Irinotecan (Campto^{R}) Pharmacokinetics and Metabolism in Patients with Elevated Serum Bilirubin Levels

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Abstract: In this study, we have calculated and reported the pharmacokinetics of irinotecan and its active metabolite, SN-38, in patients with increased plasma bilirubin levels. Four patients suffering from metastatic colorectal cancer (CRC) with high bilirubin levels (0.7 to 15 mg/dl) were selected for our study. These patients were being treated by CPT -11 (Irinotecan) in the hospital setup. To all four patients, CPT-11 was administered as a 60 min IV- infusion (180 mg/m^2, total dose 339 ± 32 mg). Blood samples were collected at 0, 15, 30, 45, 60, 90,120, 180, 240, 300 and 360 minutes after the drug administration. The drug and its pharmacologically active metabolite, SN38 were quantified in these samples by an HPLC method. The blood level profiles were analyzed for their PK behavior by Kinetica® software system. SN 38 levels were found to be decreasing with increasing bilirubin values. The possible rationalizing for lower SN38 levels with elevated bilirubin levels might be some liver impairment which slows the metabolic conversion of irinotecan into SN-38.

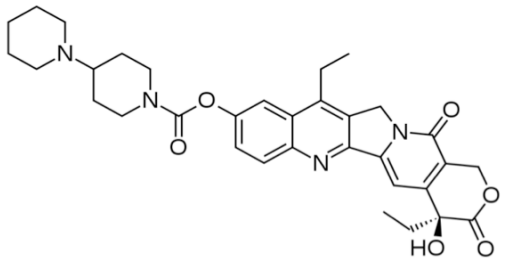
Keywords: Irinotecan (CPT-11), SN-38, Bilirubin, liver impairment, Pharmacokinetics, DLT.

INTRODUCTION

Colorectal cancer is among the most common malignancies in the Western World. Although 50% of patients can be cured by surgery alone, the outcome is poor in high-risk patients (Dukes stages B2 and C) despite adjuvant chemotherapy with 5-fluorouracil (5-FU)-based regimens. CPT-11 (irinotecan) with a unique mechanism of action is famous as a promising agent for the treatment of colorectal cancer [1].

Irinotecan (CPT-11; 7-ethyl-10-[4(-1-piperidino)-1-piperidino] carbonyloxycamptothecin) is a semisynthetic derivative of the natural alkaloid camptothecin. It belongs to the class of antineoplastic agents called topoisomerase–linhibitors. Camptothecins interact specifically with the enzyme topoisomerase I, which relieves torsional strain in DNA by inducing reversible single-strand breaks.

In Japan, Chemotherapy- naïve patients with advanced non-small cell lung cancer have shown a response rate of 32% to 34% with CPT-11 monotherapy, which has increased to 43% to 54% using CPT-11 in combination with cisplatin. CPT 11 is also active in small cell lung cancer; with a response rate of 47% when used as a single agent in patients previously treated with cisplatin in these tumors also CPT 11 combination therapy with Cisplatin gives an average response rate of 85%.



CPT-11 activity has also been reported in phase II trials of patients with squamous cell carcinoma of the uterine cervix or skin, and in those with cancer of the ovary, stomach, or pancreas and in patients with lymphoma [2].

Preclinical *in vitro* activity of CPT-11 has been noted against xenografts derived from ependymoma, childhood and adult high-grade astrocytoma, and medulloblastoma [3] CPT 11 is indicated as first-line therapy (with 5-fluorouracil and leucovorin) for metastatic colorectal cancer (CRC) [4].

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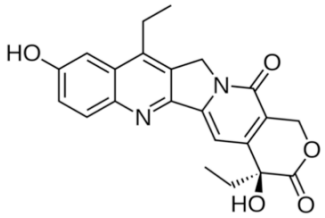
ISSN: 1929-2260 / E-ISSN: 1929-2279/16

Structure of Irinotecan

CPT-11 undergoes excessive biotransformation in humans by different metabolic routes and needs activation into its pharmacologically active metabolite SN-38 (7-ethyl 10-hydroxy-camptothecin) by the human carboxylesterase enzyme (hCES2). This SN-38 then exerts its cytotoxic effects by intermediate forms of drug-stabilized covalent DNA–topoisomerase-I complexes. All metabolic pathways are sensitive for drug-drug interactions [5]. Irinotecan is mainly eliminated by the liver and, to a lesser extent, by the kidneys. Irinotecan's active metabolite, SN-38, is glucuronidated by the hepatic isoenzyme uridine diphosphate glucuronosyltransferase (UGT1A1) into SN -38-ß-D-glucuronide.



Structure of SN-38

The administration of chemotherapeutic agents to cancer patients with co existing hepatic dysfunction requires careful consideration. There are a variety of ways in which liver impairment can affect drug kinetics, including changing the intrinsic hepatic clearance of drugs, reducing hepatic metabolic capacity, and altering the biliary excretion of drugs. In addition, low serum albumin levels due to liver impairment, lead to increased fractions of free drug, and portal hypertension can affect drug absorption [6].

The serum total bilirubin level is the most commonly used marker to assess the need for chemotherapy dose adjustments. Thus, there may happen many potential hazards in administrating cancer chemotherapeutic agents to patients with impaired hepatic function.

A separate phase I study has confirmed that irinotecan dose reductions are required in patients with liver impairment. In the study twelve patients with hyperbilirubinemia (median serum bilirubin: 2.1 mg/dL, range: 1.0-5.5 mg/dL) were given irinotecan on an every-3-week schedule. Three of five patients developed DLT at a dose of 145 mg/m^2, and zero of seven patients developed DLT at a dose of 115 mg/m^2. Two of the DLTs were neutropenia and one was worsening liver function. There were no episodes of dose-limiting diarrhea in patients with an increased bilirubin level. The study concludes that as the patients with elevated bilirubin being treated with irinotecan can have an increased risk of toxicity; a dose reduction is recommended [6].

MATERIAL AND METHODS

Four patients who had different high bilirubin levels and were being treated by CPT -11 (Irinotecan) were selected for our case study. Informed consents from patients and permission from Institutional review board had been taken. In this case report, we describe the therapeutic drug monitoring (TDM) of irinotecan in patients with differently elevated levels of bilirubin with the aim to gain additional information on potentially necessary pharmacokinetics parameters. All selected patients were administered CPT-11 as a 60 min IV - infusion (180 mg/m^2, total dose 339 ± 32 mg). The patients demographics were mean age of 62 years, mean body weight of 67 kg, mean height of 162 cm, and mean body surface area of 1.70 m^2.

Sample Collection

After administration of irinotecan infusion, 5ml post dose blood samples were collected at time of 0,15,30,45,60,90,120,180,240,300, and360minutesin order to elucidate the pharmacokinetics of CPT 11 for different elevated levels of bilirubin. Blood samples were collected into sodium citrate impregnated tubes.

After centrifugation at 2500 rpm for 5 minutes to remove blood cells, 2 ml of the plasma were stored at –80°C until analysis. Sample clean-up and analysis needs to be performed within two weeks to avoid further formation of SN-38 from CPT-11 due to activity of hCEs even when frozen [7].

Sample Cleanup

One ml of plasma sample was mixed with 3.0 ml of a mixture of ice-cold acetonitrile / methanol (1:1, v / v %) and vortexed for 2 min. After protein precipitation, the sample was centrifuged in a cool centrifuge for 3 min at 10,000 rpm (4^{o}C). From the supernatant, an aliquot of 1000 µl was acidified with 20 µl of phosphoric acid (8.5 %) to shift the equilibrium from the carboxylate to the lactones form of the compounds, vortexed for 1 min and put into the auto sampler microvial [7].

Chromatography

Plasma concentration levels of CPT-11 and SN-38 at different time intervals were quantified by isocratic reversed-phase HPLC using a highly sensitive fluorimetric detection [8, 9].

Pharmacokinetic Parameters Calculation

Pharmacokinetic parameters for both Irinotecan and SN-38 were calculated using Kinetica®(Kinetica, version 5.1; Waltham, MA 02454), evaluating different PK parameters such as AUC(ng/mL*min), Cmax(ng/mL), Tmax(min), $T_{1/2}$(min), MRT(min) and Clearance (ml/h).

C_{max}= Peak plasma concentration [ng/ml]

T_{max}= time of peak plasma concentration[min]

AUC_{tot}= Area under the concentration-time curve [ng/mL*min]

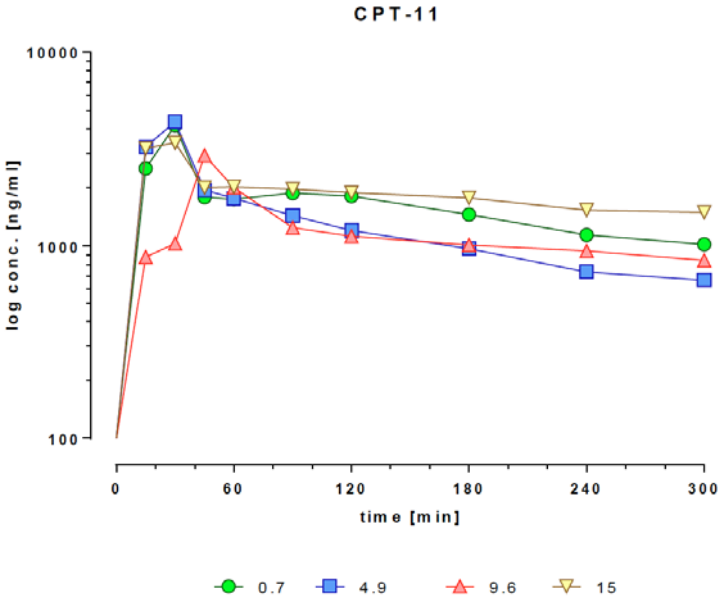


Figure 1: Log Plasma Concentration Time Curve of CPT-11 in patients having different high Bilirubin Levels, after receiving CPT-11.

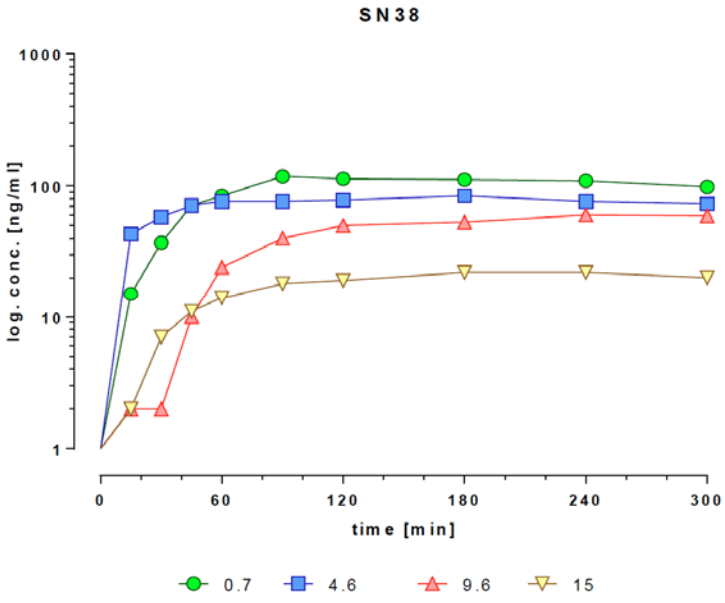


Figure 2: Log-Plasma Concentration Time Curveof SN-38in patients having different high Bilirubin Levels, after receiving CPT-11.

$AUMC_{0-t}$ = Area Under first moment concentration curve (min)^2*(ng/ml)

$t_{1/2}$el = half life of terminal elimination [min]

MRT = Mean residence time [min]

Clearance= Total body clearance [mL/h]

RESULTS

Due to our sensitive assay, plasma concentrations of SN-38 were measurable over the whole time period of the investigation. The measured plasma concentration levels of irinotecan and SN-38 at different bilirubin levels (Figures **1** and **2**). The pharmacokinetic parameters of Irinotecan and SN-38 are summarized in Table **1A** and **1B**. Our main findings are the clearance of irinotecan that decreases with increasing bilirubin levels in four different patients (Tables **1A**, **1B**). The plasma concentration v/s time levels of SN-38 decreases linearly upon bilirubin levels increment at 60 and 180 minutes (Figure **3**).

AUC values of irinotecan increase upon bilirubin levels increment, which is in contrary to the SN-38 AUC values. The ratio of AUCs of SN 38: AUC of Irinotecan decreases upon bilirubin levels increment (Table **2**).

DISCUSSION

By conducting this study, we found that the clearance of irinotecan is decreasing remarkably with the increase in bilirubin levels. The fact behind this

Table 1A: Pharmacokinetic Parameters of Irinotecan Calculated through Kinetica®

Pharmacokinetic Parameters of Irinotecan				
	Bilirubin mg/dl			
IRINOTECAN Dose; mg PK Parameter; 312.54mg	**S0.7**	**S4.9**	**S9.6**	**S15**
C_{max}; ng/mL	4192	4368	2936	3405
T_{max}; min	30	30	45	30
AUC_{0-t}; (min)*(ng/ml)	548242	438317	396291	637826
AUC_{tot}; ng/mL*min	898353	926494	948079	1310100
$\%AUC_{extra}$;	38.9725	52.6908	58.2006	51.3148
$AUMC_{0-t}$; $(min)^2$*(ng/ml)	8.25E+07	5.69E+07	6.34E+07	1.02E+08
$t_{1/2}$; min	264.884	540.38	483.941	381.902
MRT; min	381.108	661.912	682.741	545.426
Clearance; mL/h	20874.2	20240.2	19779.4	1.43E+04
Vd; ml	132950	262989	2.30E+05	1.31E+05

Table 1B: Pharmacokinetic Parameters of SN-38 Calculated through Kinetica®

Pharmacokinetic Parameters of SN-38				
	Bilirubin mg/dl			
SN-38 PK Parameter;	**S0.7**	**S4.9**	**S9.6**	**S15**
C_{max}; ng/mL	118	84	60	22
T_{max}; min	90	180	240	180
AUC_{0-t}; (min)*(ng/ml)	34160.7	25810.5	16075.5	6356.61
AUC_{tot}; ng/mL*min	94338.6	59296.7	60484.1	14358
$\%AUC_{extra}$;	63.7893	56.4723	73.422	55.7276
$AUMC_{0-t}$; $(min)^2$*(ng/ml)	6.64E+06	4.77E+06	3.48E+06	1.28E+06
$t_{1/2}$; min	460.848	378.042	581.252	322.608
MRT; min	724.103	591.82	937.579	549.213

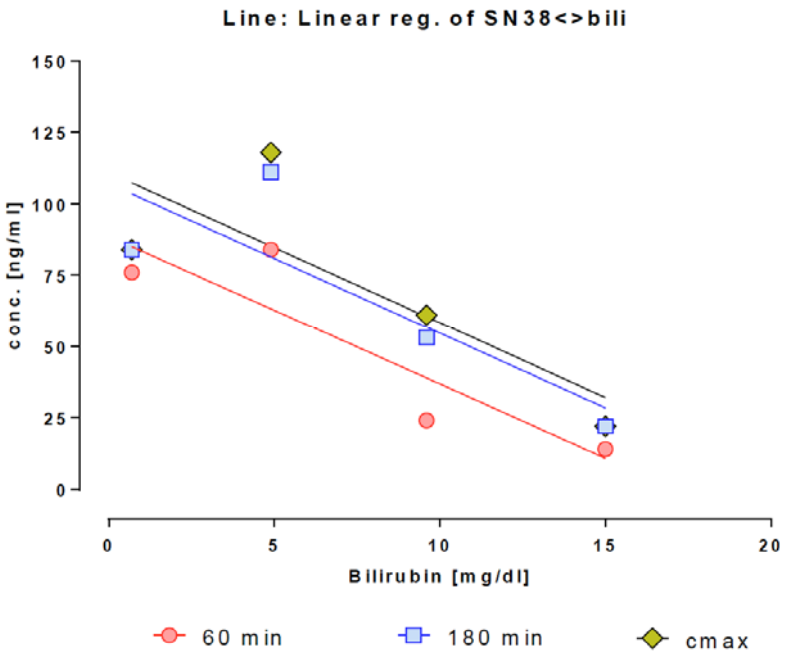


Figure 3: Plasma concentrations of SN-38 at 60 min (red circles) and 180 min (blue squares) after start of irinotecan infusion depending on bilirubin levels in patients.

Table 2: AUC Ratio of SN-38 to Irinotecan

AUC Ratio of SN-38 to Irinotecan				
Bilirubin Levels	**0.7**	**4.9**	**9.6**	**15**
AUC $_{SN38}$	307375	234659	16075.5	6356.61
AUC $_{Irinotecan}$	548242	438317	396291	637826
Ratio of SN-38:Irinotecan	0.561	0.535	0.041	0.010

relation can be some extent of liver impairment in the patients with such high levels of bilirubin. In these patients the irinotecan plasma levels are found to be high but not of SN-38 as SN-38 is conjugated and detoxified to SN-38-glucuronide by UGT1A1 [10] which may not be available due to liver impairment. This finding can also be equivalent to the results that pretreatment of rats with non-enzyme-inducing antiepileptic drug, valproic acid increased the AUC of SN-38, presumably by inhibiting the glucuronidation of SN-38 [11]. These results have now been confirmed in clinical trials of CPT-11 in patients with glioma [12].

Elevated serum bilirubin levels not only indicate reduced hepatic functioning but maythemselves influence the pharmacokinetic behavior of irinotecan and SN38. Both compounds are bound to plasma protein (e.g. albumin) and may be competitively displaced by bilirubin. As a result of this, the unbound fractions of irinotecan are enhanced, resulting in a higher systemic exposure of the drug [13]. Conjugation of SN38 takes place in various tissues by enzymes of the UGTfamily 22 [14], the same enzyme family which conjugates with thebilirubin [15]. Bilirubin and SN38 are conjugated to their respective glucuronides by the same hepatic enzyme so high levels of unconjugated serum bilirubin may be predictive of lack of these enzymes increasing the risk of irinotecan toxicity. Irinotecan (Campto^{R}) is mainly eliminated by the liver and, to a lesser extent, by the kidneys. Considering the higher bilirubin levels, 3 of four patients seem to be hepatically impaired and so their liver converts irinotecan into SN-38 to a limited extent giving a reason to why irinotecan clearance seems to be reduced. The findings also indicate that conjugation of SN-38 is also limited in hepatically impaired patients [13]. Patients with high conjugated bilirubin levels (1.0–5.5 mg/dL) had significantly lower irinotecan and SN38 clearance ($p< 0.05$), and a normal AUC of SN38 while receiving only 35–50% of usual irinotecan dosage [5]. In a phase I study of patients with liver dysfunction, hyperbilirubinemia resulted in lower biliary excretion of

irinotecan and thus higher drug exposure, leading to DLTs of febrile neutropenia and diarrhea [16]. In liver impairment hepatic enzymes (UGT family) will be partially available for the conjugation of both bilirubin and SN-38 .So the drug will be at a higher systemic exposure and thereby having decreased clearance, plasma levels of our samples indicate the same mechanism from 0.7 mg/dl to 4.9 mg/dl concentration. But at higher concentration like 9.6 and 15 mg/dl SN-38 levels decreases, because at this level of physiological impairment liver is unable to convert irinotecan into SN-38.

CONCLUSION

Results of this case study suggest the need to consider the dosage adjustment of irinotecan in patients with liver impairment or higher plasma bilirubin levels. Further pharmacokinetic studies can lead us to develop a rational dosage of irinotecan for treating the patients with colorectal malignancy and coexisting liver impairment. This case study will be beneficial in designing future studies to evaluate a rational dosage of irinotecan.

As can be seen from Figure **3**, amount of serum bilirubin can be correlated with plasma concentrations of total SN-38: increasing bilirubin levels lead to decreased SN-38 plasma concentrations.

60 min: Y = -5,19*x+89 (R = 0.90); 180 min: Y = -5,25*X + 107 (R=0.84).

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Received on 04-02-2016 Accepted on 19-02-2016 Published on 15-04-2016

DOI: http://dx.doi.org/10.6000/1929-2279.2016.05.02.4