In vitro partition of irinotecan (CPT-11) in human volunteer blood: the influence of concentration, gender and smoking

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We have performed *in vitro* incubations of blood from male and female volunteers, smokers and non-smokers, with irinotecan at a gradient of different concentrations in order to investigate changes of partition between red blood cells (RBCs), total plasma and the free fraction. Since irinotecan (CPT-11) is not metabolized *in vitro*, there is no data available on its active metabolite SN-38. After extraction and sample pre-treatment, a validated high-performance liquid chromatography method followed by fluorescence detection was used to determine the concentration of the drug in the different blood constituents. The partition ratio [the concentration in the erythrocytes divided by the concentration in plasma (E/P)] was calculated. The partition ratio of CPT-11 varied from 0.7 to 2.8, reflecting its relatively high affinity for the erythrocyte, probably because of its only moderate plasma protein binding (65%). The partition ratios increased significantly with higher whole-blood concentrations, favoring uptake in the erythrocytes when plasma protein binding is saturated. No gender difference was detected, but we found relatively more CPT-11 in the erythrocytes of non-smokers compared to smokers. The incorporation of drugs into the RBC pool may be important for transportation to tumor tissue and efficacy. Smoking can have a significant influence on drug partition in the blood. *Anti-Cancer Drugs* 16:893–895

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Introduction

Anti-cancer drugs and/or their metabolites are generally reactive substances, and are capable of inducing changes in cellular systems. In the case of blood cells, changes may even result in altered pharmacokinetics if the compartment affected is sufficiently large. Red blood cells (RBCs, i.e. erythrocytes) are most susceptible to temporary changes following exposure to anti-cancer drugs. As they have by far the largest cell volume and surface area when compared with other cellular components of the blood, they can induce alterations in pharmacokinetics [1].

Irinotecan (CPT-11) is a semisynthetic water-soluble camptothecin, which is extensively metabolized in the liver and converted by the enzyme carboxyl esterase to 7-ethyl-10-hydroxycamptothecin (SN-38), the cytotoxic activity of which is at least 100-fold greater than that of CPT-11 *in vitro* [2]. Both CPT-11 and SN-38 are prone to metabolic interconversion between an active lactone form and an inactive carboxylate form. This reversible process is dependent on the pH and the presence of binding proteins in the systemic circulation. Only the lactone form is able to pass cell membranes, including those of blood cells [3].

CPT-11 has activity in leukemia, lymphoma, and several solid tumors such as colorectal, lung, ovarian, cervical, pancreatic, stomach and breast cancer. In phase I trials, most responses were observed at the highest dose levels, indicating a clear dose–response relationship with this drug [4].

The plasma protein binding of CPT-11 and SN-38 is 65 and 95%, respectively. Since it is generally accepted that only the fraction of drug unbound in plasma partitions into RBCs [5,6], one would expect relatively more CPT-11 in the erythrocytes compared with SN-38 *in vivo*. *In vitro*, no SN-38 is present, since the metabolism of CPT-11 takes place in the liver and in the intestinal wall. Thus far data of RBC versus plasma partitioning of CPT-11 are limited, so we performed *in vitro* incubations at different concentration gradients in volunteer blood. We also compared these ratios with those obtained during *in vivo* studies with two different oral formulations of CPT-11 [7,8].

Methods

CPT11 incubations

Standard reference CPT-11 was kindly provided by Rhône-Poulenc-Rorer (Paris, France) at a concentration of 2 mg/ml. Stock solutions of 100 µg/ml were made by dissolving 50 µl of the basic solution after reconstitution in 950 µl NaCl 0.9% and stock solutions of 1 µg/ml were made by dissolving 10 µl of the first stock solution in

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990 µl NaCl 0.9%. Spiked plasma and RBC samples were prepared by addition of calculated amounts of the stock solutions to 100 µl of drug-free plasma and RBCs, resulting in calibration standards of 2, 5, 20, 50, 100 and 200 ng/ml CPT-11. Pools of quality control samples for CPT-11 (and SN-38) were prepared in human plasma and whole blood at the concentrations of 10, 75, 150 and 750 ng/ml.

Blood (12 ml) was collected from eight volunteers (two female smokers, two female non-smokers, two male smokers and two male non-smokers) into EDTA tubes and divided into 12 Eppendorf cups. Calculated amounts of CPT-11 were added to the cups to produce five different concentrations: 5, 50 and 500 ng/ml, and 5 and 50 µg/ml. After a 1 h incubation at 37°C, half the samples at 0.04% CO_2 (room air) and the other half at 20% CO_2, plasma and RBCs were separated by the measurement of sediment (MESED) device [9]. The different CO_2 conditions are important, as the physiology of the RBC is influenced *in vivo* by changes in CO_2 tension between the systemic and pulmonary circulations. All samples were frozen at −20°C until further analysis.

Sample pre-treatment and high-performance liquid chromatography (HPLC)

We used a method which was modified from an assay published previously [10]. HPLC was used and validated for the simultaneous determination of CPT-11 and its metabolite SN-38 in human plasma and RBCs. Camptothecin was used as an internal standard. As sample pre-treatment, 100 µl of HPLC-grade methanol was added to 500 µl of plasma together with 100 µl of internal standard solution. After vortexing, the solution was heated to 40°C for 15 min. An additional 200 µl of triethylamine acetate buffer was then added to the solution, which was centrifuged at 14 000 *g* for 5 min. Finally, the supernatant was filtered over a 0.2-µm PVDF HPLC filter and 20 µl was injected into the HPLC system. To 100 µl of RBC, 400 µl of distilled water and 100 µl of internal standard were added. After 5 min, the solution was vortexed, mixed and centrifuged at 14 000 *g* to precipitate the cell debris. The supernatant was treated the same way as described for plasma. Separation was achieved on a Waters (Milford, MA) Symmetry 300 C8 reversed-phase column (25 cm × 4.6 cm, 5 µm). The mobile phase consisted of 72% triethylamine acetate buffer (pH 5.2) and 28% acetonitrile at a flow rate of 1.5 ml/min. CPT-11 and camptothecin were detected by fluorescence with excitation and emission wavelengths of 369 and 424 nm, respectively. SN-38 was detected by fluorescence with respective excitation and emission wavelengths of 367 and 534 nm. The limits of quantitation of CPT-11 and SN-38 were 0.5 and 0.25 ng/ml, respectively. Within-run and between-run precisions were less than 10%, and average accuracies were 90–110%.

Results

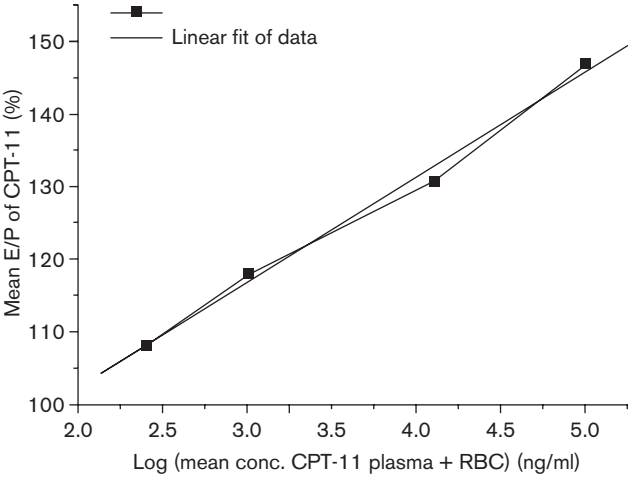
The partition ratio [the concentration in the erythrocytes divided by the concentration in plasma (E/P)] of CPT-11 varied between 0.7 and 2.8. The means of the partition ratios per incubation concentration were plotted against the mean whole-blood concentrations (RBC plus plasma) as shown in Figure 1. In the range studied, there was a linear relationship between the fraction of CPT-11 in the RBCs and the whole-blood concentration ($p = 0.0034$).

Partition ratios of CPT-11 were not different between men and women, and the percentage of CO_2 present during incubation had no effect. Surprisingly, we found relatively more CPT-11 in the erythrocytes of non-smokers, compared with smokers, suggesting a major impact of cigarette smoking on the affinity of the RBC for CPT-11 (Fig. 2, unpaired *t*-test on the whole group: $p = 0.015$).

Discussion

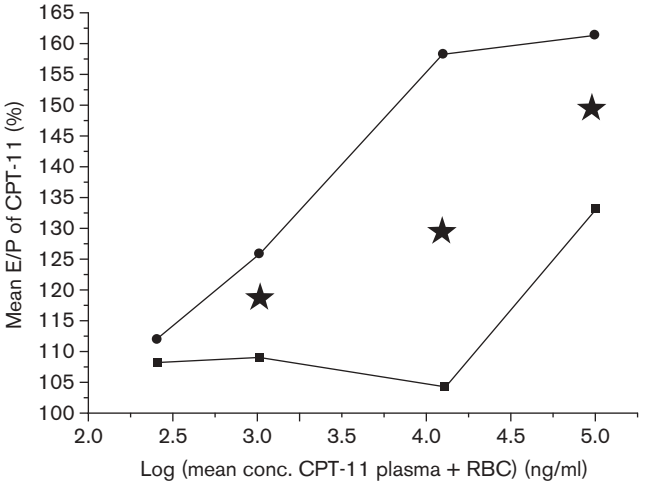
One of the primary goals of measuring drug concentrations is to relate the measurement to pharmacologic response and toxicity. However, only unbound drug can pass through cell membranes, because protein-bound drugs are usually too large. Accordingly, the unbound drug concentration and/or the cellular drug levels are more closely related to the activity of the drug than is the total plasma concentration. The camptothecin analogs, including CPT-11, share a pH- and protein-dependent reversible conversion between the pharmacologically active lactone form and its lactone-ring-opened carboxylate form. Since only the active lactone form is able to diffuse across cell membranes, RBCs can play an important role

Fig. 1



Mean E/P versus log total blood concentration of CPT-11 in human volunteer blood. Linear regression analysis of the relationship is described as $y = 72.26 + 1.45x$ ($p = 0.0034$).

Fig. 2



Mean E/P versus log total blood concentration of CPT-11 for volunteer non-smokers (circles) and smokers (squares). Significant differences between male and female E/P are indicated by $*p<0.05$ (Student's *t*-test, unpaired).

in its pharmacokinetic behavior. Our *in vitro* experiments aimed to determine the partition ratios of CPT-11 between RBCs and plasma. Since CPT-11 is not metabolized *in vitro*, there is no data on SN-38.

The partition ratios of CPT-11 vary between 0.7 and 2.8, indicating that the RBC has a relatively high affinity for CPT-11. This is compatible with a plasma protein binding of 65% for the parent drug, indicating a relatively large free fraction, able to diffuse into the RBCs [11]. In the range studied, there was a linear relationship between the quantity of CPT-11 in the RBCs and the whole-blood concentration ($p = 0.0034$). This phenomenon was also observed during incubation studies with docetaxel and gemcitabine [12]. This concentration-dependent rise in partition ratios of CPT-11 was not detected during our phase I studies with an oral formulation of CPT-11 [7,8], because the concentrations reached *in vivo* after oral dosing were much lower than the incubation concentrations in this study, which were based on the concentration range seen after i.v. administration. If the incubation concentrations above 500 ng/ml are excluded, this concentration effect is also lost.

The RBCs of smokers only load CPT-11 at much higher incubation concentrations compared with those of non-smokers. Perhaps arylamines in cigarette smoke, and other highly reactive intermediates present in the blood of smokers, prevent RBCs from binding CPT-11 [13,14]. In conclusion, the RBC pool contains relatively more CPT-11 compared with plasma, certainly at higher incubation concentrations. Erythrocytes can play an important role in the transport of drugs because of their number and long lifetimes (120–140 days). The importance of the cellular uptake of cytotoxic drugs has also been recognized with respect to tumor kill. The incorporation of drugs into RBCs may occur by passive diffusion, active transport or linkage to RBC surface proteins. They may be transported to tumor tissue and mobilized from the erythrocytes by different active or passive transport mechanisms. The higher concentrations of CPT-11 in the erythrocytes of non-smokers, compared with smokers, could help us understand the poorer response of smokers to chemotherapy. However, the clinical relevance of these findings is still unclear and needs further investigation.

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