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Research Article

STUDY ON *IN VIVO* RELEASE AND *IN VIVO* ABSORPTION OF CAMPTOTHECIN-LOADED POLYMERIC NANOPARTICLES: LEVEL A *IN VITRO* - *IN VIVO* CORRELATION

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ABSTRACT

Objectives: The aim of this study was to develop an *in vitro* - *in vivo* correlation (IVIVC) for the prepared Camptothecin (CPT)-loaded polymeric nanoformulation.

Methods: In this study, CPT-loaded polymeric nanoformulation was prepared by nanoprecipitation method using containing poly (methacrylic acid-co-methyl methacrylate) (polymer), poloxamer 188 (non-ionic surfactant), and β-cyclodextrin (stabilizer). *In vitro* release rate data were obtained from prepared polymeric nanoformulation using the USP apparatus type 2. A single-dose, crossover pharmacokinetic study for the nanoformulation was carried out in six albino rats. These data were used as the basis for the IVIVC model development.

Results: The plasma concentration of CPT was estimated by high-performance liquid chromatography. The pharmacokinetic parameters were calculated from the plasma concentration of CPT and time data. Furthermore, the deconvolution of the *in vivo* concentration-time data was performed using Wagner–Nelson method to estimate the *in vivo* drug release profile.

Conclusion: Therefore, a level A IVIVC was developed for CPT-loaded polymeric nanoformulation between dissolution percentage and intestinal absorption in rats. The simplest way to demonstrate a correlation is to plot the percentage absorbed *in vivo* versus the percentage released *in vitro* at the same time.

Keywords: Camptothecin, *In vitro - in vivo* correlation, Wagner–Nelson, Dissolution, Pharmacokinetics.

INTRODUCTION

Camptothecin (CPT) naturally occurring quinolone alkaloids shows a significant anticancer activity with a broad spectrum of human malignancies. CPT is an inhibitor of the DNA-replicating enzyme topoisomerase I which is believed to act by stabilizing a topoisomerase I-induced single strand break in the phosphodiester backbone of DNA, thereby preventing relegation [1]. Despite of its promising activity, the clinical applications are hampered by its poor water solubility, low stability in physiological medium, severe systemic toxicity, and low antineoplastic activity [2]. Accordingly, a novel drug delivery system is imperative to overcome the internal defects. In recent years, nanostructured materials such as nanoparticles have been considered as potential carriers for hydrophobic drug delivery that may resolve the aforementioned problems [3].

A key goal in the pharmaceutical development of dosage forms is a good understanding of the *in vitro* and *in vivo* performance of the dosage forms. One of the challenges of biopharmaceutics research is correlating *in vitro* drug release information of drug formulations to the *in vivo* drug profiles [4]. *In vitro - in vivo* correlations (IVIVC) play an important role in reducing the drug development time and optimization of the formulation. A good correlation is a tool for predicting *in vivo* results based on *in vitro* data [5]. The IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development [6].

The IVIVC is a mathematical relationship between *in vitro* properties of a dosage form with its *in vivo* performance. For oral dosage forms, the *in vitro* release is usually measured and considered as dissolution rate. The relationship between the *in vitro* and *in vivo* characteristics can be expressed mathematically by a linear or nonlinear correlation [7]. However, the plasma concentration cannot be directly correlated to the *in vitro* release rate; it has to be converted to the *in vivo* release or absorption data, either by pharmacokinetic compartment model analysis or by linear system analysis [6].

IVIVC definitions

Food and Drug Administration (FDA) definition of IVIVC (FDA, 1997)

The IVIVC has been defined by the FDA as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response."

In general, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed [8]. However, the correlation between the *in vitro* dissolution rate and *in vivo* absorption rate does not always exit. Nevertheless, making an accurate prediction of *in vivo* performance based on *in vitro* dissolution is not a straight forward process and many traps could involuntarily bias the predictions. Thus, the main objective of the IVIVC is to serve as a surrogate for *in vivo* bioavailability and to support biowaivers [9].

Levels of IVIVC

There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C. The concept of correlation level is based on the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form [10].

Level A correlation

The IVIVC that correlates the relationship between the entire *in vitro* and *in vivo* profiles for its regulatory relevance and is called the level A correlation. This level of correlation is generally linear and represents a point-to-point relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from the dosage form [6,11].

The purpose of level A correlation is to define a direct relationship between *in vivo* data such that measurement of *in vitro* dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form. In this context, the model refers to the relationship between the *in vitro* dissolution of an extended release dosage form and an *in vivo* response such as plasma drug concentration or amount of drug absorbed.

Level B correlation

A level B IVIVC is based on the principles of statistical moment analysis. In this level of correlation, the mean *in vitro* dissolution time of the product is compared to either mean *in vivo* residence time or the mean *in vivo* dissolution time. The level B correlation does not uniquely reflect the actual *in vivo* plasma level curves because a number of different *in vivo* curves will produce similar mean residence time values [6,11].

Level C correlation

A Level C correlation relates a single dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to a pharmacokinetic parameter such as area under the curve (AUC), t_{max}, or C_{max}. This is the weakest level of correlation relationship between absorption, and dissolution is established since it does not reflect the complete shape of plasma drug concentration-time curve, which is the critical factor that defines the performance of a drug product [6,11].

Multiple level C correlations

This level refers to the relationship between one or more pharmacokinetic parameters of interest (C_{max}, AUC, or any other suitable parameters) and the amount of drug dissolved at several time point of dissolution profile. Multiple point level C correlation may be used to justify biowaivers provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest [6,11] (Table 1).

METHODS

Formulation of CPT-loaded polymeric nanoparticles

About 100 mg of poly (methacrylic acid-co-methyl-methacrylate) polymer with 10 mg of CPT were dissolved in 10 ml of dimethyl sulfoxide. The prepared organic phase was transferred at once into 500 ml beaker containing 50 mg of β-cyclodextrin, 100 mg of poloxamer 188, and 20 ml of distilled water under mechanical stirring (Remi, India) at 500 rpm. Polymeric nanoparticles were formed spontaneously, but the stirring process is continued for 50 minutes to aid the size reduction and to evaporate the residual solvents (Table 2). The fabrication experiments were performed in triplicate.

***In vitro* evaluation**

In vitro drug release of CPT from polymeric nanoparticles was evaluated by dialysis bag diffusion technique. The prepared CPT-loaded polymeric nanoformulation (weight equivalent to 10 mg of drug) was placed in a cellulose dialysis bag (cutoff 12 000; HIMEDIA, Mumbai, Maharashtra, India) and sealed at both ends. The dialysis bag was immersed in the receptor compartment containing dissolution medium maintained at 37°C±0.5°C with a rotating speed of 100 rpm [12]. The release characteristic of CPT from the prepared nanoparticle formulation was investigated using USP dissolution apparatus 2 (Electrolab, Mumbai, Maharashtra, India). To achieve simulated gastrointestinal transit condition, the release profile of nanoformulation was studied with the dissolution medium of changing pH at various time intervals. Initially, the dissolution medium was maintained at pH 1.2 with 350 ml of 0.1N HCl for 0-2 hrs. At the end of the second hour, the pH of the dissolution medium was raised to 4.5 by the addition of 250 ml of solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH and the total volume of dissolution medium was 600 ml. At the end of the 4th hour, pH of medium was raised to 7.4 by addition of 300 ml of phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) [13,14]. At predetermined time intervals, 5 ml of sample was withdrawn and replaced with fresh dissolution media. The collected samples were filtered through 0.45 µm membrane filter (Millipore). After appropriate dilution, the concentration of drug in the sample was analyzed using high-performance liquid chromatography (HPLC).

Table 1: Various parameters used in IVIVC depending on the level

Level	A	B	C
In vitro	Dissolution curve	Statistical moments: MDT	Disintegration time, time to have 10%, 50%, 90% dissolved, dissolution rate, dissolution efficiency
In vivo	Input (absorption) curves	Statistical moments: MRT, MAT, etc.	C_{max}, T_{max}, K_a, time to have 10%, 50%, 90% absorbed, AUC (total or cumulative)

IVIVC: *In vitro - in vivo* correlation, AUC: Area under the curve, MDT: Mean dissolution time, MAT: Mean absorption time, MRT: Mean residence time

***In vivo* evaluation**

In vivo evaluation of the prepared CPT-loaded polymeric nanoformulations was assessed in rats randomly distributed into two groups of six rats in each group. Rats in group 1 received pure CPT suspension (5 mg/kg), and group 2 rats received CPT encapsulated polymeric nanoformulation (5 mg/kg) with the help of cannula after anesthetizing for a very short period with diethyl ether. The animal experiment was performed as per the protocol approved by the Institutional Animal Ethics Committee (160/1999/CPCSEA; Proposal Number 975; Approved on 07.02.2013). The blood samples (0.5 ml) were collected from the retro-orbital plexus under mild ether anesthesia into heparinized microcentrifuge tubes (containing 20 µl of 1000 IU heparin/ml of blood) at 0 minute 1, 2, 4, 8, 10, 12, 24, and 36 hrs after drug administration. After each sampling, 1 ml of dextrose-normal saline was administered to prevent changes in the central compartment volume and electrolytes. Plasma samples were obtained by centrifugation of each blood sample at 3000 rpm at 4°C for 10 minutes and was stored at −20°C, and the concentration of drug was determined by HPLC analysis [15]. The pharmacokinetic parameters such as area under the plasma concentration-time curve (AUC_{0-t}), maximum plasma concentration (C_{max}), and the time taken to reach the maximum plasma concentration (T_{max}) were determined from plasma concentration data.

IVIVC

The level A IVIVC, the point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate, was studied. The procedure of developing an IVIVC consisted of the following steps: Calculation of cumulative *in vitro* dissolution rate, calculation of cumulative *in vivo* absorption rate from concentration-time data obtained by Wagner–Nelson method and modeling the relationship between *in vivo* absorption rate and *in vitro* dissolution rate.

Wagner–Nelson is a mass equation which allows calculation of the absorption in the case of the one compartment model as stated in guidelines [8,16]. This equation uses observed concentrations (C[t]), AUC, and apparent elimination rate constant determined from the data (ke) as presented in Equation 1.

$$A\% = \frac{C(t) + ke \times AUC_0^t}{ke \times AUC_0^\infty} \times 100$$

This equation exhibits a domain from 0% to 100%. In some cases, when the Wagner–Nelson equation is used, a flip-flop model could exist, especially in case of sustained release formulations where the absorption rate is much lower than the elimination rate. In this case, the terminal decreasing of the plasma concentration curve, which normally reflects the elimination rate (ke), becomes a reflection of actual absorption rate (ka), while the initial increasing part of the curve, which normally reflects the absorption rate (ka), is the actual representation of the elimination rate (ke).

Table 2: Fabrication of Camptothecin-loaded polymeric nanoparticles

Trials	A (mg)	B (mg)	C (mg)	D (mg)	E (ml)	F (ml)	G (min)	H (rpm)	J	K	L
1	10	100	50	100	10	20	50	500	At once	Or. to Aq.	Blade

A: Concentration of drug, B: Concentration of poly (methacrylic acid-co-methyl-methacrylate), C: Concentration of β–cyclodextrin, D: Concentration of Poloxamer 188, E: Volume of organic phase, F: Volume of aqueous phase, G: Stirring time, H: Stirring speed, J: Mode of addition, K: Process, L: Stirring mode, Or.: Organic phase, Aq.: Aqueous phase

Table 3: Pharmacokinetic parameters of Camptothecin after oral administration of free drug and nanoformulation (5 mg/kg) in rats

Parameter	Pure Camptothecin suspension	Camptothecin nanoformulation
T_{max} (h)	0.5±0.02	12±0.42***
C_{max} (ng/ml)	135.15±12.56	197.75±16.25***
AUC (ng/hrs/ml)	187.80±58.26	1826.52±76.18***
AUMC (ng/hrs/ml)	286.25±24.26	22873.3±85.12***
$t_{1/2}$ (h)	0.86±0.03	3.26±0.25***
Ke (h^{-1})	0.95±0.02	0.08±0.01***
MRT (h)	1.52±0.25	20.07±1.42***
Cl (ml/h/kg)	0.02±0.03	0.001±0.02***

The values are represented as mean±SD; (n=6); ***Indicates that the result is highly significant at p<0.001. SD: Standard deviation, IVIVC: *In vitro - in vivo* correlation, MRT: Mean residence time, AUMC: Area under the first moment curve

However, the simplest way to demonstrate a correlation is to plot the percentage absorbed *in vivo* (obtained by Wagner–Nelson Method) versus the percentage released *in vitro* at the same time [8].

RESULTS AND DISCUSSION

Fabrication of plain and CPT-loaded polymeric nanoparticles

CPT-loaded poly (methacrylic acid-co-methyl-methacrylate) nanoparticles were prepared based on the principle of nanoprecipitation under the influence of stirring. In nanoprecipitation method, the solvent stream contains CPT and poly (methacrylic acid-co-methyl-methacrylate) in water miscible organic solvent dimethyl sulfoxide, and antisolvent stream contains poloxamer 188 as a surfactant and β-cyclodextrin as a stabilizer in water. Addition of solvent stream into the antisolvent stream results in the miscibility of dimethyl sulfoxide with water, which leads to the increase in the polarity of dimethyl sulfoxide, which in turn decreases the solubility of the polymer. However, nucleation of polymer gets initiated when the equilibrium concentration surpasses the solubility threshold of the polymer. Stirring process aid the size reduction of polymer at the initial stage but in the later stages, anionic nature of polymer provided anionic charge to the nanoparticle surface and higher number of likely charged nanoparticles repels each other and creates an electrostatic repulsive force and maintains the nanoparticles in Brownian motion, which is expected to overcome the Van der Waals attractive force arising from induced dipole-dipole interaction between nanoparticles and gravitational force, thereby stabilize the nanoformulation by preventing the aggregation.

In vitro dissolution profiles of the prepared CPT-loaded polymeric nanoparticles are presented in Fig. 1. The prepared CPT-loaded polymeric nanoparticles showed 98.22 % of drug release. *In vitro* drug release from the drug-loaded polymeric nanoparticles was assessed in simulated gastrointestinal conditions. The pH condition used was pH 1.2 for 2 hrs (stomach), pH 4.5 for 2 hrs (duodenum) followed by pH 7.4 (distal ileum and colon) for the remaining period of the study using a USP dissolution test apparatus (Apparatus type 2). The drug release was found to be less than 5% up to 4 hrs and the drug release increased when the pH of the medium was adjusted to 7.4.

The plasma concentration-time profiles for the CPT-loaded polymeric nanoparticles are presented in Table 3. The pharmacokinetic

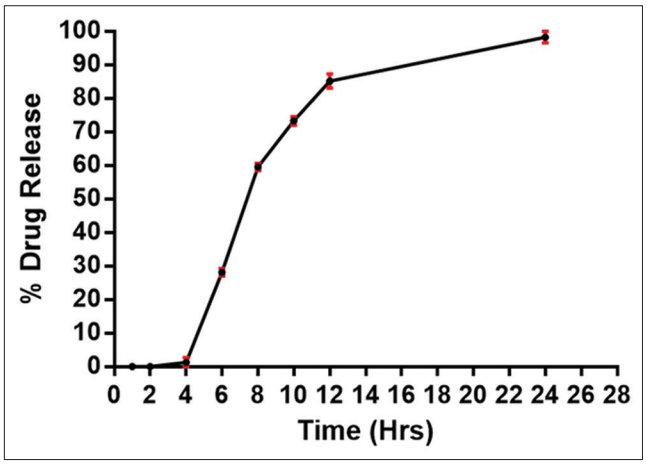

Fig. 1: *In vitro* release profile of Camptothecin-loaded polymeric nanoparticles. The values are represented as mean±standard deviation (n=6)

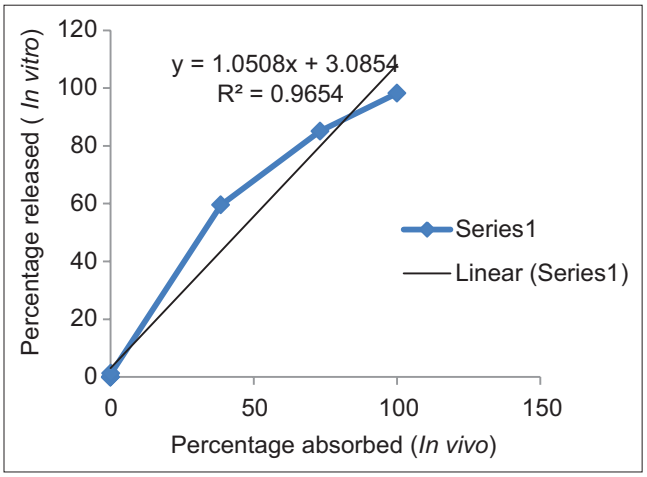

Fig. 2: *In vitro - In vivo* correlation of Camptothecin-loaded polymeric nanoparticles

parameters of the mean concentration-time profile of the prepared CPT-loaded polymeric nanoparticles were estimated.

The pharmacokinetic profiles of the prepared CPT-loaded polymeric nanoformulations showed a significant difference from the pharmacokinetic profiles of free CPT suspension. The AUC of CPT in rats treated with nanoparticles was 1826.52±76 ng/h/ml, which was significantly improved (***p<0.001) compared with that of free CPT suspension (187.80±58 ng/h/ml). The improved AUC of CPT nanoparticles is due to more uptake of CPT in the intestine from the nanoformulation.

The feasibility of developing the level A correlation for CPT-loaded polymeric nanoparticles formulations was evaluated by plotting the percentage of fraction dissolved *in vitro* with respect to the percentage of fraction absorbed *in vivo* (Fig. 2). There was a good correlation between the *in vitro* and *in vivo* cumulative release profiles. A consistent

correlation (r^2>0.965) was observed between *in vitro* and *in vivo* profiles. The correlation quality depends solely on the quality of the data. The proposed method demonstrates a schema for developing IVIVC using data from biostudies conducted during formulation development.

CONCLUSION

As the objective of the study is to develop the IVIVC mathematical model to describe the relationship between the *in vitro* fraction dissolved and *in vivo* fraction absorbed, the level A IVIVC was developed and showed a best-fit relationship between *in vitro* dissolution and *in vivo* absorption data for CPT-loaded polymeric nanoparticles formulation.

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