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ORIGINAL ARTICLE

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Comparative brain tissue distribution of camptothecin and topotecan in the rat

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Abstract *Purpose*: The primary objective of this investigation was to compare the extent of brain distribution of the lactone and the carboxylate forms of camptothecin (CPT) and topotecan (TPT) in awake freely moving rats. *Methods*: The plasma concentration-time profiles of the lactone and the carboxylate forms of CPT and TPT were determined simultaneously after a single i.v. administration of the lactone form of each drug. Also, the brain extracellular fluid (ECF) concentration-time profiles were characterized utilizing the microdialysis technique. This technique allowed serial sampling of the brain ECF in awake rats. *Results*: CPT-lactone in plasma declined bi-exponentially with a terminal half-life of 102 ± 25.2 min. During the elimination phase, the plasma concentration of CPT-carboxylate was approximately ten times the concentration of CPT-lactone. The brain ECF to plasma distribution ratio measured as the ratio of the AUC in the brain ECF to the AUC in plasma was 0.51 ± 0.08 for CPT-lactone, and 0.26 ± 0.21 for CPT-carboxylate. The terminal half-life for TPT-lactone was 64.0 ± 9.4 min. During the elimination phase, the TPT-carboxylate concentration was higher than that of TPT-lactone but the carboxylate to lactone concentration ratio was much lower than that of CPT. The brain ECF to plasma distribution ratio was 0.38 ± 0.12 for TPT-lactone, and 0.21 ± 0.06 for TPT-carboxylate. *Conclusions*: CPT and TPT are distributed to the brain ECF most probably by passive diffusion across the blood-brain barrier. Although the brain ECF to plasma distribution ratio for CPT-lactone was higher than that for TPT-lactone, the brain ECF concentrations of TPT-lactone were significantly higher than the CPT-lactone brain ECF concentrations. The relatively high brain ECF to plasma distribution ratio of these two drugs makes them potential candidates for first-line treatment of CNS tumors.

Key words Camptothecin · Topotecan · Pharmacokinetics · Brain distribution · Microdialysis

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Introduction

Camptothecin (CPT) is a naturally occurring alkaloid isolated from the Chinese plant *Camptotheca acuminata* while topotecan (TPT) is a water-soluble semisynthetic derivative of CPT [27]. Both CPT and TPT are potent inhibitors of DNA topoisomerase I [15, 16], an effect that causes cell death due to the formation of cleavable complexes which can lead to DNA strand breaks [15–18, 28]. Camptothecins in general have been shown to possess antineoplastic activity against a wide variety of tumors including central nervous system (CNS) tumor xenografts [9, 10, 13].

CPT and TPT have an α-hydroxy-δ-lactone ring that can undergo reversible pH-dependent hydrolysis which leads to ring opening and the formation of the carboxylate form [29]. The carboxylate forms of CPT (CPT-carboxylate) and TPT (TPT-carboxylate) predominate under physiological conditions (pH 7.4) while the lactone forms (CPT-lactone and TPT-lactone) predominate under acidic conditions. The closed lactone ring is a required structural feature that allows CPT and TPT to interact with their target enzyme topoisomerase I. This makes the lactone structure of these drugs responsible for their biological activities [14, 26], and indicates that the effectiveness of these drugs will depend on the concentration of the biologically active lactone form at the site of action.

The primary challenge in the treatment of CNS neoplasms is to deliver the effective chemotherapeutic agent in its active form in a sufficient concentration to its

site of action. The major obstacles for the distribution of cytotoxic drugs into the CNS are the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (blood-CSF barrier) [7]. Direct introduction of antineoplastic agents into the CSF by intrathecal injection can achieve high CSF drug concentrations, but a sharp concentration drop between the CSF and the brain parenchyma has been observed [5]. These facts demonstrate the importance of developing strategies for improving the brain delivery of cytotoxic drugs and also for identifying effective antineoplastic agents with higher brain to plasma distribution ratios.

The CSF distribution of TPT has been studied in children during constant rate i.v. infusion [1], and in nonhuman primates after intravenous (i.v.) and intrathecal administration [3, 4, 25]. The CSF to plasma distribution ratio of TPT-lactone in children ranges from 0.1 to 0.59 after a 24-h i.v. infusion and from 0.11 to 0.86 after a 72-h i.v. infusion [1]. The average CSF to plasma distribution ratio of TPT-lactone has been found to be 0.3 in nonhuman primates which is higher than that of other antineoplastic agents such as etoposide and doxorubicin [3]. The CSF distribution of CPT after i.v. CPT-carboxylate administration has been found to be negligible [12], and there is absolutely no information about the brain tissue distribution of CPT and TPT.

The primary objective of this investigation was to compare the brain ECF distribution of CPT and TPT after a single i.v. bolus administration of each drug in awake freely moving rats. This was achieved by simultaneously studying the plasma and brain ECF pharmacokinetics of the lactone and the carboxylate forms of each drug after a single i.v. administration. Serial brain ECF concentrations of these cytotoxic drugs were determined utilizing the microdialysis technique. This is the first study to investigate the time-course of the brain tissue distribution of CPT and its derivatives.

Materials and methods

Chemicals and drugs

CPT-sodium (NSC-100880) and TPT (NSC-609699) were kindly provided by the National Cancer Institute (Bethesda, Md.) and were used as supplied. Liposyn III 20% was obtained from Abbott Laboratories (North Chicago, Ill.) and sodium heparin (1000 U/ml) from Elkins-Sinn (Cherry Hill, N.J.). Acetonitrile, methanol and chloroform were supplied from Burdick and Jackson Laboratory (Muskegon, Mich.). Monobasic sodium phosphate, and monobasic ammonium phosphate were obtained from J.T. Baker (Phillipsburg, N.J.). All solvents were of HPLC grade and chemicals were of reagent grade.

Plasma and brain ECF pharmacokinetics experiment

Animal preparation

Male Wistar rats weighing 300–350 g, purchased from Simonsen Laboratories (Gilroy, Calif.), were maintained on a 12-h light/dark cycle with Purina chow pellets and water *ad libitum* for at least 7 days before use in experiments. All operating procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985) and were approved by the institutional animal care and use committee. Surgical preparation was performed in two phases under aseptic conditions while the rats were anesthetized. Brain surgery for the placement of the microdialysis guide cannula was performed first, followed 5 days later by cannulation of the femoral artery and the femoral vein.

A guide cannula (CMA/Microdialysis, Acton, Mass.) was inserted with the aid of a stereotaxic apparatus (Model 963 David Kopf Instrument, Tujunga, Calif.) into the brain cortex while the animal was fully anesthetized. The position of the guide cannula was identified according to the coordinates of the rat brain atlas (AP +2 mm from pregma, ML ± 2 mm, DV −2 mm from dura) [23]. The guide cannula was fixed to the skull with acrylic dental cement with the help of small stainless steel screws attached to the skull. The skin was closed and the rat was allowed to recover for at least 5 days. After the recovery period, the femoral artery and femoral vein were cannulated according to procedures described previously in detail [19]. The cannulae were passed through a tether attached to an anchor button sutured in the back of the neck of the rat. The femoral vein and femoral artery cannulae were connected to a three-channel swivel. The microdialysis probe (CMA-12; CMA/Microdialysis, Acton, Mass) was inserted into the guide cannula and the probe inlet was connected to the third channel of the swivel.

The rats were allowed to recover for at least 2 days before the experiment. During the recovery period, the femoral vein and femoral artery cannulae were filled with heparinized saline (50 U/ml) to avoid blood clotting. Also, the microdialysis probe was continuously perfused with simulated CSF (5 m*M* KCl, 120 m*M* NaCl, 1.4 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 5 m*M* glucose, and 0.2 m*M* phosphate-buffered saline to adjust the pH to 7.4 and the sodium concentration to 120.7 m*M*) at a rate of 1 µl/min. This recovery period after the insertion of the microdialysis probe was necessary to allow enough time for the tissue surrounding the tip of the probe to heal before the actual microdialysis experiment [2].

Drug administration

CPT and TPT solutions for i.v. administration were freshly prepared just before administration. An accurately weighed amount of the drug (5 mg) was dissolved in 0.5 ml i.v. fat emulsion Liposyn III 20%. The fat emulsion was acidified by the addition of 0.5 ml McIlvaine's buffer (pH 2.2), and the mixture was vortexed for 10 min. This acidification step was necessary to make sure that the drugs were entirely in the biologically active lactone form. Just before drug administration, an aliquot of the drug solution for i.v. injection was diluted (1:100 000) with phosphate buffer, pH 7.4, and the resulting solution was injected immediately into an HPLC system used for drug analysis. All the injected solutions showed a single peak corresponding to the lactone form of the drug. This was an important confirmation step to ensure that all the administered drug was in the active lactone form.

Pharmacokinetic experiment

Two groups each of six rats were used to investigate the plasma and brain pharmacokinetics of CPT and TPT, one group for CPT and the second for TPT. Each rat received a single i.v. dose of 10 mg/kg of drug. The drug solution for i.v. injection was prepared as described above and was administered slowly via the femoral vein cannula over a period of 15 min. Ten blood samples each of 0.1 ml were collected through the femoral artery cannula at 5, 35, 65, 95, 125, 155, 185, 215, 245, and 305 min after drug administration into heparinized tubes. The effluent of the microdialysis probe was collected every 30 min directly into HPLC autosampler vials, and 20 µl was injected immediately into the HPLC system for drug determination. This sampling schedule was chosen so that brain microdialysis samples and blood samples could be analyzed immediately after collection.

Stability of the drugs in the simulated CSF

The stability of the lactone and the carboxylate forms of CPT and TPT in the simulated CSF solution used in the brain microdialysis experiment was investigated. This experiment was performed to determine the extent of conversion from one form of the drug to the other during the microdialysis sample collection period. Three different drug solutions of the carboxylate, the lactone and a mixture of both (carboxylate + lactone) were prepared by spiking three different aliquots of simulated CSF solutions with known quantities of the appropriate drug form to produce a final concentration of approximately 40 ng/ml in each solution. The resulting drug solutions in simulated CSF were analyzed for the concentration of both forms of the drug over a period of 1 h. Drug analysis was achieved by direct injection into the HPLC system as described below. This experiment was performed in triplicate.

Sample analysis

The sample preparation procedures were similar for CPT and TPT. Blood was centrifuged immediately after collection for 2 min and then 50 µl plasma was transferred to a clean tube. Plasma was treated with 200 µl of a methanol acetonitrile mixture (1:2 v/v), vortexed for 10 s to precipitate the plasma protein, and then 50 µl phosphate buffer, pH 7.4, was added. The mixture was centrifuged for 2 min and then 20 µl of the supernatant was injected immediately into the HPLC system. The time from sample collection to sample injection into the HPLC system did not exceed 5 min to decrease the possibility of conversion of one form of the drug to the other.

The mobile phase for CPT analysis consisted of 25% v/v acetonitrile in 50 m*M* monobasic ammonium phosphate, and the flow rate was 0.5 ml/min. Separation was achieved using a Supelcosil ABZ + plus column (25 × 0.21 cm, 5 µm; Supelco, Bellefonte, Pa.). The column effluent was monitored using an RF-535 Shimadzu fluorescence detector (Shimadzu Scientific Instrument, Columbia, Md.). The detector was set at an excitation wavelength of 370 nm and an emission wavelength of 435 nm. The peak heights were calculated using an HP 3395 electronic integrator (Hewlett Packard, Palo Alto, Calif.). The retention times for CPT-carboxylate and CPT-lactone under the described chromatographic conditions were 4.2 min and 10.7 min, respectively. The chromatographic conditions for TPT analysis, were similar except that the percent of acetonitrile in the mobile phase was 14% and the fluorescence detector was set at an excitation wavelength of 380 nm and an emission wavelength of 520 nm. The retention times for the carboxylate and the lactone forms of TPT under the described chromatographic conditions were 5.0 min and 11.6 min, respectively.

Different standard curves were constructed for the lactone and the carboxylate forms of each drug. This was because the fluorescence emission-concentration relationship is different for the lactone and the carboxylate forms of each drug. Also this allowed us to determine the extent of conversion from one form of the drug to the other during the sample preparation procedures. The drug concentrations in unknown samples were determined by comparing the peak heights in the unknown samples with the peak heights obtained in the standard curve. The standard curves were linear over the entire concentration range (10–2000 ng/ml for CPT-lactone and CPT-carboxylate and 40–2000 ng/ml for TPT-lactone and TPT-carboxylate) with a coefficient of determination > 0.998. This method was sensitive enough to quantitate CPT-lactone and CPT-carboxylate at concentrations as low as 10 ng/ml and TPT-lactone and TPT-carboxylate at concentrations as low as 40 ng/ml in 50 µl plasma samples. The inter- and intraday coefficients of variation of these analytical procedures were lower than 15%.

The microdialysis probe effluent was analyzed for drug content immediately after collection by direct injection into the HPLC system using the chromatographic conditions describe above. The drug concentration in the probe effluent was determined by comparing the peak heights in the unknown samples with the peak heights obtained in the standard curve constructed by spiking the simulated CSF with known quantities of the drug in the range 2–1000 ng/ml. Again, separate standard curves were constructed for the carboxylate and the lactone forms of each drug. The brain ECF concentration of each form of the drug was calculated from the probe effluent concentration after correction for probe recoveries. The probe recoveries for each form of each drug were determined from in vitro microdialysis experiments [20]. The average recoveries of the microdialysis probe were 23.1 ± 3.4, 24.9 ± 2.1, 28.5 ± 4.2, and 24.6 ± 2.4 for CPT-carboxylate, CPT-lactone, TPT-carboxylate, TPT-lactone, respectively.

Pharmacokinetic analysis

The plasma concentration-time profile in each animal was fitted to a two-compartment pharmacokinetic model by nonlinear regression analysis using the reciprocal of the measured concentration ($1/y$) as the weighting function. The pharmacokinetic parameters including the distribution and elimination half-lives, volume of distribution and the total body clearance (TBC) for the drugs were determined by nonlinear regression analysis utilizing PCNONLIN version 4.0 (SCI statistical consultant, Edgwood, ky.). The areas under the curve (AUC) of each drug moiety from time 0 to 5 h after drug administration (AUC_{0-5}) were calculated by the linear trapezoidal rule and extrapolated to time infinity to obtain $AUC_{0-\infty}$ [11]. The brain ECF to plasma distribution ratio was determined from the brain ECF to plasma AUC ratio of each drug form.

Results

Analysis of the lactone standards of both CPT and TPT did not show any measurable peak for the carboxylate form, and the same was true for the carboxylate standards where no measurable peaks for the lactone form were observed. This indicates that the sample preparation procedures did not result in significant interconversion of the drug forms. The drug stability experiment showed that there was no significant conversion of lactone to carboxylate and vice versa during the microdialysis sample collection period for either CPT or TPT. Since the microdialysis samples were collected every 30 min, the mean residence time for each sample in the collection vial was 15 min. The two drugs CPT and TPT were chemically stable in the buffered simulated CSF solution, but a decrease in the concentration of one form of the drug resulted in an increase in the concentration of the other form. The sum of the amounts of the lactone and the carboxylate forms of the drug at any time during the entire experiment period accounted for the initial amount of the drug added.

The average decrease in the CPT-lactone concentration after 15 min was 4% while the average decrease in the CPT-carboxylate concentration was 3% for the solutions containing the individual forms of the drugs. The ratio CPT-carboxylate/CPT-lactone increased by 4% in the solution containing the mixture of both CPT forms during the same period of time. For TPT, the average decrease in the TPT-lactone concentration was 7% and in the TPT-carboxylate concentration was 6% in the solutions containing the individual forms of TPT. The ratio TPT-carboxylate/TPT-lactone increased by 3% in the solution containing the mixture of both TPT forms during the first 15 min. This stability experiment was performed at room temperature because this is the temperature at which the probe effluent was collected.

The plasma concentration-time profile of CPT-lactone declined biexponentially after i.v. administration with a distribution half-life of 9.6 ± 1.84 min and an elimination half-life of 102 ± 25.1 min. The pharmacokinetic parameters of CPT-lactone obtained from fitting its plasma profile to the two-compartment pharmacokinetic model are summarized in Table 1. CPT-carboxylate appeared rapidly in the plasma with the highest concentration observed in the first sample drawn 5 min after CPT-lactone administration. The plasma concentration-time profile of CPT-carboxylate declined in parallel with the profile of the lactone, but the carboxylate concentrations were on average about ten times the CPT-lactone concentrations during the elimination phase. The ratio of the total AUC of CPT-carboxylate to that of CPT-lactone in plasma was 1.91 ± 0.49. Representative examples of the plasma concentration-time profiles of CPT-lactone and CPT-carboxylate after i.v. administration of 10 mg/kg CPT-lactone are shown in Fig. 1A.

Both CPT-lactone and CPT-carboxylate appeared in the brain ECF with the maximum concentrations achieved between 30 and 60 min for both forms of the drug. The brain ECF concentration of the two drug forms declined in parallel with the plasma profiles. However, the concentration of the CPT-lactone in the brain ECF was always higher than the concentration of CPT-carboxylate. The brain ECF to plasma distribution ratios measured as the ratio of the brain ECF AUC to the plasma AUC were 0.51 ± 0.08 and 0.26 ± 0.21 for CPT-lactone and CPT-carboxylate, respectively. Representative examples of the brain ECF concentration-time profiles of CPT-lactone and CPT-carboxylate are shown in Fig. 1B.

After i.v. administration of TPT, the plasma concentration-time profile of TPT-lactone declined biexponentially with a distribution half-life of 9.5 ± 2.3 min and an elimination half-life of 64.0 ± 9.4 min. The pharmacokinetic parameters of TPT-lactone are summarized in Table 1. TPT-carboxylate appeared in the plasma with the highest concentration observed 35 min after TPT-lactone administration. This indicates a slower rate of conversion of TPT-lactone to TPT-carboxylate than of CPT-lactone. The plasma concentration-time profile of TPT-carboxylate declined in parallel with the profile of TPT-lactone with TPT-carboxylate concentrations remaining higher than TPT lactone concentrations during the elimination phase. However, the TPT-carboxylate/TPT-lactone plasma ratio during the elimination phase was not as high as the CPT-carboxylate/CPT-lactone plasma concentration ratio. The ratio of the total AUC of TPT-carboxylate to that of TPT-lactone in plasma was 0.90 ± 0.46. Representative examples of the plasma concentration-time profiles of TPT-lactone and TPT-carboxylate after administration of 10 mg/kg TPT-lactone are shown in Fig. 2A.

TPT-lactone and TPT-carboxylate appeared in the brain ECF with the maximum concentrations achieved

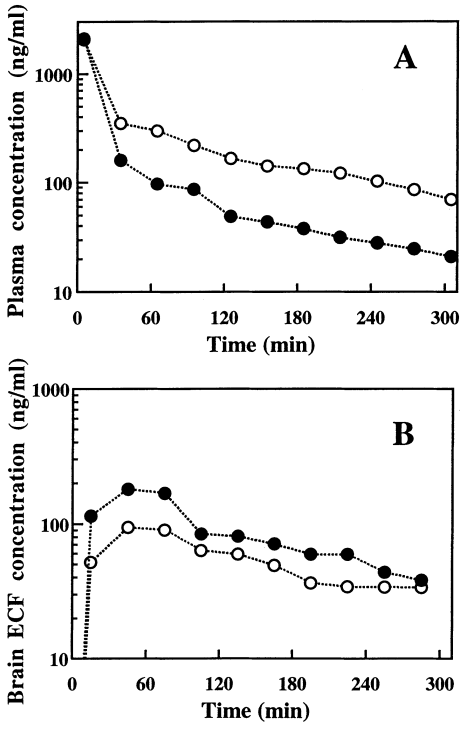

Fig. 1A,B A representative example of the plasma concentration-time profile (**A**) and the brain ECF concentration-time profile (**B**) of (●) CPT-lactone and (○) CPT-carboxylate in a rat after i.v. administration of 10 mg/kg CPT-lactone

Table 1 Pharmacokinetic parameters of CPT-lactone and TPT-lactone in rats after a single i.v. bolus doe of 10 mg/kg. All the presented parameters are for the lactone form of the drug. Values are means ± SD ($n = 6$). ($\alpha_{t1/2}$ distribution half-life, $\beta_{t1/2}$ elimination half-life, V_c volume of the central compartment, Vd_β volume during the elimination phase, *TBC* total body clearance, *AUC* area under the curve)

Parameter	Camptothecin	Topotecan
$\alpha_{t1/2}$ (min)	9.6 ± 1.84	9.4 ± 2.34
$\beta_{t1/2}$ (min)	102 ± 25.2	64.0 ± 9.4
V_c (l/kg)	3.65 ± 0.54	1.7 ± 0.41
Vd_β (l/kg)	25.0 ± 4.6	6.38 ± 2.0
TBC (ml/min)	59 ± 6.3	22.1 ± 4.8
AUC_{plasma} (μg min/ml)	68.1 ± 8.7	186 ± 62.7
$AUC_{brain\ ECF}$ (μg min/ml)	34.2 ± 4.1	71.6 ± 39.2
$AUC_{brain\ ECF}/AUC_{plasma}$	0.51 ± 0.08	0.38 ± 0.12
Lactone/carboxylate plasma ratio	1.91 ± 0.49	0.90 ± 0.46

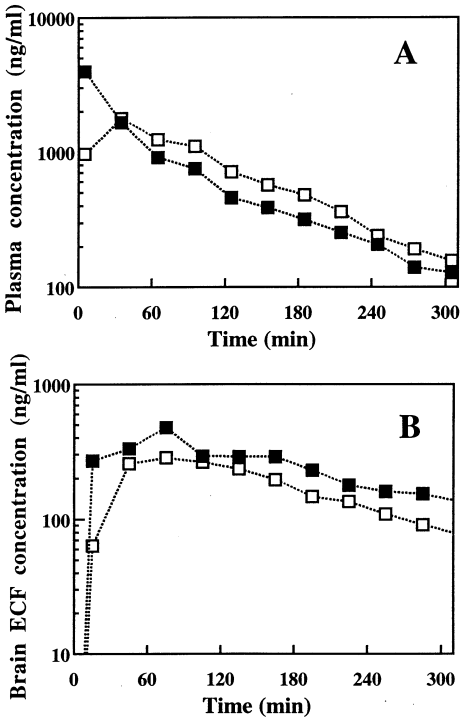

Fig. 2A,B A representative example of the plasma concentration-time profile (**A**) and the brain ECF concentration-time profile (**B**) of (■) TPT-lactone and (□) TPT-carboxylate in a rat after i.v. administration of 10 mg/kg TPT-lactone

between 60 and 90 min for both forms of the drug. The brain ECF concentration of the two drug forms declined in parallel with each other and both paralleled the corresponding profiles in plasma. The concentrations of TPT-lactone in the brain-ECF were always higher than the concentrations of TPT-carboxylate. The brain-ECF to plasma distribution ratios measured as the ratio of the brain ECF AUC to the plasma AUC were 0.38 ± 0.12 and 0.21 ± 0.06 for TPT-lactone and TPT-carboxylate, respectively. Representative examples of the brain ECF concentration-time profiles of TPT-lactone and TPT-carboxylate are shown in Fig. 2B.

Discussion

Investigation of the pharmacokinetics of CPT and its derivatives requires analytical procedures that can accurately quantitate both the lactone and the carboxylate forms of the drug in biological fluids. The sample preparation procedures should not cause conversion of one form of the drug to the other. This second requirement complicates tissue distribution experiments of this group of drugs because tissue sample preparation requires lengthy procedures that can result in significant conversion of one form of the drug to the other. In the current investigation the analytical procedures allowed simultaneous determination of the lactone and the carboxylate forms of both CPT and TPT with minimal interconversion between the forms.

The current study is the first study in which the brain tissue distribution of CPT and TPT has been investigated, which is important to determine the effectiveness of these drugs in treating CNS tumors. The microdialysis technique used to study the brain ECF distribution was ideal for these drugs because the effluent of the microdialysis probe was injected directly into the HPLC system immediately after collection. The stability of CPT and TPT in the simulated CSF showed that the ratio between the two drug forms did not change significantly during the sample collection period. This is despite the fact that the pH of the simulated CSF was 7.4, and at this pH the equilibrium between the two drug forms is shifted toward the carboxylate form [8, 24]. This can be explained by the fact that simulated CSF solution does not include protein and the conversion of the lactone form to the carboxylate form proceeds at a much slower rate in the absence of protein [6, 24]. This minimal conversion of one form of the drug to the other makes the microdialysis technique a suitable tool for studying the tissue distribution of CPT and its derivatives.

The plasma concentration-time profile of CPT-lactone declined biexponentially after i.v. administration. CPT-lactone was rapidly converted to CPT-carboxylate in plasma as reported previously [24]. The highest carboxylate concentration observed in the sample was obtained 5 min after of CPT-lactone administration. Although the entire dose was in the form of CPT-lactone as confirmed by the HPLC analysis of the injection solution, the AUC of CPT-carboxylate was twice the AUC of CPT-lactone. During the elimination phase an equilibrium between the lactone and carboxylate forms of CPT was established and both forms declined at the same rate. CPT was predominantly in the carboxylate form during the elimination phase with the carboxylate concentration about ten times the lactone concentration. The protein binding of CPT-carboxylate in rat plasma is much higher than the plasma protein binding of CPT-lactone [24]. This is similar to the relative protein binding of the two CPT forms reported in humans [21]. The higher affinity of CPT-carboxylate to plasma protein shifts the equilibrium towards the carboxylate form and can explain the high carboxylate to lactone concentration ratio during the terminal phase in our rat experiments.

The brain-ECF concentration of CPT-lactone was higher than the concentration of CPT-carboxylate despite the higher plasma CPT-carboxylate concentration. There may be several explanations for this higher CPT-lactone concentration in the brain ECF. CPT-lactone is more lipophilic that CPT-carboxylate and this higher lipophilicity may be responsible for the higher brain ECF distribution. It is also possible that CPT-lactone is distributed into the brain ECF and CPT-carboxylate results from conversion of the CPT-lactone to the carboxylate form in the brain ECF. The finding of significant distribution of CPT into the brain ECF does not contradict previous reports of negligible CPT CSF distribution after CPT-carboxylate administration [12].

This is because after CPT-carboxylate administration the plasma CPT-lactone concentration is very low [24], and the less lipophilic CPT-carboxylate has limited brain distribution.

The plasma concentration-time profile of TPT-lactone declined biexponentially after i.v. administration. The conversion of TPT-lactone to TPT-carboxylate in plasma was slower than the conversion of CPT-lactone, with the maximum TPT-carboxylate concentration observed 35 min after TPT-lactone administration. The plasma profile of TPT-carboxylate declined in parallel with the profile of the lactone. During the elimination phase an equilibrium was established between the two TPT forms with the carboxylate concentration always slightly higher than the lactone concentration. The average TPT-carboxylate to TPT-lactone AUC ratio was 0.90 which is significantly lower than the ratio for CPT. This can be explained by the significantly lower protein binding of TPT-carboxylate than of CPT-carboxylate [22]. Overall, TPT had a lower TBC than CPT primarily because of a significantly smaller volume of distribution of TPT. This resulted in significantly higher concentrations of TPT in the plasma compared with CPT after administration of the same dose.

The brain-ECF concentration of TPT-lactone was higher than the concentration of TPT-carboxylate despite the higher plasma TPT-carboxylate concentrations. This resulted from the higher lipophilicity of TPT-lactone than of TPT-carboxylate. This significant penetration of TPT-lactone into the brain ECF is approximately similar to the penetration of TPT-lactone into the CSF in children [1], and to its penetration into the CSF in nonhuman primates [3, 25]. This high brain-ECF penetration suggests that TPT-lactone is distributed into the brain tissues across the BBB. This is because, if the drug is distributed only into the CSF and then diffuses across the brain tissues, a sharp drop in drug concentration is usually observed in brain tissues [5].

This investigation showed for the first time that CPT is distributed to brain tissues after a single i.v. administration. Also, TPT is distributed to brain tissues in addition to its CSF distribution reported previously [1, 3, 25]. The brain-ECF to plasma distribution ratios of the pharmacologically active CPT-lactone and TPT-lactone were 0.51 ± 0.08 and 0.38 ± 0.12, respectively. Despite the lower brain ECF to plasma distribution ratio for TPT-lactone, its AUC in brain ECF which reflects the absolute concentrations was nearly twice as much that of CPT-lactone in brain ECF. This resulted from the significantly higher plasma TPT-lactone concentration than plasma CPT-lactone concentration. The brain ECF to plasma distribution ratios of CPT and TPT are much higher than the CNS distribution of other chemotherapeutic agent. Compared to CSF concentrations, the brain ECF concentrations should be a better indicator of the brain tumor concentrations. So, the results obtained in the current investigation indicate that these drugs are very good candidates for the treatment of CNS tumors.

In summary, the microdialysis technique followed by immediate sample analysis was shown to be suitable for the simultaneous determination of the lactone and the carboxylate forms of CPT and its derivatives in tissue samples with minimal experimental errors. Both CPT and TPT are distributed to the brain ECF most probably by distribution across the BBB. The relatively high brain ECF to plasma distribution ratio of these two drug makes them suitable for the treatment of CNS tumors.

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