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SHORT COMMUNICATION

Whole-brain radiation fails to boost intracerebral gefitinib concentration in patients with brain metastatic non-small cell lung cancer: a self-controlled, pilot study

Luo Fang[1,2] · Xiaojiang Sun[2,3] · Yu Song[1] · Yiwen Zhang[1] · Fanzhu Li[4] · Yaping Xu[2,3] · Shenglin Ma[5] · Nengming Lin[6]

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Abstract
Purpose Whole-brain radiation therapy (WBRT) is generally considered as an efficient strategy to improve blood–brain barrier (BBB) permeability by damaging BBB structure and is therefore, used as a promising pretreatment of chemotherapy. However, the impact of radiotherapy on leaky BBB is still controversial for the reason that BBB of metastatic brain tumor lesion had been breached by tumor metastasizing. Herein, we conducted a self-controlled study to evaluate the effect of WBRT on the permeability of BBB in non-small cell lung cancer (NSCLC) patients with brain metastases (BM).
Method A prospective self-controlled research was performed. Radiation-naive NSCLC patients with BM were enrolled and treated with gefitinib for 2 weeks, and then concurrently combined with WBRT for 2 weeks. Plasma and cerebrospinal fluid (CSF) before and after WBRT were collected on day 15 and 29 after the initiation of gefitinib treatment. The concentrations of gefitinib in these samples were measured by HPLC.
Results Three patients were enrolled and evaluated. The concentrations of gefitinib in plasma and CSF pre-WBRT were comparable to those of post-WBRT. Consequently, no significant change was noted in the CSF-to-plasma ratios of gefitinib before and after WBRT (2.79 ± 1.47 vs. 2.35 ± 1.74 %, $p = 0.123$).
Conclusions The WBRT may not affect the BBB permeability by determining the concentration of gefitinib in NSCLC patients with BM.

Keywords Gefitinib · Blood–brain barrier · Whole-brain radiation therapy · Non-small cell lung cancer · Brain metastases

✉ Shenglin Ma
mashenglin@medmail.com.cn

✉ Nengming Lin
lnm1013@163.com

1 Laboratory of Clinical Pharmacy, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China

2 Zhejiang Key Laboratory of Diagnosis and Treatment Technology on Thoracic Oncology (Lung and Esophagus), Hangzhou, Zhejiang, China

3 Department of Radiation Oncology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China

4 College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China

5 Department of Radiation Oncology, Affiliated Hangzhou Hospital of Nanjing Medical University, Hangzhou, Zhejiang, China

6 Institute for Individualized Medicine, Hangzhou First People's Hospital, Hangzhou, Zhejiang, China

Introduction

Metastatic malignant brain tumor is characterized as a type of extreme poor-prognosis cancer in NSCLC [1]. The response of both cytotoxic drugs and molecular-targeted agents for intracranial lesions has been hampered by the blood–brain barrier (BBB) because most antineoplastic agents are difficult to cross the BBB massively to reach an efficient concentration [2, 3]. For instance, gefitinib, a small molecular-targeted agent in non-small cell lung cancer (NSCLC) with brain metastasis (BM) [4–6], is exposed in brain with an inferior level [7, 8]. Thus, to obtain a satisfied clinical outcome, tremendous strategies are exploited to enhance the permeability of BBB. Radiation is considered as a feasible strategy to optimize drug delivery into

brain [9–12]. Nowadays, there is a tendency that the radiation can be adopted as a pretreatment to open BBB then followed by systematic therapy to achieve high intracranial drug concentration [11], whereas most studies focused on primary intracranial tumor, where BBB was intact and radiation could induce BBB tight junction damage and lead to improved penetration [9, 13, 14]. The researches on patients with BM were rarely reported, and the effect of radiation on the BBB of secondary brain tumor remained unclear. In addition, BBB in metastatic lesions was morphologically heterogeneous. In contrast to primary lesions, BBB of metastatic lesions was not integral but breached [15–17]. Drug penetration is greatly improved following the process of tumor metastasizing into brain [7]. For the reason that BBB of metastatic lesions was already damaged, the efficiency of WBRT on the further opening of BBB would be limited. Herein, we designed a self-controlled prospective study to observe the impact of WBRT on the BBB penetration of gefitinib in BM-NSCLC patients.

Patients and methods

Patients

Adult radiation-naive patients (aged ≥18 years) with histologically confirmed diagnosis of BM-NSCLC, with at least one measureable lesion on brain magnetic resonance imaging, were eligible. Other eligibility criteria included a life expectancy of ≥12 weeks, without brain surgery or other intracranial diseases, ECOG PS of 0–3, adequate hematological parameters, hepatic, and renal function. Patients who received combined therapy (e.g., osmotic agents or traditional Chinese medicines) which may have any potential impact on the penetration behavior of gefitinib were excluded. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by the Ethics Committee of Zhejiang Cancer Hospital (No: zjzlyy-[2010]-26), and written informed consents were obtained from all patients before the collection of samples.

Treatment

Gefitinib was administered orally at a daily dose of 250 mg for 4 weeks, mono-therapy during the first 2 weeks and then concurrently combined with WBRT (30 Gy/10 F/2 W) during the last 2 weeks. The WBRT was administrated with a daily dose of 3 Gy to a total dose of 30 Gy. The treatment schedule was five fractions per week for 2 weeks. Adjuvant therapy, including antiemetics, diuretics, analgesics, and parenteral nutrition support were given when indicated. No other chemotherapeutic agents were administered during the course of the study. The schedule of treatment is shown in Fig. 1.

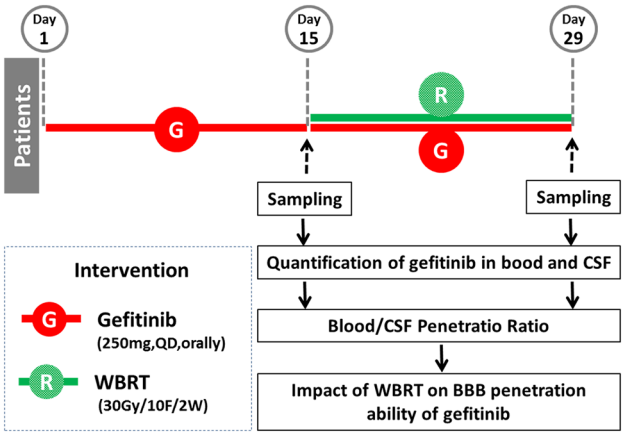

Fig. 1 Intervention schedule for treatment and sampling

Sample collection and bioanalysis

Blood and CSF samples of pre-WBRT were collected on day 15 before the beginning of WBRT. And the samples of post-WBRT were collected on day 29. All samples were obtained just before the administration of gefitinib at the first treatment cycle. The schedule of sampling is shown in Fig. 1. Then, the samples were pretreated and analyzed according to the previously validated methods [18]. Plasma and CSF were isolated by centrifugation at 5000 rpm at 4 °C for 10 min. Subsequently, the gefitinib in plasma and CSF was extracted by liquid–liquid extraction and solid-phase extraction, respectively. Afterward, the concentrations of gefitinib were assayed using a validated high-performance liquid chromatography (HPLC) method, as described previously [18]. The separations were carried out on a HPLC system (Agilent 1100, USA) equipped with a Zorbax Elipse XDB C_{18} column (5 μm, 4.6 × 150 mm, Agilent, USA).

Statistics processing

A paired-samples *t* test was used to evaluate the differences of penetration behavior between the pre-WBRT and post-WBRT, and a two-tail p value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS (version 20; IBM Corporation, Armonk, NY).

Results

Patient characteristics

Eight Chinese patients with BM from NSCLC were enrolled from February to November in 2010. Three participants were eligible and evaluable, and five were excluded because of the suspended therapy or refusing to supply CSF

Table 1 Clinical characteristics of the patients

Characteristics	Patients		
	No. 1	No. 2	No. 3
Age	70	43	64
Gender	Male	Female	Male
Histology of primary lesion	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
Differentiated phenotypes	Low	Low	Middle
Performance status	2	1–2	2
Smoking	Never	Never	Former
No. of brain metastases	2	1	1
Location of the lesion	Right frontal lobe, light temporo-parietal junction	Right frontal lobe	Right cerebellum
Initial size of brain metastases	22 mm × 19 mm, 14 mm × 13 mm	4 mm × 3 mm	20 mm × 15 mm
Complicated disease	Hypertension	NCD	Hypertension, gastric ulcer
Concurrent therapy	Captopril, nitrendipine, piracetam, furosemide	Omeprazole, compound amino acid injection, fat emulsion injection	Irbesartan, omeprazole, amifostine, ondansetron compound amino acid injection, fat emulsion injection
Prior chemotherapy	No	GP regimen (4 cycles), docetaxel mono-therapy (3 cycles)	GP regimen (4 cycles)

GP regimen gemcitabine plus cisplatin, *NCD* no complicated disease

Table 2 Plasma and cerebrospinal fluid concentrations of gefitinib

Patients	CSF (ng/mL)		Plasma (ng/mL)		CSF-to-plasma ratio (%)	
	Pre-WBRT	Post-WBRT	Pre-WBRT	Post-WBRT	Pre-WBRT	Post-WBRT
No. 1	5.72	3.16	302.29	272.82	1.89	1.16
No. 2	1.91	1.61	95.43	103.79	2.00	1.55
No. 3	4.81	4.58	107.2	105.13	4.49	4.35
Average	4.15 ± 1.99	3.12 ± 1.48	168.31 ± 116.18	160.58 ± 97.21	2.79 ± 1.47	2.35 ± 1.74
p value	0.310		0.564		0.123	

samples in WBRT combined course. The characteristics of enrolled patients are shown in Table 1. All of them had measurable metastatic lesion in brain from NSCLC. Their ages ranged from 43 to 70. Two patients had been treated with 1 or 2 regimens of systemic chemotherapy for NSCLC before the occurrence of brain metastases.

Concentrations of gefitinib in plasma and CSF

The trough concentrations of gefitinib in plasma and CSF are summarized in Table 2. The intracranial level of gefitinib in all patients ranged from 1.16 to 5.72 ng/mL. The average CSF-to-plasma concentration ratios before and after WBRT were 2.79 ± 1.47 and 2.35 ± 1.74 %, respectively.

Discussion

It was reported that WBRT could induce focal BBB disruption and improve its permeability [19]. However, we found that WBRT failed to improve the penetration efficiency of gefitinib crossing blood to brain in NSCLC patients with brain metastases. It may be attributed to the damaged BBB of metastatic lesions. In general, the radiation breaks down BBB by progressively decreasing the density of endothelial cells and altering integrity of tight junctions [20–23]. However, previous studies have emphasized that the BBB in metastatic lesions was not intact but leaky [15–17]. Both endothelial cell and astrocytes contributed to tight junction of BBB throughout the metastatic deposits were already disrupted [16]. Even in early metastatic brain tumors (larger than 0.5 mm), the BBB is verified as an abnormal situation [17]. Further studies indicated that the intracranial level of gefitinib was significantly higher in the patients with BM than in non-BM patients [7]. It suggested that BBB of BM patients was already opened. Accordingly, subsequent radiotherapy may not have great impact on the morphology of BBB. Other than osmotic means, vasoactive substances, and focused ultrasound, radiation was not considered as a confirmed method of BBB disruption due

to lack of abundant clinical evidence [19, 24–26]. Similarly, few researches focused on the impact of radiation on BBB permeability of small molecular-targeted antineoplastic drug in metastasis lesions [7, 8]. Moreover, the two researches were both retrospective studies, and the permeability of gefitinib before and after WBRT was evaluated on different patients, respectively. Consequently, bias was inevitably induced by the unpaired penetration information of pre-WBRT with that of post-WBRT. So, we developed this self-controlled research to verify the role of WBRT by comparing the permeability of pre-WBRT to that of post-WBRT in same individual.

Although BBB in metastatic lesions is more permeable than in primary lesion, the intracranial level of gefitinib remains low. The level of gefitinib in CSF disclosed in this study was consistent with the reported study [7, 8] and was lower than the value of 50 % EGFR inhibitor concentration (IC_{50}) of gefitinib (8.9 ng/mL, in vitro) [27]. The intact BBB acts as a highly selective physical barrier and depends on two protective systems, not only tight junction structure but also efflux transporters. The efflux transporters expressed in endothelial cells act as active guarders by transporting drugs from brain to vessel. Thus, the ability of chemical compound to cross BBB is not only relying on its ideal characteristics, such as low molecular weight and nonpolar, but also determined by whether being substrates of efflux pumps or not. Gefitinib is a substrate to P-glycoprotein (P-gp) which is one of the important efflux transporters highly expressed in BBB. There is a preliminary evidence that radiotherapy do not modulate P-gp expression in human malignant glioma in vivo [28].

Previous research suggested that combined therapy of gefitinib and WBRT would confer better clinical outcome than either treatment alone [6]. However, the mechanism of enhanced therapeutic efficacy is unclear. And it is hypothetically attributed to the intrinsically antiproliferative, proapoptotic, and radiosensitizing effects of gefitinib in cancer cells [29, 30], or the improved gefitinib concentration within the brain. In this study, we found that WBRT would not improve the ability of gefitinib crossing BBB. Although the result of our present study still needs further validation on the basis of larger analysis pool, it provides novel insights into the understanding of TKI efficacy after WBRT.

However, our conclusion was limited to the small sample size. For the reason that many Chinese patients worried about the trauma caused by lumbar puncture, only three patients were willing to take this test. In other words, we tried to reduce bias by better design, such as self-controlled design and strict inclusion criteria, to eliminate interference of other factors that may affect BBB potentially, and to increase the power of our result.

As discussed above, the preliminary result of this prospective research suggested that the impact of WBRT on CSF–plasma penetration of gefitinib in the CNS metastatic NSCLC patients may be overestimated. And we expected that the result would inspire a bigger size research with excellent designs to validate this observation.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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