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Intratumoral concentrations of imatinib after oral administration in patients with glioblastoma multiforme

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Abstract

Background—Imatinib, an orally administered tyrosine kinase inhibitor of PDGF receptor, c-abl and c-kit, is currently in clinical trials to assess its efficacy in malignant gliomas. Although imatinib does not readily penetrate an intact blood-brain barrier (BBB), the extent to which it distributes into regions of high grade gliomas where the BBB is compromised has not been determined.

Methods—Patients with recurrent high-grade gliomas for whom repeat surgical tumor debulking was clinically indicated received imatinib mesylate 600 mg orally once a day for seven days prior to surgery. Tissue samples were collected from different regions of the tumor and the approximate location of these samples was determined using frameless stereotactic neuronavigation. Plasma samples were obtained immediately before and after the resection. The concentration of imatinib in the plasma and tumor samples was determined using high performance liquid chromatography with mass spectrometric detection.

Results—Eleven tumor samples were obtained from three patients with recurrent glioblastoma multiforme. The median concentration of imatinib in these 11 tumor specimens was 1.34 µg/g (range, 0.21–4.31 µg/g) and the median tumor-to-plasma ratio was 0.71 (range, 0.28–3.03).

Conclusion—These findings suggest that imatinib can reach intratumoral concentrations similar to those or higher than in plasma in regions of glioblastoma where the BBB is disrupted as indicated by contrast enhancement on magnetic resonance imaging.

Keywords

Imatinib mesylate; intratumoral concentration; glioblastoma multiforme; malignant glioma

Introduction

Imatinib mesylate is a selective tyrosine kinase inhibitor of PDGF receptor, c-kit and c-abl tyrosine kinases that has been approved for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). Evidence from preclinical studies suggests that imatinib may also have activity against malignant gliomas [1]. Several clinical

studies investigating the use of imatinib mesylate in the treatment of patients with malignant glioma have been completed [2-5]. As imatinib has been shown to penetrate an intact blood-brain barrier (BBB) poorly [6-8], concerns remain as to whether this agent achieves therapeutic concentrations within the primary brain tumors. This study was conducted to assess the distribution of imatinib within contrast enhancing regions of high grade gliomas where the integrity of the BBB is compromised. This important question has not previously been addressed.

Patients and Methods

Study design

This research study was reviewed and approved by the Institutional Review Board of The Johns Hopkins University. Written informed consent was obtained from each participating patient. Patients with biopsy proven high-grade glioma (WHO grade III or IV) with radiographic evidence of recurrent disease who were scheduled to undergo repeat resection were eligible for this study. The eligibility criteria also included age ≥ 18 years, Karnofsky Performance Score of ≥ 60 , Mini Mental Status Exam (MMSE) score of ≥ 15 , and a scheduled MRI/wand study planned prior to surgery. Patients were excluded from this study if they had comorbidities precluding therapy with imatinib mesylate, previous adverse reactions to imatinib mesylate, concurrent medications with known clinically significant interactions with imatinib mesylate such as enzyme inducing antiepileptic drugs (EIAED) or if they were pregnant, lactating, or had taken any investigational drugs within the previous 28 days.

The participants consented to take imatinib mesylate 600 mg orally once a day for seven consecutive days prior to surgery, with the last dose taken one day prior to surgery to achieve a steady-state of the imatinib concentration in plasma. Imatinib mesylate, provided in 100 mg pills, was provided by Novartis Pharmaceuticals.

Tumor samples were obtained intraoperatively and their location was determined using frameless stereotactic neuronavigation (BrainLAB, Munich, Germany). Blood samples were obtained to determine the concentration of imatinib in plasma shortly before and after the tissue samples were resected. The tumor tissue and plasma samples were promptly stored at -80°C until assayed.

Determination of imatinib in plasma and brain tumor tissue

A validated analytical method based upon reversed-phase high performance liquid chromatography with mass spectrometric detection (LC/MS) was used to determine the concentration of imatinib in plasma, as previously described, with minor modifications [9]. Benzoylimatinib (4-[(4-methyl-1-piperazinyl)methyl]-N-(4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]benzoylamino]phenyl]benzamide) was synthesized for use as the internal standard by derivatizing imatinib free base (10-mg) with excess benzoyl chloride (70- μL) in pyridine (1-mL). Frozen plasma and tissue samples were thawed at ambient temperature.

Tissue samples were homogenized as previously described and prepared for LC/MS analysis in the same manner as plasma samples [10]. Each tissue sample was rinsed three times with ice-cold phosphate buffered saline, gently blotting with filter paper between each rinsing, and weighed in a microcentrifuge tube. After adding ice-cold water at a volume equivalent to 10-times the tissue weight, assuming a density of 1.0 g/cm^3 , the tissue was homogenized for 4-min using an Ultra-Turrax T8 disperser with an S8N-5G dispersing element (IKA Works, Inc., Wilmington, NC). The homogenate was sonicated for 5-min, subjected to three freeze-thaw cycles to completely lyse cells, and centrifuged (12,000 g, 10-min).

Plasma and tissue homogenate (100- μ L) were spiked with 5- μ L of the internal standard working solution (20- μ g/mL in acetonitrile) and vigorously mixed for 30 sec with 295- μ L of acetonitrile to precipitate proteins. After centrifuging at 12,000 *g* for 5-min, 50- μ L of the supernatant was diluted with 200- μ L of 0.1% formic acid; 100- μ L of the solution was loaded on a 15-cm $\times$ 4.6-mm I.D. Luna 5- μ m C18(2) HPLC column preceded by a 4-mm $\times$ 3-mm I.D. Luna C18 precolumn (Phenomenex, Torrance, CA). Chromatography was performed at ambient temperature using a binary mobile phase, delivered at 1.0-mL/min, composed of 0.1% formic acid in water and 0.1% formic acid in methanol. The percentage of methanol was increased linearly from 30% at the beginning of the run to 80% over 6-min. Imatinib and the internal standard eluted at approximately 4.4 min and 5.3 min, respectively. The column was then flushed with 95% methanol for 3-min and equilibrated with the initial composition of the mobile phase for an additional 3-min before the next injection.

Flow from the column was directly introduced into the atmospheric pressure ionization-electrospray interface of an 1100 Series ion trap mass spectrometer (Agilent Technologies, Palo Alto, CA) from 2.5 to 6.0 min during the run and to waste at all other times. Nitrogen was used as the nebulizing gas at 50-p.s.i. and as the drying gas at a flow rate of 10-L/min and a temperature of 300°C. Operating parameters for the ion source and ion transfer optics providing optimal response for the $[M+H]^+$ ion of imatinib at m/z 494.3 were: capillary potential, -4500-V; skim 1, 42-V; capillary exit offset, 80-V; octopole, 3.0-V; octopole delta, 1.7-V; trap drive, 45.0-V; skim 2, 10.0-V; octopole rf, 300-V; lens 1, -0.5-V; lens 2, -42-V. Ion Charge Control was used to regulate the accumulation of ions in the trap (maximum accumulation time, 150-ms; maximum abundance, 30,000). Positive ion detection was performed in the normal scanning mode (m/z 480-620). Each data point in the total ion chromatogram was generated by averaging raw data from 5 scans with a rolling average of 2 scans. Extracted ion chromatograms for imatinib at m/z 494.3 and the internal standard at m/z 598.3 were integrated to provide peak areas.

Study samples were assayed together with a series of 8 calibration standards of imatinib in plasma at concentrations ranging from 0.010 to 1.00 μ g/mL and three quality control samples (0.030, 0.45, and 0.90 μ g/mL). Standard curves were constructed by plotting the imatinib-to-internal standard chromatographic peak area ratio against the known concentration of imatinib in each calibration standard. Linear least squares regression was performed with weighting in proportion to the reciprocal of the drug concentration normalized to the number of calibration standards. Values of the slope and y-intercept of the best-fit line were used to calculate the imatinib concentration in study samples. The drug concentration in tissue homogenates was multiplied by a factor of ten to provide the tissue concentration, expressed as μ g/g tissue weight, to account for the phosphate buffered saline added to prepare the homogenate. Imatinib was determined with an interday accuracy of 106.4% and a precision of 8.9% at the lowest concentration included in the calibration curves. Interday accuracy for three quality control samples ranged from 96.5-102.9% with a precision of 4.3-6.5%.

Results

A total of 14 patients were screened for participation in this study. Three of these patients met all eligibility criteria and agreed to participate. Imatinib mesylate was well tolerated and no side effects were reported that could be attributed to the study drug.

A total of 11 tumor samples and 6 plasma samples were collected from these patients. The imatinib mesylate concentration in 11 tumor samples and corresponding plasma samples are shown (Table 1). The median concentration of imatinib in these 11 tumor specimens was 1.34 μ g/g (range, 0.21-4.31 μ g/g). Forty-five percent of the tumor specimens had imatinib

concentrations higher than the average concentration of drug plasma during the resection. The tumor-to-plasma ratios were calculated to provide a relative index of drug penetration into the tumor. The median tumor-to-plasma ratio was 0.71 (range, 0.28-3.03).

The intratumoral concentrations of imatinib and tumor-to-plasma ratios demonstrated significant intra- and interindividual variability, probably reflecting local differences in the permeability of the BBB in different locations within the tumor. The frameless stereotactic neuronavigation system was used to determine the integrity of the BBB at the locations where tumor specimens were taken. The accuracy of this localization may be reduced as a result of shifting brain during the tumor resection thus providing only an approximation of the location of the tumor harvest relative to the pre-operative imaging. This analysis is further complicated by the relatively small size of the resected tumor samples and the significant heterogeneity in contrast enhancement even between closely neighboring areas within each individual tumor. Nevertheless, two tumor samples from the outer margin of the resection cavity that macroscopically resembled normal brain had the lowest measured imatinib concentration and showed the lowest contrast enhancement.

Discussion

Assessing the efficacy studies of novel agents in patients with primary brain tumors is a challenge, as MRI scans measure the extent of BBB dysfunction rather than actual tumor size. However, it becomes even more difficult to determine if a new drug is worth pursuing when the investigator has no information whether the administered agent has the potential to reach therapeutic concentrations in the brain tumor tissue. As a result, studies of the delivery of novel agents to brain tumors should precede efficacy studies as efficacy would be predicted to be poor if the drug never arrived at the target tissue.

The efficacy of imatinib in patients with primary brain tumors has been marginal. Used as a single agent in recurrent glioma, imatinib showed only minimal effect [4,5]. It was hypothesized that hydroxyurea (HU) could have an additive effect with imatinib in treatment of gliomas and that there might be an increased delivery to the brain when both agents are administered concomitantly [11]. *In vivo* experiments in mice and rats however did not show an influence of HU on the brain distribution of imatinib [12]. A recently published phase II study with 112 patients with recurrent malignant gliomas of various histologies again demonstrated only a limited antitumoral activity [5]. So far there have been no randomized controlled trials to assess the use of imatinib in the treatment of malignant glioma that could shed more light on the usefulness of the drug in this setting.

Previous reports suggest that imatinib crosses an intact BBB poorly. This is based largely on analysis of the imatinib concentration in CSF in patients receiving this drug. In patients with central nervous system relapse of chronic myelogenous leukemia treated with imatinib mesylate, levels of the drug were two logs lower in CSF than in plasma [6]. Other pharmacokinetic studies of imatinib mesylate in patients and in nonhuman primates demonstrated only marginal flux across the blood-brain barrier as well [7,8]. Furthermore, it has been shown that the brain distribution of imatinib mesylate is restricted by p-glycoprotein (p-gp or multidrug resistance protein Mdr1a), as well as likely by breast cancer resistance protein (Bcrp1), two efflux pumps expressed at the blood-brain barrier [13,14].

This study addresses the question of whether the previously described poor responses to imatinib in patients with glioblastoma were a result of inadequate delivery to the brain tumor tissue. Accrual to this study was difficult as it was purely a pharmacokinetic investigation with no expected therapeutic benefit for the patients, as they received the drug for only one week preoperatively. Furthermore, imatinib could be considered as being a “prior therapy”

or an “investigational drug”, which might render a patient ineligible for another research trial or cause an undue delay before patients could be entered on this trial. In addition, the correlation of the imatinib tumor-to-plasma ratio with intraoperative neuronavigation imaging proved more difficult than anticipated. The “region of tumor removal” determined from the MRI was large enough that there was considerable variability in the contrast enhancement, and thus BBB integrity, within this region. As the exact location of the small sample of tumor sent for analysis within this region is unspecified a precise correlation is difficult.

Nevertheless, our findings demonstrate that imatinib mesylate can reach significant intratumoral concentrations in patients with glioblastoma multiforme and a disrupted BBB. Although the number of patients and samples in this study is small, clearly all measured plasmatissue ratios were above the data previously published for CSF concentrations in the context of an intact BBB where the CSF concentration was approximately 100-times lower in the CSF as compared to plasma (0.044 $\mu\text{g/mL}$ in CSF versus 3.27 $\mu\text{g/mL}$ in plasma [6]). Interestingly, a substantial number of samples yielded intratumoral concentrations that were higher than those measured in the plasma. In the present study, patients received imatinib for seven days before surgery so that steady-state pharmacokinetic conditions were achieved, providing adequate time for imatinib to establish an equilibrium between systemic circulation and intracellular and extracellular regions in the CNS. Similar findings were suggested in a recent abstract [15]. These findings also suggest that drug concentrations within the CSF are an unreliable measure of drug levels within human brain tumors. In accordance with our findings, recent reports from similarly designed studies that assessed the drug distribution of other targeted agents (lapatinib, erlotinib, gefitinib, temsirolimus and rapamycin) demonstrated that significant intratumoral concentrations could also be reached by these drugs [16-19].

Our data suggest that the relatively limited therapeutic effect of oral imatinib in patients with high grade gliomas is likely not due to inadequate concentrations of this agent reaching the tumor tissue. Further studies of this agent might be targeted to the subgroup of patients with contrast enhancing lesions in whom the PDGF receptor tyrosine kinase is over expressed. In addition, our data raise serious questions about the therapeutic concentrations of this agent that would be achieved in the non-enhancing portions of most primary brain tumors.

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Figure 1. Example of intraoperative neuronavigation images of areas of the tumor with low (A) contrast enhancement and comparatively high (B) contrast enhancement. The numbers represent the tumor-plasma imatinib ratios of tumor samples resected from the marked areas.

Table 1

Data from analysis of 11 tumor samples obtained from 3 patients with glioblastoma multiforme (GBM). SD = standard deviation.

Tumor histology and location	Intratumoral drug concentration (µg/g)		Plasma drug concentration (µg/mL)		Tumor/Plasma ratio
	Individual sample	Average ± SD	Indiv. Sample*	Average	
Patient 1: GBM, right frontotemporal	4.31	2.87 ± 1.49	1.91	1.89	2.28
	2.96		1.88		1.57
	1.34				0.71
Patient 2: GBM, left frontotemporal	0.21	0.31 ± 0.08	0.73 0.79	0.76	0.28
	0.33				0.44
	0.28				0.37
	0.40				0.53
Patient 3: GBM, right frontoparietal	0.58	2.06 ± 1.06	0.83 1.12	0.97	0.59
	2.05				2.10
	2.64				2.71
	2.95				3.03

* The first value represents the plasma concentration from before, the second from after resection.