Themed Issue: The Role of Microdialysis in Pharmacokinetics and Pharmacodynamics
Guest Editors - Markus Mueller and Ronald J. Sawchuk

In Vivo Microdialysis for PK and PD Studies of Anticancer Drugs

Submitted: June 17, 2005; Accepted: July 12, 2005; Published: October 24, 2005

Qingyu Zhou[1] and James M. Gallo[1]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140

ABSTRACT

In vivo microdialysis technique has become one of the major tools to sample endogenous and exogenous substances in extracellular spaces. As a well-validated sampling technique, microdialysis has been frequently employed for quantifying drug disposition at the desired target in both preclinical and clinical settings. This review addresses general methodological considerations critical to performing microdialysis in tumors, highlights selected preclinical and clinical studies that characterized drug disposition in tumors by the use of microdialysis, and illustrates the potential application of microdialysis in the assessment of tumor response to cancer treatment.

KEYWORDS: microdialysis, in vivo sampling, tumors, drug distribution, pharmacokinetics

INTRODUCTION

Cancer chemotherapy is an integral component of cancer therapy. Although not considered curative, advances in drug discovery, targeted therapy, and the improved characterization of the PK (PK) and PD (PD) properties of anticancer drugs indicate drug treatment will be a critical cornerstone in the war on cancer. Drugs fail in cancer patients for numerous reasons that in some cases can be attributed to PK and PD failures such as the development of drug resistance and inadequate tumor drug concentrations.[1,2] It is generally assumed that pharmacological effects are related to the drug concentration in a target tissue. Since the relationship between plasma concentrations and pharmacological effects has been successfully defined for a variety of medications in different therapeutic classes, plasma concentrations are commonly used as a surrogate for drug concentrations at other sites.[3] However, the microenvironment within tumors is significantly different from that within normal tissues. Many factors such as heterogeneous tumor blood flow, vascular permeability, and cell density, as well as increased interstitial pressure, may hamper the penetration and delivery of drugs from plasma into the tumor and distribution within the tumor, leading to complex relationships between concentrations in plasma, interstitium, and neoplastic cells.[4-6] Therefore, given the complexities of drug accumulation in tumors, it is likely that tumor-specific drug concentration measurements will be of greater value, compared with plasma drug concentrations, as an indicator of drug action and clinical response.

Preliminary evidence has suggested that concentrations of chemotherapeutic drugs in a tumor may correlate with the response to chemotherapy.[7,8] Therefore, it would be ideal if the time course of a given drug and/or its metabolite(s) could be determined in tumors. Initially, this task was often approached by analysis of drug concentrations in tumor homogenates, which involved killing a series of animals at multiple discrete time points following drug administration.[9] Besides considerable resources needed to conduct such studies, the main drawback of this postmortem technique is that drug concentrations determined in tumor homogenates only reflect an average of vascular, interstitial, and intracellular drug concentrations, which invariably limit evaluation of site-specific phenomenon.

Recently, noninvasive techniques including positron emission tomography (PET)[10,11] and magnetic resonance spectroscopy (MRS)[12,13] have been adopted to allow the intensity and duration of tumor exposure to therapeutic agents to be monitored continuously in a single animal. However, those noninvasive alternatives are not readily available and may not offer either the desired sensitivity, or specificity.

The development of microdialysis has provided researchers with a specific tool to sample extracellular spaces for endogenous and exogenous compounds. This in vivo sampling technique is based on the passive diffusion of substances across a semipermeable membrane, which is usually fashioned into a probe so that the interior of the probe can be perfused with a suitable carrier solution.[14,15] Microdialysis was originally developed to allow in vivo sampling of neurotransmitters in the brain.[16] Over the last decade, in vivo microdialysis has found increasing application in PK and drug metabolism studies, particularly in the area of monitoring drug disposition at peripheral tissue sites; microdialysis

Corresponding Author: James M. Gallo, Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140. Tel: 215-707-9699; Fax: 215-707-9409; E-mail: jmgallo@temple.edu

probes can be placed in virtually any tissue, organ, or biological fluid, and the collected fractions can be analyzed by the use of the most suitable analytical techniques.[17,18]

There is growing interest in using microdialysis methodology to evaluate the disposition of anticancer agents in tumor xenografts and in patients with accessible tumors.[19-21] Sampling by this technique has several advantages over the postmortem and noninvasive imaging techniques mentioned above. First, continuous sampling can be performed in a single animal subject over a prolonged period, thereby allowing fewer animals to be used and minimizing the interanimal variability. Second, only extracellular protein-unbound or active drugs in the direct vicinity of neoplastic cells are sampled and measured. Free drug concentrations in the interstitial compartment determined over time can readily support the development of physiologically based PK models. Third, microdialysis sampling can be used for a broad range of substances, including the majority of small molecules and some macromolecules, in contrast with noninvasive techniques, which are often limited by low spatial resolution, high costs associated with the synthesis of radiolabeled compounds, and the performance of imaging studies.

In the following review, considerations of using microdialysis methodology for PK and PD studies are examined with an emphasis on its application to understanding drug disposition and response in solid tumors. In addition, potential advances offered by microdialysis that may be applied to problems in PK and PD research of anticancer drugs are discussed.

Considerations of Using Microdialysis Methodology for Pharmacokinetic Studies

Recovery

It has long been recognized that drug equilibration across the microdialysis membrane, separating interstitial fluid and the perfusion medium, is a dynamic process and requires a calibration technique to estimate actual interstitial fluid drug concentrations.[22,23] This combined with other factors indicates that microdialysate concentrations of a substance reflect only a fraction of the unbound concentrations in the extracellular fluid surrounding the implanted probe. The ratio of the microdialysate concentration to the actual interstitial concentration of a substance is defined as relative recovery, while the total amount recovered per time unit is termed absolute recovery.[24] Determination of absolute recovery may be enough to make valid comparisons between the data obtained. However, if accurate estimations of extracellular concentrations are desired, relative recovery has to be determined. Both in vitro and in vivo methods have been used to assess relative recovery in microdialysis experiments.[25-33] The main determinants of in vitro and in vivo recoveries are summarized in Table 1. In addition to the main factors that influence the in vitro recovery, such as membrane properties, perfusate flow rate, and physicochemical properties of the analyte, the in vivo performance of a microdialysis probe is also dependent on the surrounding tissue properties and the interaction between the analyte of interest and the tissue to be sampled.[34-36] In this regard, results from the in vivo method are more reliable and allow better estimation of actual extracellular concentrations of a given analyte as compared with those from the in vitro method.

In vivo calibration

To date, various in vivo calibration methods have been used to assess relative recovery in in vivo microdialysis experiments. Among those established methods, the simplest one is retrodialysis. This technique is based on the assumption that the mass transport efficiency over the membrane is the same in both directions, and that recovery is independent of the perfusate concentrations[32]:

$$RR = 1 - \frac{C_{out}}{C_{in}}, \qquad (1)$$

where RR is the relative recovery, C_{in} is the analyte concentration in the perfusate, and C_{out} is the analyte concentration in the dialysate.

The further elaboration of the retrodialysis method is retrodialysis using an internal reference, which consists of adding a marker to the perfusate.[27,29-31] The marker should be a compound with diffusion characteristics close to the analyte

Table 1. Determinants of In Vitro and In Vivo Recoveries of Microdialysis Probes

	Recovery	
Determinant	**In Vitro**	**In Vivo**
Probe geometry	•	•
Membrane pore size	•	•
Concentration gradient over the membrane	•	•
Flow rate of the perfusate	•	•
Temperature	•	•
pH	•	•
Binding of the substance to the membrane or outlet tubing	•	•
Physicochemical properties of the analyte	•	•
Tortuosity of the interstitial space		•
Volume of the interstitial compartment		•
Transport capacity over the cell membrane or certain physiological barriers (eg, blood-brain barrier)		•
Various release, uptake, and clearance processes		•

of interest. The recovery is determined by measuring the relative loss of the analyte diffusing from the perfusate into the extracellular fluid (ECF). Application of this method may be hindered by the availability of a substance with diffusion properties similar to the analyte, and the potential competition or interference of the internal standard with the disposition of the analyte.

A more comprehensive method for the in vivo calibration introduced by Lönnroth et al[25] is the zero net flux method, which is based on the principle that the net direction of diffusion over the microdialysis membrane follows the concentration gradient. This method, which consists of the determination of mass transport of the analyte across the microdialysis membrane as a function of perfusate concentrations, is often found to be time-consuming as it requires steady-state conditions:

$$RR = \frac{(C_{in} - C_{out})}{(C_{in} - C_m)}, \quad (2)$$

where C_m is the concentration of the extracellular fluid surrounding the dialysis probe, which is given by the intercept on the C_{in}-axis of the C_{in}-C_{out} versus C_{in} plot.

The "zero flow" method is another comprehensive method used to estimate extracellular concentrations at steady-state by extrapolation of dialysate concentrations at various flow rates to the concentration at zero flow.[37] This method possesses about the same advantages and disadvantages as the zero net flux method.

$$RR = 1 - e^{\frac{-k}{\Phi}}, \quad (3)$$

where k is a constant and Φ is the flow rate of the perfusate.

Approaches other than the above-mentioned methods have also been used for in vivo calibration of microdialysis probes[25,26,28]; however, all methods suffer one or more disadvantages. Therefore, the choice of an appropriate calibration method should be made based on the specific study design to ensure accurate estimation of the microdialysis probe recovery.

Recovery of macromolecules

The utility of the microdialysis technique is not limited to sampling small molecules. The current development of larger molecular weight cutoff microdialysis probes (up to 100 kDa) has made it possible to sample macromolecules, including certain proteins, which can act as valuable markers of drug response. However, increased porosity of the semipermeable membrane can quickly cause a solution balance problem. In other words, the membrane becomes more susceptible to water loss due to osmotic flux.[17,36] To counteract this osmotic flux, an osmotic agent can be added into the perfusate as a means to counterbalance the transmembrane hydrostatic driving pressure.

In a study by Trickler and Miller,[38] bovine serum albumin (BSA) was used as an osmotic agent in the perfusate to prevent the fluid loss from the microdialysis probe when attempting to dialysis molecules of up to 53 kDa. By adjusting the BSA content of phosphate-buffered saline (PBS) as a function of the flow rate of the perfusate, fluid loss due to convective flow out of the probe was completely abolished. Moreover, the addition of BSA to the perfusate improved the in vitro recovery of 2 cytokines being tested (ie, interleukin-1 beta [IL-1B] and rat tumor necrosis factor [TNF]) with the molecular weight of 17.3 and 53 kDa, respectively.

Another osmotic agent often used to improve recovery of macromolecules is dextran. In a study by Dabrosin et al,[39] with the addition of 4% (wt/vol) of dextran, the mean recovery of vascular endothelial growth factor (VEGF) at room temperature was 6% at a flow rate of 0.6 μL/min. The amount of VEGF present in the microdialysate collected in vivo was well above the lower limits of quantitation of an enzyme-linked immunosorbent assay (ELISA). Similarly, by using a perfusate containing Ringers Dextran 60[40] or dextran-70,[41] reliable sample volume and high analyte recovery were achieved by Sjogren et al[40] for the in vivo microdialysis determination of interleukin-6 in human dermis.

Invasiveness of Microdialysis Probe Implantation

Microdialysis is an invasive technique and has the potential to cause adverse tissue reactions that may influence the measurements and interpretation of the data. The invasiveness of peripheral microdialysis has been examined in different tissues, including dermis,[42] liver,[43] muscle, and tumor.[44] The extent of tissue damage due to probe implantation was evaluated by histological examination as well as by microdialysis delivery studies. Expected acute inflammatory response was observed in dermis, liver, and muscle, with the initial invasion of neutrophils followed by macrophages, which generally had no effect on the performance of the implanted microdialysis probes.[42-44] For intracerebral microdialysis, the perturbation in normal blood-brain barrier (BBB) transport function by the implantation of guide cannulas and/or microdialysis probes may lead to overestimation of the rate of transfer into and out of the brain.[45-47] One way to attenuate the damage may be to leave a few days for experimental animals to reestablish the barrier properties before microdialysis is conducted. Even in the event of tissue damage that could alter analyte recovery, a comparative study design should negate such effects as each treatment group would be similarly affected.

Application of Microdialysis in Investigation of Drug Disposition in Tumor

Quantification of drug delivery to the tumor tissue using microdialysis is an attractive way to assess if sufficient

amounts of anticancer drugs can reach their targets. Our previous review has provided comprehensive information on the potential applications of microdialysis in characterization of drug disposition in tumor.[19] In the present review, we provide an overview of representative studies published over the past 5 years and highlight the contributions of microdialysis technique to the development of physiologically based PK models, which may eventually predict anticancer drug concentrations in human tumors.

Preclinical Studies

Microdialysis has seen extended applications in preclinical PK studies aimed to characterize tumor disposition of both conventional and novel antineoplastic agents,[48-53] evaluate novel formulations,[54] and scrutinize potential drug-drug interactions at the site of action in a wide range of tumor models.[55,56]

Microdialysis has been used to characterize local distribution in brain tumors of 4-pyridoxate diammine hydroxy platinum (PyPt), a novel cisplatin derivative, using rats implanted with 9L glioma.[53] In this study, 2 microdialysis probes were placed so that microdialysates from ECF in tumor and normal brain tissues could be collected simultaneously. By using this dual-probe approach, tissue disposition of the drug in both tumor and neighboring normal regions can be examined in the same brain, and comparative drug disposition data obtained from the same animal. The result of this study showed that unbound platinum (Pt) released from PyPt insufficiently penetrated the BBB into normal interstitial space, whereas the uptake of unbound Pt in the 9L glioma was significantly higher than that in the normal brain tissue owing to the compromised BBB. Although it was not determined, it would be assumed for accurate comparison of the data from these 2 sites that implantation trauma would be equivalent in normal brain and brain tumor. Barring the technical difficulties of performing microdialysis at multiple sites in the brain, the strategy is appealing.

The ability of microdialysis sampling to selectively quantify drug disposition in tumors renders it a unique approach that can provide valuable information on drug delivery systems, drug interactions, and drug transport. Zamboni et al[54] measured the unbound (Pt) concentrations in tumor interstitium from 2 probes placed in both right and left side of the tumor after administration of cisplatin and its 2 different liposomal formulations, SPI-077 and SPI 077 B103, to 3 groups of mice bearing B16 murine melanoma tumor, respectively. The peak concentrations of total Pt measured in tumor homogenates were 2.2- to 5-fold higher after administration of SPI-077 and SPI-077 B103 than after administration of cisplatin. The time-to-peak concentrations of total Pt in tumor were longer after administration of SPI-077 (48 hours) and SPI-077 B103 (96 hours) than after administration of cisplatin (0.5 hours). However, there was no detectable unbound Pt in the tumor ECF after administration of SPI-077 and SPI-077 B103, whereas unbound Pt concentrations were detectable in the ECF of all tumors after administration of cisplatin. In addition, the peak concentrations of Pt-GG and Pt-AG DNA adducts were 3.7- and 6.2-fold lower after administration of SPI-077 and SPI-077 B103 than after administration of cisplatin. These results suggest that SPI-077 and SPI-077 B103 are able to distribute into tumor and lodge in the interstitial spaces among tumor cells but release very little unbound Pt into tumor ECF or into tumor cells to form Pt-DNA adducts. This finding was in agreement with that in a phase 1-2 study of SPI-077 in patients with inoperable head and neck cancer, showing that only 2 of 18 patients had partial responses to SPI-077 with 2 responses in 29 evaluated sites in spite of the absence of dose-limiting toxicity.[57] This study demonstrated the ability of microdialysis measurements to distinguish what material is available at the tumor and provided a mechanistic foundation to help interpret clinical observations.

Treatment with cytotoxic drugs in conjunction with angiogenic inhibitors represents a paradigm for combination of antineoplastics with different mechanisms of action and nonoverlapping toxicities to enhance responses to cancer treatment and attenuate the development of drug resistance. Some studies have reported that angiogenic agents may decrease tumor uptake of the co-administrated anticancer drugs by reducing microvessel density and capillary permeability,[55,58,59] whereas others have shown that antiangiogenic therapy may enhance cytotoxic chemotherapy by restoring capillary architecture and decreasing interstitial fluid pressure and hypoxia.[56,60] Several publications from the laboratory of J. Gallo have reported the use of microdialysis in evaluating the potential drug interactions between angiogenic inhibitors and cytotoxic drugs at the target site.[55,56,58,59] Based on the previous finding that the interstitial fluid concentration of temozolomide (TMZ), a DNA-alkylating agent, was significantly decreased in the presence of TNP-470, an antiangiogenic agent, in a rat C6 glioma subcutaneous (sc) model,[59] a further study was performed using xenograft models that differentially expressed VEGF to evaluate the TMZ:TNP-470 drug interaction in both sc and intracerebral gliomas.[55] Probe calibration in vivo for this study was achieved by the method of zero-flow. Consistent with the previous study, it was shown that, in both the sc and intracerebral VEGF-overexpression tumor models, TNP-470 treatment produced significant reductions in TMZ tumor concentrations and tumor:plasma concentration ratios compared with control, being reduced an average of 25% and 50% in the sc and intracerebral tumors, respectively. However, in another study that was designed to extend the

same concept by the use of another angiogenic inhibitor, SU5416, a specific receptor tyrosine kinase inhibitor of VEGF receptor 2, a paradoxical effect of SU5416 on the tumor disposition of TMZ in sc and intracerebral tumors was observed [56]. In sc tumors, SU5416 treatment produced a 24% reduction in steady-state TMZ tumor concentration (C_t) values ($P < .05$) as well as 21% reductions in tumor/plasma concentration ratios (C_t/C_p; $p = 0.11$) compared with control. In intracerebral tumors, steady-state TMZ C_t and C_t/C_p ratios were significantly increased by 2-fold in the SU5416 treatment group compared with control. A possible explanation that may pertain to this differential result is the microdialysis sampling site, peripheral versus central, which may be enhanced by the ability of the dimethyl sulfoxide administration vehicle to cause tumor necrosis. Regardless of the discrepancy between the results obtained from TMZ:TNP-470 and TMZ:SU5416 drug interaction investigations, the importance of using microdialysis to evaluate drug interactions between antineoplastics at the target site has been established. Based on the important information, additional work is needed to develop PK-PD strategies to identify optimal combination regimens of angiogenesis inhibitors and cytotoxic drugs.

Clinical Studies

The clinical use of microdialysis technique to evaluate drug disposition in tumors is of considerable interest with the recognition that insufficient drug penetration into the interstitium of solid tumors represents a rate limiting step in clinical response to chemotherapy. However, relatively few studies have hitherto been performed in a clinical setting owing to both ethical and methodological considerations. Nonetheless, microdialysis has been used to evaluate the tumor disposition of some commonly used anticancer drugs, including 5-fluorouracil (5-FU),[7] capecitabine,[20] cisplatin,[61] dacarbazine,[62] and methotrexate,[63,64] in patients with accessible tumors, such as breast cancer, melanoma, and oral cancer.

In a study of Mader et al,[20] microdialysis probes were implanted into a cutaneous metastasis and subcutaneous connective tissue to evaluate the interstitial tissue PKs of capecitabine and its metabolites in breast cancer patients with skin metastases. Probe calibration for capecitabine and 5-FU was performed in both healthy and tumor tissues according to an in vivo retrodialysis procedure. The recovery of capecitabine was found to be similar to that of 5-FU in both tissues. The exposure to capecitabine in the malignant lesions was significantly higher compared with plasma, whereas the area under the curve (AUC) of all other metabolites including 5′-deoxy-5-fluorocytidine (DFCR), 5′-deoxy-5-fluorouridine (DFUR), and 5-FU did not differ significantly between metastatic tumors and plasma. These results were in accordance with those obtained previously by Schüller et al, who measured 5-FU levels in tumor biopsies and reported a mean tumor/plasma ratio of 21 for 5-FU in patients with colorectal tumors.[65] With regard to tissue exposure at the extracellular level, there was no significant difference between malignant and healthy tissues, suggesting little selectivity among both types of tissues.

Microdialysis has been employed to compare a standard high-dose intra-arterial (IA) cisplatin chemotherapy with a novel treatment approach, so-called crystalline cisplatin embolization, in which cisplatin is administered as a highly concentrated cisplatin crystalline suspension in combination with intravenous infusion of sodium thiosulfate (STS), a cisplatin neutralizer, in patients with oral cancer.[61] Tumor concentrations of both free cisplatin and STS were determined using microdialysis. Probe recoveries were only estimated in vitro because as the authors mentioned, prolongation of tumor dialysis was not tolerable for the patients due to restrictions of speaking, drinking, and eating during the dialysis period. It has been found in this study that crystalline cisplatin embolization yielded ~5-fold higher tumor cisplatin levels than the standard IA perfusion. Moreover, in contrast to cisplatin IA perfusion, cisplatin embolization appeared to meet 2 PK prerequisites for optimal response and low toxicity on the basis of the systemic and intratumoral molar STS/cisplatin ratios, which should be greater than 500 outside the tumor to neutralize cisplatin, and lower than 100 within tumors to avoid a loss of tumor cell killing.[66] It was concluded that this study provided a PK basis for the superselective high-dose IA cisplatin regimen combined with intravenous STS for neoadjuvant treatment of oral cancer and suggests that the novel cisplatin embolization approach is superior to IA cisplatin perfusion.

To examine if the therapeutic failure in tumor therapy is attributed to an impairment of transcapillary drug transfer into solid tumor, Joukhadar et al[62] employed microdialysis sampling for measuring dose intensity of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide (AIC) in cutaneous malignant melanoma metastases as well as subcutaneous adipose tissue after intravenous administration of dacarbazine at doses of 200 to 1000 mg/m^2 in patients with metastatic malignant melanoma (MMM). Microdialysis probe recovery of dacarbazine was determined using in vivo retrodialysis method. The results of this study showed that AUC values for dacarbazine and AIC measured in plasma correlated closely with corresponding AUC values measured in the ECF of MMMs, suggesting favorable tumor penetration characteristics of dacarbazine and its active metabolite AIC. This finding indicates that the poor response to antineoplastic therapy with dacarbazine may be to the resistance of melanoma cells at a molecular level, and not necessarily an inability of dacarbazine and AIC to penetrate into the interstitium of MMM.

Pharmacokinetic Modeling

Traditional PK models concern themselves with the disposition of a large mass of drug in the entire body, while disposition of little mass in a specific site is not described. Heterogeneous tumor drug distribution further diminishes the hope that kinetics of drug in plasma will parallel that in tumor. Therefore, a PK model including a tumor compartment that is relatively closely associated with the pharmacological effect may provide enhanced insight not only into drug transport but also into pertinent PK-PD relationships.

Zamboni et al[50] proposed a 2-compartment model with uncoupled distribution of the plasma and tumor compartments, which was used to fit to the plasma and tumor ECF concentration-time profiles of unbound Pt after cisplatin administration to tumor-bearing mice. The authors stated that it was impossible to obtain accurate estimates of the rate constant describing the movement of drug into the tumor ECF or the volume of the ECF by modeling the plasma and tumor distribution simultaneously owing to the 100- to 1000-fold difference between the rate constants describing systemic and tumor disposition of unbound Pt. Therefore, the tumor disposition of drug was modeled separately from the systemic disposition. The following differential equation was used to define the rate of change of unbound Pt concentration in tumor ECF:

$$\frac{dC_{ECF}}{dt} = \left(\frac{k_{P-ECF}}{V_{ECF}}\right) \cdot A_p \; - \; k_{ECF-P} \cdot C_{ECF}, \qquad (4)$$

where C_{ECF} is the concentration of unbound Pt in the tumor ECF, $k_{P\text{-}ECF}$ is the rate of transfer of unbound Pt from plasma into tumor ECF, $k_{ECF\text{-}P}$ is the rate constant of transport of unbound Pt out of tumor ECF, V_{ECF} is the volume of tumor ECF, and A_p is the fitted exponential function describing the amount of unbound Pt in the plasma.

Another model proposed by Zamboni et al[67] was a 3-compartment PK model, which consisted of a central, a peripheral, and a tumor compartment and was used to describe topotecan plasma and tumor ECF disposition in a human neuroblastoma model. In this study, serial sacrifice designs were used to collect blood samples, while serial microdialysate samples were collected over a time period of 5 hours from freely-moving mice bearing human neuroblastoma xenografts. Plasma concentrations of topotecan were modeled simultaneously with the tumor ECF concentrations. The volume of the tumor compartment was set at 5 mL/m^2 as estimated by volume displacement.

Physiologically based hybrid PK models have been recently developed by the Gallo group.[68] Such models coupled a forcing function, an equation describing the plasma drug concentration-time profile, with a model describing drug disposition in tumors. The great potential of this hybrid model approach lies in its ability to predict drug disposition in human tumors based on the preclinical (tumor disposition) and clinical (forcing function) data. Of the 2 hybrid models proposed in this study, the hybrid model derived from a preclinical study of temozolomide disposition in tumors was able to include a 3-compartment tumor model that depicted vascular, interstitial fluid, and intracellular subcompartments due to the availability of tumor ECF drug concentrations. This structure introduces tumor blood flow and physiologic volumes into the model that provides not only a means to assess how these parameters impact on drug disposition but that also can be readily replaced with human data when the hybrid model is applied to humans.

Application of Microdialysis in Assessment of Chemotherapeutic Response

Microdialysis sampling can be employed to assess chemotherapeutic responses or toxicities by monitoring changes in the concentration of one or more endogenous compounds in the target tissue. In a clinical study by Castejon et al,[69] microdialysis was used to determine the levels of free 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in blood after administration of cisplatin in cancer patients as 5-HT is known to be involved in the production of emesis associated with cisplatin treatment. The previously determined in vivo recoveries of the intravenous microdialysis probes for 5-HT and 5-HIAA averaged 98.6%.[70] The results from this study showed that the increases in free 5-HIAA levels in blood were associated with increases in urinary 5-HIAA, and these increases occurred at times at which acute emesis is known to develop after cisplatin. Although similar results were not found for 5-HT, it is suggested that the circulating 5-HIAA levels represent the metabolism of 5-HT either within the gut or on its passage through the liver after being released from the gut, and thus can be used as a marker of free 5-HT within the intestinal wall that is involved in triggering the emetic response after cisplatin chemotherapy.

Although microdialysis is at its best for small molecules, the availability of microdialysis probes with higher molecular weight membrane cutoff has made it possible to sample biologically relevant macromolecules. Garvin and Dabrosin[71] examined the effect of tamoxifen on the secretion of VEGF in a mouse model of human MCF-7 breast cancer using microdialysis to sample VEGF in the tumor interstitium, where VEGF is biologically active. The results from this study showed that tamoxifen significantly decreased the extracellular VEGF in solid MCF-7 tumors but increased intracellular VEGF. Microdialysis played a critical role in this study compared with the other techniques, such as western blot of the tumor homogenate and immunohistochemistry of tumor sections, which were unable to detect extracellular VEGF specifically. The same approach has

also been successfully applied in a clinical study to investigate the variability of VEGF in normal human breast tissue in vivo during the menstrual cycle.[72] Given the fact that the extracellular space is the bioactive site for the majority of growth factors associated with tumor growth, using microdialysis to sample growth factors at extracellular spaces opens the possibility to monitor tumor response to growth factor-targeting chemotherapy. Moreover, if the time course of such responses could be quantified by the use of microdialysis and integrated with the PK profiles of the corresponding drug, it would help to define the PK-PD relationship, which is essential for the rational design of drug administration regimens in cancer patients.

CONCLUSIONS

The ability of in vivo microdialysis to measure the chemical composition of the ECF has provided an important tool enabling the determination of tumor drug concentration-time profiles. The information on drug disposition in tumors may help to define not only drug transport but also pertinent PK-PD relationships that could aid to select appropriate drug candidates and to design optimal dosing regimens. It is hoped that with the increasing acceptance of microdialysis as a well-validated sampling technique, the application of microdialysis in the development of anticancer drugs will become the norm. The application of microdialysis for evaluating tumor response to chemotherapy has not been fully established as yet. Nonetheless, with the commercial availability of large molecular weight cut-off microdialysis probes, microdialysis approach is expected to offer exciting potential for defining concentration-effect relationships for anticancer drugs.

REFERENCES

1. Galmarini CM, Galmarini FC. Multidrug resistance in cancer therapy: role of the microenvironment. *Curr Opin Investig Drugs*. 2003;4:1416-1421.

2. Hryniuk WM. The importance of dose intensity in the outcome of chemotherapy. In: *Important Adv Oncol.* 1988:121-141.

3. Masson E, Zamboni WC. PK optimisation of cancer chemotherapy: effect on outcomes. *Clin Pharmacokinet*. 1997;32:324-343.

4. Yuan F. Transvascular drug delivery in solid tumors. *Semin Radiat Oncol*. 1998;8:164-175.

5. Jain RK. Delivery of molecular and cellular medicine to solid tumors. *Adv Drug Deliv Rev*. 2001;46:149-168.

6. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res*. 2004;64: 3731-3736.

7. Müller M, Mader RM, Steiner B, et al. 5-fluorouracil kinetics in the interstitial tumor space: clinical response in breast cancer patients. *Cancer Res*. 1997;57:2598-2601.

8. Hunz M, Jetter A, Wilde S, et al. Plasma and tissue PKs of epirubicin in nine patients with primary breast cancer. *Eur J Clin Pharmacol*. 2001;57:A31.

9. Garimella TS, Ross DD, Eiseman JL, et al. Plasma PKs and tissue distribution of the breast cancer resistance protein (BCRP/ABCG2) inhibitor fumitremorgin C in SCID mice bearing T8 tumors. *Cancer Chemother Pharmacol*. 2005;55:101-109.

10. Meikle SR, Matthews JC, Brock CS, et al. PK assessment of novel anti-cancer drugs using spectral analysis and positron emission tomography: a feasibility study. *Cancer Chemother Pharmacol*. 1998;42:183-193.

11. Fischman AJ, Alpert NM, Babich JW, Rubin RH. The role of positron emission tomography in PK analysis. *Drug Metab Rev*. 1997;29:923-956.

12. Jynge P, Skjetne T, Gribbestad I, et al. In vivo tissue PKs by fluorine magnetic resonance spectroscopy: a study of liver and muscle disposition of fleroxacin in humans. *Clin Pharmacol Ther*. 1990;48:481-489.

13. Artemov D, Solaiyappan M, Bhujwalla ZM. Magnetic resonance pharmacoangiography to detect and predict chemotherapy delivery to solid tumors. *Cancer Res*. 2001;61:3039-3044.

14. Benveniste H, Huttemeier PC. Microdialysis - theory and application. *Prog Neurobiol*. 1990;35:195-215.

15. Ungerstedt U. Microdialysis - principles and applications for studies in animals and man. *J Intern Med*. 1991;230:365-373.

16. Benveniste H. Brain microdialysis. *J Neurochem*. 1989;52:1667-1679.

17. Elmquist WF, Sawchuk RJ. Application of microdialysis in PK studies. *Pharm Res*. 1997;14:267-288.

18. Garrison KE, Pasas SA, Cooper JD, Davies MI. A review of membrane sampling from biological tissues with applications in PKs, metabolism and PDs. *Eur J Pharm Sci*. 2002;17:1-12.

19. Chu J, Gallo JM. Application of microdialysis to characterize drug disposition in tumors. *Adv Drug Deliv Rev*. 2000;45:243-253.

20. Mader RM, Schrolnberger C, Rizovski B, et al. Penetration of capecitabine and its metabolites into malignant and healthy tissues of patients with advanced breast cancer. *Br J Cancer*. 2003;88:782-787.

21. Brunner M, Muller M. Microdialysis: an in vivo approach for measuring drug delivery in oncology. *Eur J Clin Pharmacol*. 2002;58:227-234.

22. Johnson RD, Justice JB. Model studies for brain dialysis. *Brain Res Bull*. 1983;10:567-571.

23. Ungerstedt U. Measurement of transmitter release by intracerebral dialysis. In: Marsden CA, ed. *Measurement of Neurotransmitter Release In Vivo.* Chichester, UK: John Wiley & Sons; 1984:81-105.

24. Ståhle L. On mathematical models of microdialysis: geometry, steady-state models, recovery and probe radius. *Adv Drug Deliv Rev*. 2000;45:149-167.

25. Lönnroth P, Jansson P, Smith U. A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol*. 1987;253:E228-E231.

26. Ståhle L. Drug distribution studies with microdialysis. I. Tissue dependent difference in recovery between caffeine and theophylline. *Life Sci*. 1991;49:1835-1842.

27. Larsson CI. The use of an "internal standard" for control of the recovery in microdialysis. *Life Sci*. 1991;49:PL73-PL78.

28. Menacherry S Jr, Hubert W Jr, Justice JB Jr. In vivo calibration of microdialysis probes for exogenous compounds. *Anal Chem*. 1992;64:577-583.

29. Yokel RA, Allen DD, Burgio DE, McNamara PJ. Antipyrine as a dialyzable reference to correct differences in efficiency among and within sampling devices during in vivo microdialysis. *J Pharmacol Toxicol Methods*. 1992;27:135-142.

30. Van Belle K, Dzeka T, Sarre S, Ebinger G, Michotte Y. In vitro and in vivo microdialysis calibration for the measurement of carbamazepine and its metabolites in rat brain tissue using the internal reference technique. *J Neurosci Methods*. 1993;49:167-173.

31. Wang Y, Wong SL, Sawchuk RJ. Microdialysis calibration using retrodialysis and zero-net flux: application to a study of the distribution of zidovudine to rabbit cerebrospinal fluid and thalamus. *Pharm Res*. 1993;10:1411-1419.

32. Le Quellec A, Dupin S, Genissel P, Saivin S, Marchand B, Houin G. Microdialysis probes calibration: gradient and tissue dependent changes in no net flux and reverse dialysis methods. *J Pharmacol Toxicol Methods*. 1995;33:11-16.

33. Clement R, Malinovsky JM, Dollo G, Le Corre P, Chevanne F, Le Verge R. In vitro and in vivo microdialysis calibration using retrodialysis for the study of the cerebrospinal distribution of bupivacaine. *J Pharm Biomed Anal*. 1998;17:665-670.

34. Ståhle L. Drug distribution studies with microdialysis. I. Tissue dependent difference in recovery between caffeine and theophylline. *Life Sci*. 1991;49:1835-1842.

35. Stenken JA. Methods and issues in microdialysis calibration. *Anal Chim Acta*. 1999;379:337-358.

36. Chaurasia CS. In vivo microdialysis sampling: theory and applications. *Biomed Chromatogr*. 1999;13:317-332.

37. Jacobson I, Sandberg M, Hamberger A. Mass transfer in brain dialysis devices - a new method for the estimation of extracellular amino acids concentration. *J Neurosci Methods*. 1985;15:263-268.

38. Trickler WJ, Miller DW. Use of osmotic agents in microdialysis studies to improve the recovery of macromolecules. *J Pharm Sci*. 2003;92:1419-1427.

39. Dabrosin C, Margetts PJ, Gauldie J. Estradiol increases extracellular levels of vascular endothelial growth factor in vivo in murine mammary cancer. *Int J Cancer*. 2003;107:535-540.

40. Sjogren F, Svensson C, Anderson C. Technical prerequisites for in vivo microdialysis determination of interleukin-6 in human dermis. *Br J Dermatol*. 2002;146:375-382.

41. Rosdahl H, Ungerstedt U, Henriksson J. Microdialysis in human skeletal muscle and adipose tissue at low flow rates is possible if dextran-70 is added to prevent loss of perfusion fluid. *Acta Physiol Scand*. 1997;159:261-262.

42. Ault JM, Riley CM, Meltzer NM, Lunte CE. Dermal microdialysis sampling in vivo. *Pharm Res*. 1994;11:1631-1639.

43. Davies MI, Lunte CE. Microdialysis sampling for hepatic metabolism studies: impact of microdialysis probe design and implantation technique on liver tissue. *Drug Metab Dispos*. 1995;23:1072-1079.

44. Palsmeier RK, Lunte CE. Microdialysis sampling of tumors for study of the metabolism of antineoplastic agents. *Cancer Bull*. 1994;46:58-66.

45. Westergren I, Nystrom B, Hamberger A, Johansson BB. Intracerebral dialysis and the blood-brain barrier. *J Neurochem*. 1995;64:229-234.

46. Morgan ME, Singhal D, Anderson BD. Quantitative assessment of blood-brain barrier damage during microdialysis. *J Pharmacol Exp Ther*. 1996;277:1167-1176.

47. Groothuis DR, Ward S, Schlageter KE, et al. Changes in blood-brain barrier permeability associated with insertion of brain cannulas and microdialysis probes. *Brain Res*. 1998;803:218-230.

48. Joukhadar C, Klein N, Mader RM, et al. Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. *Cancer*. 2001;92:2190-2196.

49. Johansen MJ, Thapar N, Newman RA, Madden T. Use of microdialysis to study platinum anticancer agent PKs in preclinical models. *J Exp Ther Oncol*. 2002;2:163-173.

50. Zamboni WC, Gervais AC, Egorin MJ, et al. Inter- and intratumoral disposition of platinum in solid tumors after administration of cisplatin. *Clin Cancer Res*. 2002;8:2992-2999.

51. Dukic SF, Kaltenbach ML, Heurtaux T, Hoizey G, Lallemand A, Vistelle R. Influence of C6 and CNS1 brain tumors on methotrexate PKs in plasma and brain tissue. *J Neurooncol*. 2004;67:131-138.

52. Leggas M, Zhuang Y, Welden J, Self Z, Waters CM, Stewart CF. Microbore HPLC method with online microdialysis for measurement of topotecan lactone and carboxylate in murine CSF. *J Pharm Sci*. 2004;93:2284-2295.

53. Tokunaga Y, Nakashima M, Sasaki H, et al. Local distribution into brain tumor and PKs of 4-pyridoxate diammine hydroxy platinum, a novel cisplatin derivative, after intracarotid administration in rats with 9L malignant glioma: simultaneous brain microdialysis study. *Biol Pharm Bull*. 2000;23:1491-1496.

54. Zamboni WC, Gervais AC, Egorin MJ, et al. Systemic and tumor disposition of platinum after administration of cisplatin or STEALTH liposomal-cisplatin formulations (SPI-077 and SPI-077 B103) in a preclinical tumor model of melanoma. *Cancer Chemother Pharmacol*. 2004;53:329-336.

55. Ma J, Pulfer S, Li S, Chu J, Reed K, Gallo JM. PD-mediated reduction of temozolomide tumor concentrations by the angiogenesis inhibitor TNP-470. *Cancer Res*. 2001;61:5491-5498.

56. Ma J, Li S, Reed K, Guo P, Gallo JM. PD-mediated effects of the angiogenesis inhibitor SU5416 on the tumor disposition of temozolomide in subcutaneous and intracerebral glioma xenograft models. *J Pharmacol Exp Ther*. 2003;305:833-839.

57. Harrington KJ, Lewanski CR, Northcote AD, et al. Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. *Ann Oncol*. 2001;12:493-496.

58. Devineni D, Klein-Szanto A, Gallo JM. In vivo microdialysis to characterize drug transport in brain tumors: analysis of methotrexate uptake in rat glioma-2 (RG-2)-bearing rats. *Cancer Chemother Pharmacol*. 1996;38:499-507.

59. Devineni D, Klein-Szanto A, Gallo JM. Uptake of temozolomide in a rat glioma model in the presence and absence of the angiogenesis inhibitor TNP-470. *Cancer Res*. 1996;56:1983-1987.

60. Teicher BA, Dupuis NP, Robinson MF, Emi Y, Goff DA. Antiangiogenic treatment (TNP-470/minocycline) increases tissue levels of anticancer drugs in mice bearing Lewis lung carcinoma. *Oncol Res*. 1995;7:237-243.

61. Tegeder I, Brautigam L, Seegel M, et al. Cisplatin tumor concentrations after intra-arterial cisplatin infusion or embolization in patients with oral cancer. *Clin Pharmacol Ther*. 2003;73:417-426.

62. Joukhadar C, Klein N, Mader RM, et al. Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. *Cancer*. 2001;92:2190-2196.

63. Ekstrom PO, Andersen A, Saeter G, Giercksky KE, Slordal L. Continuous intratumoral microdialysis during high-dose

methotrexate therapy in a patient with malignant fibrous histiocytoma of the femur: a case report. *Cancer Chemother Pharmacol*. 1997;39:267-272.

64. Müller M, Brunner M, Schmid R, et al. Interstitial methotrexate kinetics in primary breast cancer lesions. *Cancer Res*. 1998;58:2982-2985.

65. Schüller J, Cassidy J, Dumont E, et al. Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol*. 2000;45:291-297.

66. Abe R, Akiyoshi T, Baba T. Inactivation of cis-diamminedichloroplatinum (II) in blood by sodium thiosulfate. *Oncology*. 1990;47:65-69.

67. Zamboni WC, Houghton PJ, Hulstein JL, et al. Relationship between tumor extracellular fluid exposure to topotecan and tumor response in human neuroblastoma xenograft and cell lines. *Cancer Chemother Pharmacol*. 1999;43:269-276.

68. Gallo JM, Vicini P, Orlansky A, et al. PK model-predicted anticancer drug concentrations in human tumors. *Clin Cancer Res*. 2004;10:8048-8058.

69. Castejon AM, Paez X, Hernandez L, Cubeddu LX. Use of intravenous microdialysis to monitor changes in serotonin release and metabolism induced by cisplatin in cancer patients: comparative effects of granisetron and ondansetron. *J Pharmacol Exp Ther*. 1999;291:960-966.

70. Paez X, Hernandez L. Plasma serotonin monitoring by blood microdialysis coupled to high-performance liquid chromatography with electrochemical detection in humans. *J Chromatogr B Biomed Sci Appl*. 1998;720:33-28.

71. Garvin S, Dabrosin C. Tamoxifen inhibits secretion of vascular endothelial growth factor in breast cancer in vivo. *Cancer Res*. 2003;63:8742-8748.

72. Dabrosin C. Variability of vascular endothelial growth factor in normal human breast tissue in vivo during the menstrual cycle. *J Clin Endocrinol Metab*. 2003;88:2695-2698.