The Effect of P-glycoprotein on Paclitaxel Brain and Brain Tumor Distribution in Mice

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ABSTRACT

It may be inferred from the presence of P-glycoprotein (Pgp) in brain capillaries that this drug efflux pump is a factor in limiting the penetration of certain agents into brain tumors. However, by contrast with normal brain capillaries which constitute the blood–brain barrier, brain tumor capillaries are compromised or “leaky,” and the extent to which Pgp expression in brain tumor neovasculature retains its capacity to limit drug penetration has not been determined. To address this question, we studied the normal brain and brain tumor distribution of paclitaxel (PAC), a known Pgp substrate, using steady-state PAC dosing regimens in wild-type and Pgp knockout (mdr1a−/− and mdr1b−/−) mice bearing an intracerebral B-16 melanoma. At comparable steady-state PAC plasma concentrations of ~5 μg/ml, steady-state PAC brain concentrations in Pgp knockout mice were ~3-, 1.8-, and 1.7-fold greater in left brain, right brain, and brain tumor, respectively, than in wild-type mice and statistically different (P < 0.05) in each brain region. Determination of the steady-state brain/plasma concentration ratios or partition coefficients, which take into account any differences in plasma concentrations between each group, indicated a similar pattern as did the absolute brain concentrations. It is concluded that even in the neovasculature of brain tumors, Pgp has the facility to limit drug penetration, although somewhat less so than in normal brain.

INTRODUCTION

Pgp is an established factor in the pharmacokinetics of anticancer agents (1–6). As determined from studies of Pgp-deficient mice, and from investigations of the effects of Pgp inhibitors on normal rodents and humans, Pgp is capable of reducing the p.o. bioavailability, and facilitating the hepatobiliary excretion, of drugs that are substrates of the pump, such as PAC and digoxin (2, 7–10). The involvement of Pgp in these processes is based on its expression in gut enterocytes and bile canalicula. Another site at which Pgp could affect the distribution of anticancer agents is the brain, a site at which the pump is known to function as a component of the BBB (1, 2, 11). Expression of Pgp at the apical surfaces of brain capillary endothelial cells has been determined to limit the penetration into this organ of agents, such as ivermectin, cyclosporin A, and loperamide (1, 2, 11). However, by comparison with the numerous studies on the effect of Pgp on the p.o. bioavailability, hepatobiliary excretion, and tissue distribution of drugs, relatively little is known about the role that Pgp plays in the penetration of anticancer agents into the brain. At least two reports suggest that Pgp can limit the penetration of vinblastine into normal brain of wt mice, by comparison with Pgp ko mice (1, 3). Although these studies neither characterized the complete time course of vinblastine in the mice, nor accounted for the differences in plasma drug concentrations that occur as a result of loss of Pgp function in liver, the results suggested that Pgp influenced normal brain penetration of vinblastine (1, 3). Another unsettled issue regarding Pgp, and of direct significance to cancer treatment, is whether the pump is able to reduce the penetration of anticancer agents into brain tumors. The notion that Pgp might function in this capacity is supported by studies showing that the pump is expressed in brain tumor neovasculature (12–14). However, on the other hand, tumor blood capillaries are known to be “leaky,” a feature that is thought to contribute to the enhanced penetration of anticancer agents into brain tumors (15), and it is possible that under these pathological circumstances, the activity of the pump may be markedly diminished. These issues pertaining to the function of Pgp in anticancer drug penetration into normal brain and brain tumor also have a bearing on whether Pgp inhibitors have a role in treating brain cancers.

Studies of PAC distribution into the CNS of both rodents and humans have indicated cerebrospinal fluid, and normal brain concentrations are only a small fraction of concomitant plasma concentrations (16–18). Limited data from patients have indicated higher PAC brain tumor concentrations than in normal brain consistent with the disruption of the BBB (17). These investigations did not address the role of Pgp as a determinant of PAC distribution into the CNS and, thus, led support to our investigation that sought to characterize the role of Pgp in PAC distribution, not only in normal brain, but also in brain tumors.

MATERIALS AND METHODS

Chemicals. PAC was obtained from Mead-Johnson (Taxol injection; Bristol-Myers Co., Princeton, NJ). The Pgp [Ab-1] antibody was purchased from Oncogene (Boston, MA). FVB parental and mdr1a/b−/− ko mice were provided by Taconic (Germantown, NY). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

In Vivo Intracerebral Tumor Models. To establish a syngeneic mouse brain tumor model, three cell lines (i.e., B-16, A20, and 4C8) were screened for PAC expression and in vivo growth characteristics. The B-16 mouse melanoma cell line was kindly provided by Dr. Klein-Szanto, Fox Chase Cancer Center. The A20 mouse glioma cell line was kindly provided by the Brain Tumor Research Center, University of California (San Francisco, CA), and the 4C8 cell line was obtained from Dr. Linda Parysek, Children’s Hospital of Philadelphia. Western blot analyses were completed to assess Pgp expression in each cell line using KB-3–1 and KB-C2 human epidermoid carcinoma cell lines, provided by Dr. G. Kruh, Fox Chase Cancer Center, as Pgp-positive and -negative controls, respectively.

Each cell line (i.e., B-16, A20, and 4C8) was evaluated for their ability to form brain tumors in male FVB mice (25–30 grams). Mice were anesthetized with an i.p. dose (0.1 ml/10 grams body weight) of a 3:2:1 (volume:volume:volume) mixture of ketamine hydrochloride (10 mg/ml):acepromazine maleate (1 mg/ml):xylazine hydrochloride (2 mg/ml), secured in a stereotaxic apparatus, and had implanted 3 μl of a tumor cell suspension (106 cells/ml) into the right (2.5 mm lateral from the bregma) thalamic region at a depth of 3 mm. The hole was sealed with bone wax, and the skin was sutured. The mice were then returned to cages and received a standard rat diet and water ad libitum. Animals were monitored daily for symptoms and body weight.

Pharmacokinetic Studies. The goal of the pharmacokinetic investigations was to compare PAC brain and brain tumor distribution in wt and Pgp ko mice under steady-state conditions. Both wt and Pgp ko mice were implanted with B-16 melanoma cells as described above. In ~10 days, animals showed CNS...
PACLITAXEL BRAIN AND BRAIN TUMOR DISTRIBUTION IN MICE

RESULTS AND DISCUSSION

The area of membrane transporters is increasing in importance as more and more transporters are discovered and their potential role as drug efflux pumps is uncovered. The pharmacokinetic investigations that have been completed have considered the role of transporters in drug distribution that is calculated as either the ratio of tissue:plasma concentrations in normal brain and brain tumor. In addition, our results may be relevant to PAC brain and tumor distribution in humans because the steady-state PAC exposures and Cremophor EL on brain distribution were nullified. In addition, our results may be relevant to PAC brain and tumor distribution in humans because the steady-state PAC concentrations of ~5 μg/ml are within the range of maximum plasma concentrations obtained after different administration regimens of PAC to patients (26).

A pivotal motivation to conduct this investigation was to compare brain and brain tumor drug concentrations so as to determine whether Pgp altered drug distribution under conditions when the BBB was compromised by a tumor. To accomplish this comparison, PAC concentrations were measured under steady-state conditions at 180 min in three brain regions from each animal, normal right and left brain and brain tumor. It has been observed that “normal” brain samples collected in the ipsilateral hemisphere as the brain tumor can
show enhanced permeability depending on the proximity of the sample to the tumor (27). It was, therefore, anticipated that normal right (the hemisphere of the tumor) brain PAC concentrations would be higher than normal left brain, yet less than brain tumor. Fig. 2 illustrates the mean (±SD) brain and brain tumor PAC concentrations in both wt and Pgp ko groups in the same brain region was statistically significant (P < 0.05). Normal left brain PAC concentrations were ~3-fold greater (P < 0.05) in the Pgp ko mice (0.31 ± 0.09 μg/gram) compared with wt mice (0.11 ± 0.06 μg/gram), in accord with the notion that Pgp limits PAC penetration into brain. Right brain PAC concentrations were ~1.8-fold greater (P < 0.05) in the ko mice (0.33 ± 0.06 μg/gram) compared with the wt mice (0.18 ± 0.11 μg/gram), a difference that suggests the presence of the brain tumor in the right hemisphere did modulate drug penetration in the grossly defined normal brain region. The comparison of brain tumor PAC concentrations in wt (0.9 ± 0.67 μg/gram) and Pgp ko (1.56 ± 0.5 μg/gram) groups revealed an ~1.7-fold increase (P < 0.05) in the Pgp-deficient mice. This value was roughly comparable with the differences observed between normal brain in the wt and Pgp ko mice, suggesting that even under the conditions in brain tumor, in which enhanced drug penetration is readily apparent compared with normal brain, expression of Pgp was a limiting factor.

Comparisons of PAC concentrations within either the Pgp wt or ko groups indicated that PAC brain tumor concentrations were statistically greater (P < 0.05), ranging from ~5- to 7.5-fold higher, than either right or left brain PAC concentrations, consistent with the known “leakiness” of brain tumors. Right and left brain PAC concentrations were not statistically different from one another. Therefore, the analysis of PAC brain concentrations indicated that the rank order of concentrations was brain tumor > right brain > left brain in both animal groups, consistent with the effect of brain tumors on the BBB and that Pgp ko mice have between 1.7- and 3-fold greater PAC concentrations than in the corresponding brain region of wt mice.

Although steady-state PAC plasma concentrations enable direct comparisons of brain concentrations, analysis of partition coefficients, calculated as the ratio of steady-state tissue/plasma PAC concentrations, provides the most accurate assessment of drug distribution because any differences in plasma concentrations are accounted for in the ratio. Fig. 3 shows the mean (±SD) partition coefficients for each brain region in both wt and Pgp ko mice. The pattern of partition coefficient values with respect to both brain region and genetic type is similar to the pattern observed for PAC brain concentrations. There was a slight decrease in the differences between wt and Pgp ko mice in each brain region. These differences were 2.3-fold in left brain (0.023 ± 0.017 wt versus 0.054 ± 0.021 Pgp ko) and 1.6-fold in both right brain (0.036 ± 0.024 wt versus 0.059 ± 0.023 Pgp ko) and brain tumor (0.18 ± 0.134 wt versus 0.28 ± 0.112 Pgp ko) and were statistically different (P < 0.05) in both left and right brain but not in brain tumor (P = 0.09). The high variability of the partition coefficients in brain tumors may likely be attributed to the heterogeneous
nature of the tumor vasculature because PAC concentrations were more variable in brain tumor than in plasma and no doubt contributed to the lack of statistical significance (P = 0.09) between the brain tumor groups. The primary inferences from the partition coefficient data are that the presence of the brain tumor slightly augments, but does not abolish, the role of Pgp on PAC distribution in the tumor.

Investigations of drug penetration into brain tumors indicate that both physiological variables (i.e., blood flow, membrane integrity, and hypoxia), as well as drug-specific parameters (i.e., molecular weight and lipophilicity), are important determinants (28). Given the complexity of energy-dependent, plasma membrane-based systems for effluxing anticancer agents, and the poor results of chemotherapy in treating brain tumors, it is important to understand the extent to which ABC transporters are determinants of drug distribution into brain tumors. This is the first investigation that has addressed this question by simultaneously characterizing the role of Pgp on drug uptake into normal brain and brain tumors. Using wt and Pgp gene-disrupted mice under steady-state conditions, a number of important findings was made. We found that, with regard to a prototypical natural product anticancer agent (PAC), the BBB is compromised in brain tumors, leading to higher drug concentrations. The results also showed that under conditions in which the impact of Pgp on plasma levels is minimized, Pgp expression influences PAC concentrations in brain tumor, as well as in normal brain. Finally, we demonstrate the value of using a syngeneic brain tumor model to explore mechanisms underlying anticancer drug disposition in brain.

REFERENCES

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