Cancer Chemother Pharmacol (2011) 67:1465–1469
DOI 10.1007/s00280-011-1555-6

SHORT COMMUNICATION

Erlotinib efficacy and cerebrospinal fluid concentration in patients with lung adenocarcinoma developing leptomeningeal metastases during gefitinib therapy

Takeshi Masuda · Noboru Hattori · Akinobu Hamada · Hiroshi Iwamoto · Shinichiro Ohshimo · Masashi Kanehara · Nobuhisa Ishikawa · Kazunori Fujitaka · Yoshinori Haruta · Hiroshi Murai · Nobuoki Kohno

Received: 24 September 2010 / Accepted: 10 January 2011 / Published online: 28 January 2011

Abstract
Purpose We have treated patients with non-small-cell lung cancer (NSCLC) who developed leptomeningeal metastases (LM) during gefitinib therapy, and then found symptomatic improvement following treatment change to erlotinib. Based on this experience, we wondered whether erlotinib could be detected in cerebrospinal fluid (CSF) when it was used for NSCLC patients with LM. This study was conducted to determine erlotinib concentrations in CSF and assess responses to erlotinib in patients with NSCLC developing LM during gefitinib therapy.
Methods Three advanced NSCLC patients with LM that developed during gefitinib therapy were treated with erlotinib. On day 28 after the initiation of erlotinib treatment, plasma and CSF were obtained and the concentrations of erlotinib in these samples were measured. Eastern Cooperative Oncology Group (ECOG) performance status (PS) and neurologic symptoms were determined.
Results Erlotinib CSF penetration was 6.3% ± 6.1% (mean ± SD). In cases 1 and 2, we observed improvements in ECOG PS and neurologic symptoms. In case 3, cytological improvement was seen in the CSF. In each patient, deletion of exon 19 or exon 21 L858R mutation of the epidermal growth factor receptor (*EGFR*) gene was detected in carcinoma cells from the CSF.
Conclusions We report on 3 patients with NSCLC who had developed LM during gefitinib treatment and showed clinical improvements following change to erlotinib therapy. In all cases, small but measurable penetration of erlotinib into CSF was observed. Because *EGFR* mutations were detected in all cases, we suggest that erlotinib is a therapeutic option for LM carcinoma cells with *EGFR* mutations.

Keywords Non-small-cell lung cancer · Leptomeningeal metastases · Erlotinib · Gefitinib · Cerebrospinal fluid

T. Masuda · N. Hattori (✉) · H. Iwamoto · S. Ohshimo · M. Kanehara · N. Ishikawa · K. Fujitaka · Y. Haruta · H. Murai · N. Kohno
Department of Molecular and Internal Medicine, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8551, Japan
e-mail: nhattori@hiroshima-u.ac.jp

A. Hamada
Department of Clinical Pharmaceutical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

Introduction

Leptomeningeal metastases (LM) occur in approximately 4–15% of all patients with cancer [1]. The median survival of patients without treatment is about 4–6 weeks [2], and patients in general suffer from deterioration of performance status (PS) [3]. The occurrence of LM is increasing because of advances in the treatment of non-small-cell lung cancer (NSCLC) and neuroimaging. While radiotherapy, systemic chemotherapy, and intrathecal chemotherapy with methotrexate, thiopeta, or cytarabine have all been used to treat LM, standard therapy that prolongs survival has not been established [4]. Systemic cytotoxic chemotherapy has no efficacy against LM [5], but there have been reports that LM of NSCLC responds to erlotinib and gefitinib [6–9]. In particular, there are reports observing that erlotinib was effective for LM after the failure of gefitinib treatment [6, 7]. In our hospital, we also treated some patients with NSCLC who had developed LM during gefitinib therapy and then showed

symptomatic improvement following the administration of erlotinib. Although a previous study measured the concentration of erlotinib in the cerebrospinal fluid (CSF) of NSCLC patient with LM following a weekly high-dose administration [10], the concentrations of erlotinib in such patients during daily administration have not been investigated. Therefore, we conducted this study on NSCLC patients with LM that occurred during gefitinib therapy, who were then treated with erlotinib, to determine erlotinib concentrations in the CSF and to assess patient responses to erlotinib.

Patients and methods

Patients

Three patients with NSCLC who had developed LM during gefitinib therapy were treated with erlotinib in our hospital from December 2008 to April 2010. The dose of erlotinib was 150 mg/day for all patients, but was reduced to 100 mg/day in cases 1 and 2 because of the occurrence of grade 3 skin rash. Written informed consent for genetic analysis was obtained from each patient at the time of diagnosis.

Response and survival assessment

The response of LM to treatment was assessed by ECOG PS grading, neurologic symptoms, and CSF examination. The overall survival duration was defined as the period between the diagnosis of LM and death.

Mutational analysis

The specimens obtained by transbronchial lung biopsy in 2 patients and the cells collected from CSFs from all the patients were analyzed for *EGFR* mutations (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Screening for *EGFR* mutations was performed by the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PNA-LNA PCR) clamp-based test, and if amplifications in exons 18–21 of the EGFR gene were detected, direct sequencing of the products was performed [11].

Cytological analysis of cells in CSF

CSF was collected under sterile conditions by allowing free flow of CSF under gravity. Between 0.5 and 5.0 ml of CSF was obtained from each procedure. Two cytospin smears from each sample were prepared, and one was stained with Papanicolaou stain and the other with Peppenheim stain. The stained smears were examined by light microscopy for morphological details and to count the number of carcinoma cells.

Plasma and CSF erlotinib sampling and bioanalysis

Once appropriate consent was obtained, blood and CSF were collected simultaneously 24 h after intake of erlotinib on day 28 of therapy. Concentrations of erlotinib in plasma and CSF were determined by a validated high-performance liquid chromatography method, as described previously [12].

Results

Patient characteristics

Patient characteristics and clinical courses are summarized in Table 1. Their ages ranged from 42 to 58 years at the time of LM diagnosis. All of them had metastatic disease in the brain parenchyma before LM developed and had received whole brain radiotherapy (WBRT). Two patients had been treated with 2 or 3 regimens of systemic chemotherapy before the occurrence of LM. All patients developed LM while taking gefitinib. Disease outside the central nervous system had been well controlled in all patients during gefitinib therapy. The interval from the diagnosis of NSCLC to the occurrence of LM ranged from 10 to 65 months, with a mean of 32.3 months.

Table 1 Patient characteristics

Case	Age/sex	Histology	WBRT	Prior CTx	Initial response to gefitinib	*EGFR* mutation in TBLB sample	*EGFR* mutation in CSF cells
1	42/Male	Ad	Yes	Yes	PR	Exon 19 deletion	Exon 19 deletion
2	53/Male	Ad	Yes	Yes	PR	Nt	Exon 19 deletion
3	58/Male	Ad	Yes	No	PR	Exon 21 L858R	Exon 21 L858R

Ad adenocarcinoma; *TBLB* transbronchial lung biopsy; *Nt* not tested; *CSF* cerebrospinal fluid; *PR* partial response; *LM* leptomeningeal metastases; *CTx* chemotherapy; *WBRT* whole brain radiotherapy

Table 2 Response to erlotinib in patients, analysis of CSF (before and on day 28 after initiation of erlotinib), concentrations of erlotinib in CSF and plasma

Case	Change in PS	Neurologic symptoms	No. of carcinoma cells (/ml) before day28		Survival after start of E	Plasma (nM)	CSF (nM)	Penetration (%)
			Before	Day 28				
1	4 → 1	Improved	60	0	93	1,396	186	13.3
2	3 → 1	Improved	Nt	8	165	1,163	34.7	3.0
3	4 → 4	Unchanged	640	11	73	3,210	81.4	2.5

CSF cerebrospinal fluid; *PS* performance status; *No* number; *E* erlotinib; *Nt* not tested

Clinical outcomes

Assessments on day 28 after the initiation of erlotinib demonstrated improvements in PS and neurologic symptoms in cases 1 and 2 (Table 2). In case 1, improvement in CSF cytology was also observed. In case 2, cytological analysis of CSF before erlotinib treatment was not performed; therefore, changes in CSF cytology could not be evaluated. In case 3, erlotinib did not improve PS or neurologic symptoms; however, it reduced the number of carcinoma cells in CSF. Patient survival time ranged from 73 to 165 days (Table 2).

EGFR mutation analysis

In case 1, deletion in *EGFR* exon19 (E746-A750) was detected in both a lung specimen and CSF carcinoma cells (Table 1). In case 2, a deletion in exon19 (E746-A750) was also detected in cells from CSF specimens (Table 1). In case 3, a point mutation in exon 21 (L858R) was detected in both specimens obtained from the lung and CSF (Table 1). There were no second mutations, including exon 20 (T790M), observed.

Concentrations of erlotinib in plasma and CSF

The trough concentrations of total (free and protein-bound) erlotinib in plasma and CSF were 1,396 and 186 nM in case 1 (*erlotinib* 100 mg/day), 1,163 and 34.7 nM in case 2 (*erlotinib* 100 mg/day), 3,210 and 81.4 nM in case 3 (*erlotinib* 150 mg/day), respectively (Table 2). Erlotinib CSF penetration ranged from 2.5 to 13.3% (Table 2).

Discussion

In this report, we presented 3 patients who had developed LM during gefitinib (250 mg/day) therapy and demonstrated clinical or cytological improvements following a change to erlotinib (100 or 150 mg/day). In a previous study, Katayama et al. reported that 6 of 7 patients with gefitinib-resistant brain metastases or LM responded to erlotinib [6]; however, they did not determine the concentrations of erlotinib in CSF. In this study, we determined the concentrations of erlotinib in plasma and CSF and confirmed that there was a small but measurable penetration of erlotinib into CSF (penetration, 6.3% ± 6.1%: mean ± SD). The amount of erlotinib CSF penetration was similar to the amount reported in a recent study [13]. These observations suggest that erlotinib may be a therapeutic option for LM developing during gefitinib treatment.

The differences in LM responses to gefitinib and erlotinib may be explained by the higher erlotinib CSF concentrations than gefitinib CSF concentrations. The trough concentration of gefitinib in CSF was reported to be 6.2 nM under high-dose administration (500 mg/day) [14], and it was much lower than the trough concentration of erlotinib (ranging from 34.7 to 186 nM under administration of 100 or 150 mg/day) in this study. To our best knowledge, there has been only 1 case report of carcinomatous meningitis, in which gefitinib penetration was determined to be less than 1% [8]. This value is much lower than the penetration of erlotinib measured in this study (ranging from 2.5 to 13.3%). Although the oral bioavailabilities are reported as almost equal between erlotinib and gefitinib [15, 16], the standard doses for gefitinib (250 mg/day) and erlotinib (150 mg/day) are known to be not biologically equivalent. For daily administration of erlotinib at 150 mg in two groups consisting of 3 or 10 subjects, the maximum concentration (*Cmax* mean ± SD) and area under the curve (*AUC* mean ± SD) were reported to reach 2,120 ± 1,520 ng/ml and 38,420 ± 29,550 ng/h/ml in the former group and 1,737 ± 777 ng/ml and 26,500 ± 12,780 ng/h/ml in the latter group, respectively [17]; as reported in a previous study [18], administration of 700 mg is probably needed for gefitinib to gain similar Cmax and AUC levels (mean ± % coefficient of variation (CV): 2,146 ng/ml ± 34% and 36,077 ng/h/ml ± 43%, respectively), although the relationships between dose and Cmax, or AUC are found not to be linear. In addition, the half maximal inhibitory concentration (IC_{50}) values of erlotinib were reported to be significantly lower than those

of gefitinib for both wild-type and mutant EGFR [19–21], suggesting a difference in inhibitory effects on NSCLC cells between erlotinib and gefitinib. Actually, high-dose administration of gefitinib (1,250 mg/day) was reported to be effective for LM in a patient with NSCLC harboring an EGFR mutation [14]. Taken all these observations together, we can speculate that the CSF concentrations of gefitinib must have been lower compared with those of erlotinib determined in the studied patients and this caused the difference in responses to LM between gefitinib and erlotinib.

Although the CSF erlotinib concentration was much lower than the concentration in plasma, treatment with erlotinib yielded symptomatic and/or cytologic improvements in the LM patients of this study. This outcome may be explained by the difference in the amounts of erlotinib bound to proteins in CSF and plasma. In plasma, approximately 93% of erlotinib is bound to proteins such as albumin or alpha-1 acid glycoprotein [22]. The protein concentration in the CSF of our patients was about 100 times lower than the concentration in plasma; therefore, we can speculate that the CSF concentration of free erlotinib was sufficient to control LM in our patients. On the other hand, the protein-bound proportion of gefitinib in plasma is reported as approximately 97% [23], similar to but a little higher than that of erlotinib. The lower concentration under administration of the standard dose and a little higher protein-bound proportion in plasma may also explain the lower penetration of gefitinib into CSF than erlotinib.

In conclusion, we reported on 3 NSCLC patients who had developed LM during gefitinib treatment and who showed clinical improvements following the change to erlotinib. In all cases, a small but measurable penetration of erlotinib into CSF was observed. The fact that *EGFR* mutations were detected in CSF carcinoma cells in all the patients indicates that erlotinib is a therapeutic option for LM with *EGFR* mutations.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS). Nobuoki Kohno has received a research grant from Chugai Pharmaceutical Co., Ltd.

Conflict of interest All other authors have no conflict of interest.

References

1. Taillibert S, Laigle-Donadey F, Chodkiewicz C, Sanson M, Hoang-Xuan K, Delattre JY (2005) Leptomeningeal metastases from solid malignancy: a review. J Neurooncol 75:85–99
2. Grossman SA, Krabak MJ (1999) Leptomeningeal carcinomatosis. Cancer Treat Rev 25:103–119
3. Ceresoli GL, Reni M, Chiesa G, Carretta A, Schipani S, Passoni P, Bolognesi A, Zannini P, Villa E (2002) Brain metastases in locally advanced nonsmall cell lung carcinoma after multimodality treatment: risk factors analysis. Cancer 95:605–612
4. O'Meara WP, Borkar SA, Stambuk HE, Lymberis SC (2007) Leptomeningeal metastasis. Curr Probl Cancer 31:367–424
5. Chamberlain MC, Kormanik P (1998) Carcinoma meningitis secondary to non-small cell lung cancer: combined modality therapy. Arch Neurol 55:506–512
6. Katayama T, Shimizu J, Suda K, Onozato R, Fukui T, Ito S, Hatooka S, Sueda T, Hida T, Yatabe Y, Mitsudomi T (2009) Efficacy of erlotinib for brain and leptomeningeal metastases in patients with lung adenocarcinoma who showed initial good response to gefitinib. J Thorac Oncol 4:1415–1419
7. Yi HG, Kim HJ, Kim YJ, Han SW, Oh DY, Lee SH, Kim DW, Im SA, Kim TY, Kim CS, Heo DS, Bang YJ (2009) Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are effective for leptomeningeal metastasis from non-small cell lung cancer patients with sensitive EGFR mutation or other predictive factors of good response for EGFR TKI. Lung Cancer 65:80–84
8. Fukuhara T, Saijo Y, Sakakibara T, Inoue A, Morikawa N, Kanamori M, Nakashima I, Nukiwa T (2008) Successful treatment of carcinomatous meningitis with gefitinib in a patient with lung adenocarcinoma harboring a mutated EGF receptor gene. Tohoku J Exp Med 214:359–363
9. Kim MK, Lee KH, Lee JK, Choi JH, Hyun MS (2005) Gefitinib is also active for carcinomatous meningitis in NSCLC. Lung Cancer 50:265–269
10. Clarke JL, Pao W, Wu N, Miller VA, Lassman AB (2010) High dose weekly Erlotinib achieves therapeutic concentrations in CSF and is effective in leptomeningeal metastases from epidermal growth factor receptor mutant lung cancer. J Neurooncol 99:283–286
11. Nagai Y, Miyazawa H, Huqun TanakaT, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M, Hagiwara K (2005) Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 65:7276–7282
12. Lepper ER, Swain SM, Tan AR, Figg WD, Sparreboom A (2003) Liquid-chromatographic determination of erlotinib (OSI-774), an epidermal growth factor receptor tyrosine kinase inhibitor. J Chromatogr B Analyt Technol Biomed Life Sci 796:181–188
13. Togashi Y, Masago K, Fukudo M, Terada T, Fujita S, Irisa K, Sakamori Y, Kim YH, Mio T, Inui K, Mishima M (2010) Cerebrospinal fluid concentration of erlotinib and its active metabolite OSI-420 in patients with central nervous system metastases of non-small cell lung cancer. J Thorac Oncol 5:950–955
14. Jackman DM, Holmes AJ, Lindeman N, Wen PY, Kesari S, Borras AM, Bailey C, de Jong F, Janne PA, Johnson BE (2006) Response and resistance in a non-small-cell lung cancer patient with an epidermal growth factor receptor mutation and leptomeningeal metastases treated with high-dose gefitinib. J Clin Oncol 24:4517–4520
15. Swaisland HC, Smith RP, Laight A, Kerr DJ, Ranson M, Wilder-Smith CH, Duvauchelle T (2005) Single-dose clinical pharmacokinetic studies of gefitinib. Clin Pharmacokinet 44:1165–1177
16. Frohna P, Lu J, Eppler S, Hamilton M, Wolf J, Rakhit A, Ling J, Kenkare-Mitra SR, Lum BL (2006) Evaluation of the absolute oral bioavailability and bioequivalence of erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in a randomized, crossover study in healthy subjects. J Clin Pharmacol 46:282–290

17. Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, Eckhardt SG, Tolcher A, Britten CD, Denis L, Ferrante K, Von Hoff DD, Silberman S, Rowinsky EK (2001) Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. J Clin Oncol 19:3267–3279
18. Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, Miller V, Averbuch S, Ochs J, Morris C, Feyereislova A, Swaisland H, Rowinsky EK (2002) ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. J Clin Oncol 20:2240–2250
19. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129–2139
20. Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, DiOrio C, Doty J, Morin MJ, Moyer MP, Neveu M, Pollack VA, Pustilnik LR, Reynolds MM, Sloan D, Theleman A, Miller P (1997) Induction of apoptosis and cell cycle arrest by CP-358, 774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res 57:4838–4848
21. Kancha RK, von Bubnoff N, Peschel C, Duyster J (2009) Functional analysis of epidermal growth factor receptor (EGFR) mutations and potential implications for EGFR targeted therapy. Clin Cancer Res 15:460–467
22. Johnson JR, Cohen M, Sridhara R, Chen YF, Williams GM, Duan J, Gobburu J, Booth B, Benson K, Leighton J, Hsieh LS, Chidambaram N, Zimmerman P, Pazdur R (2005) Approval summary for erlotinib for treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen. Clin Cancer Res 11:6414–6421
23. Li J, Brahmer J, Messersmith W, Hidalgo M, Baker SD (2006) Binding of gefitinib, an inhibitor of epidermal growth factor receptor-tyrosine kinase, to plasma proteins and blood cells: in vitro and in cancer patients. Invest New Drugs 24:291–297