma concentration-time data. The elimination half-life ($T_{1/2}$) was determined by linear regression of the terminal portion of the plasma concentration-time data. The area under the plasma concentration-time curve from zero to the last measurable plasma concentration point (AUC_{0-t}) was calculated by the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty}=AUC_{0-t}+C_t/k_e$, where C_t is the last measurable plasma concentration and k_e is the terminal elimination rate constant.

Instrumental and chromatographic conditions

The chromatographic system consisted of an Agilent G1321 fluorescence detector with operated at an excitation wavelength of 370 nm and an emission wavelength of 470 nm. The chromatographic separation was achieved on a Gemini C18 column (150×2.0 mm, 5 µm, Phenomenex, Torrance, CA, USA) with a Security Guard C18 guard column. A mixture of acetonitrile (solvent A) and phosphate buffer, pH=4 (solvent B) = 45:55 (v/v) were used as mobile phase at a flow rate of 1 ml/min. The mobile phases were filtered through a 0.45 µm HV filter (Millipore), then degassed ultrasonically before use. The temperature of column and auto-sampler were maintained at 30 and 4°C, respectively. The chromatographic run time of each sample was 10 min.

Sample preparation

To a 500 µl aliquot of plasma sample in a 10 ml clean glass tube, 20 µl of internal standard (100 µg/ml camptothecin in acetonitrile) was added. The samples were vortexed for 1 min and 1.5 ml methanol-acetonitrile (50:50 v/v) was added. The mixture was vortex-mixed for 3 min. After centrifugation at 3,000 g for 10 min, the upper organic layer was then transferred into a clean glass tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was reconstituted in a 100 µl acetonitrile-water (50/50, v/v), and transferred to an auto-sampler vial. An aliquot of 20 µl was injected onto the HPLC system for analysis.

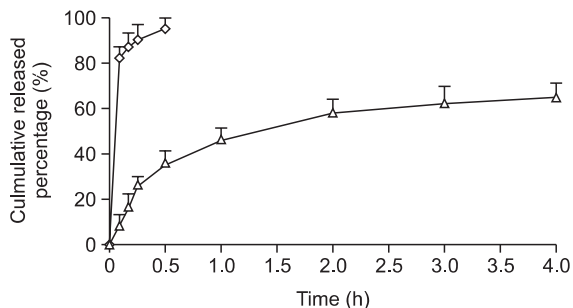


Fig. 2. *In vitro* release profiles of irinotecan suppository from three batches. Each point represents the mean value of three different experiments \pm S.D. \diamond : free irinotecan; \triangle : irinotecan suppository.

Methodology for validation

A thorough and complete method validation of irinotecan in rabbits plasma was validated for selectivity, sensitivity, linearity, precision and accuracy, recovery and stability.

Test for selectivity was carried out in 6 different lots of blank plasma (with heparin sodium as anticoagulant), processed by the same extraction protocol and analysed to determine the extent to which endogenous plasma components may contribute to the interference at the retention time of analytes and internal standard.

The linearity of the method was determined by analysis of standard plots associated with a eight-point standard calibration curve ($y=ax+b$, where y is the peak area ratio) using linear regression analysis with reciprocal of the drug concentration as a weighing factor ($1/x^2$) for irinotecan. The regression equation for the calibration curve was also used to back-calculate the measured concentration at each QC level. The peak area ratio values of calibration standards were proportional to the concentration of the analytes in plasma over the range tested. Intra-batch and inter-batch (on five consecutive days) accuracy and precision were evaluated at three different concentrations levels in five replicates for both the analytes. Mean values were obtained for calculated and expressed in terms of %bias and coefficient of relative standard deviation (%RSD), respectively.

Recovery of the analytes from the extraction procedure was performed at three different concentrations levels. It was evaluated by comparing peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (quality control working solutions spiked in extracted plasma).

Stability experiments were performed to evaluate the analyte stability in stocks solutions and in plasma samples under different condition, simulating the same conditions, which occurred during study sample analysis. Stock solution stability was performed at room temperature and at 4°C by comparing area response of stability sample of analytes and internal standard with the area response of sample prepared from fresh stock solutions. The results should be within the acceptable limit of $\pm 10\%$ change for stock solution stability experiment.

Statistical analysis

The results were expressed as mean \pm SD. ANOVA was used to test the differences between the calculated parameters using the SPSS Statistical Package (Version 10, SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant when $p < 0.05$.

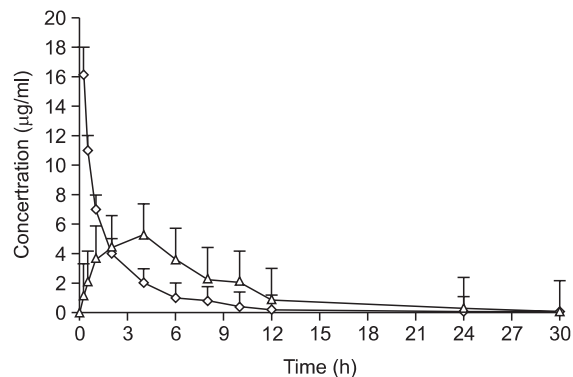


Fig. 3. Mean plasma concentrations of irinotecan in twelve rabbits after single administration (\diamond : intravenous; \triangle : rectal).

RESULTS

The preparation was a homogeneous blend and with a suitable mucoadhesive force (64 dyne/cm^2). *In vitro* drug release behavior of irinotecan suppository was studied using a paddle method. The release profiles of free irinotecan and irinotecan suppository were shown in Fig. 2. A very fast release behavior of free irinotecan was observed, whereas the cumulative release rate of irinotecan suppository was much slower followed by a sustained release. In free irinotecan group, 95% of drug were released in the first 30 min. In contrast, only 65% of drug were released from suppository in the 4 h ($p < 0.01$).

The calibration curves for irinotecan were linear from 0.2 to 50 $\mu\text{g/ml}$ with correlation coefficient $r=0.9993$, respectively across five regression curves. The equation for mean ($n=5$) of five calibration curves for the analyte were: $y=0.26x-0.0012$. The data indicated that intra-assay RSDs were between 6.3% and 9.1% for irinotecan. The inter-assay RSDs were between 3.3% and 8.3%. The overall mean recoveries for irinotecan at LQC, MQC and HQC levels were 85.2, 83.1 and 84.7%, respectively. Stock solution of irinotecan and IS were stable at room temperature for 12 h and at 4°C for 30 days with mean %change well within 7.7%. Both the analytes were found stable in controlled plasma at room temperature up to 24 h and for at least three freeze and thaw cycles. The analytes in extracted plasma samples were stable for 24 h under refrigerated condition of 4°C.

No symptoms were noted in any of the rabbits that received two irinotecan formulations. The mean plasma concentration-time profile of irinotecan after a single intravenous or rectal administration in the rabbits is illustrated in Fig. 3. Table 1 lists the pharmacokinetic parameters. Following a single intravenous dose of irinotecan (50 mg/kg), the plasma irinotecan concentration demonstrated a bi-exponential decay, with a rapid decline over 15 min. C_{max} , $t_{1/2}$, $AUC_{0-30\text{h}}$ and $AUC_{0-\infty}$ were $16.1 \pm 2.7 \mu\text{g/ml}$, $7.6 \pm 1.2 \text{ h}$, $71.3 \pm 8.8 \mu\text{g}\cdot\text{h/ml}$ and $82.3 \pm 9.5 \mu\text{g}\cdot\text{h/ml}$, respectively. Following rectal administration of 100 mg/kg irinotecan, the plasma irinotecan concentration reached a peak of $5.3 \pm 2.5 \mu\text{g/ml}$ at 4 h. The $AUC_{0-30\text{h}}$ and $AUC_{0-\infty}$ were $32.2 \pm 6.2 \mu\text{g}\cdot\text{h/ml}$ and $41.6 \pm 7.2 \mu\text{g}\cdot\text{h/ml}$, respectively. It representing $\sim 50.6\%$ of the absolute bioavailability.

Table 1. Pharmacokinetic parameters of irinotecan in rabbits

Parameter	Intravenous administration	Rectal administration
T _{max} (h)	0.25	4
C _{max} (μg/ml)	16.11 ± 2.72	5.28 ± 2.54*
t _{1/2} (h)	7.56 ± 1.24	14.41 ± 2.42*
AUC _{0-30h} (μg·h/ml)	71.31 ± 8.75	32.15 ± 6.15*
AUC _{0-∞} (μg·h/ml)	82.33 ± 9.47	41.62 ± 7.24*

Note: *shows $p > 0.05$ vs intravenous administration.

DISCUSSION

Irinotecan hydrochloric has been widely used for colorectal cancer, small cell lung cancer and treatment for solid tumors such as stomach cancer. The clinical trials proved that it can significantly prolong the patients' survival. However, the hematologic toxicity and gastrointestinal reactions severely limit the clinical application of irinotecan. Some researches have proved that the elimination method of irinotecan hydrochloric inside animal and human body is hepatic metabolism and biliary (Koizumi *et al.*, 2004). Its metabolizing enzymes include carboxylesterase (CEs), glucuronyl transferase enzymes (UGTs), cytochrome P450 (CYP3A) and B-glucuronidase enzymes. Irinotecan hydrochloric *in vivo* is likely to be influenced by carboxylesterase to be converted to the active metabolite 10-hydroxy-7-ethyl camptothecin (SN-38) (Takatori *et al.*, 2013). It can also form a non-reactive by CYP3A method.

The Japanese scholar had first successfully prepared the suppository, which had outer shell made of the matrix and the hollow can be filled with solid, liquid or suspension state of drug. It provided a suitable dosage form for the long-term treatment of asthma, diabetes, anemia, cancer and other chronic disease. It can replace some of the injections and enemas. The dose is accurate, convenient and the hollow bolt release can reduce the frequency of administration as well as increase the patients' compliance.

Mucoadhesive force is known to be dependent on the nature and the concentration of mucoadhesive polymers. The stronger the mucoadhesive force is, the more it can prevent the gelled suppositories from reaching the end of the colon, the pathway for the first-pass effect. But if the mucoadhesive force is too excessive, the suppository can damage the rectal mucous membrane (Choi *et al.*, 1998). Therefore, suppository must have the suitable mucoadhesive force. In this study the PEGs itself has a moderate bioadhesive force due to binding of the hydrophilic oxide group to oligosaccharide chains.

The results of this study showed that after the intravenous administration of irinotecan, the maximum plasma concentration was significantly higher than rectal administration ($p > 0.05$). Meanwhile, from this study we found that 1) the AUC after the rectal administration was similar with oral administration; 2) after rectal administration of irinotecan, there was nearly 50% of the absolute bioavailability, suggesting that

rectal administration may become another method of administration of irinotecan.

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