

ORIGINAL ARTICLE

Pilot study of radiofrequency hyperthermia in combination with gefitinib in gefitinib-effective patients with advanced NSCLC

Yijia Qin^{1*}, Yu Sun^{2*}, Yongmei Liu³, Yiqiao Luo¹ & Jiang Zhu³

¹ West China School of Medicine, Sichuan University, Chengdu, China

² Department of Radiation Oncology, West China Hospital, Sichuan University, Chengdu, China

³ Department of Thoracic Oncology, West China Hospital, Sichuan University, Chengdu, China

Keywords

Comprehensive therapy; EGFR-TKI; hyperthermia; NSCLC.

Correspondence

Jiang Zhu, Department of Thoracic Oncology, West China Hospital, Guoxue Lane, Chengdu 610041, China.

Tel: +86 189 8060 2256

Email: zhujiang1@medmail.com.cn

*These authors contributed equally to this work.

Received: 9 December 2015;

Accepted: 6 February 2016.

doi: 10.1111/1759-7714.12346

Thoracic Cancer 7 (2016) 422–427

Abstract

Background: Non-small-cell lung cancer (NSCLC) is the leading cause of death from cancer in China. Gefitinib is effective for patients with positive epidermal growth factor receptor gene mutation; however, acquired drug resistance counteracts the duration response. Hyperthermia is widely clinically applied in the treatment of solid tumors. This pilot study was designed to evaluate the feasibility of the combination of gefitinib and hyperthermia.

Methods: Patients newly diagnosed with advanced NSCLC were screened. Eleven patients who responded to first-line gefitinib treatment were enrolled in the study. Along with 250 mg gefitinib daily, local radiofrequency hyperthermia was administered twice a week until tumor progression was observed. The serum, heat shock protein (HSP)70, was also frequently detected during the course.

Results: The most common toxicity included skin rash (81.8%) and abnormal liver function (45.5%) when treated with gefitinib, and fatty scleroma (36.4%) was observed when combined with hyperthermia. Grade 3 side effects (skin rash) occurred in only one patient. Median progression-free survival was 22 months (95% confidence interval [CI]: 12.95–31.05 months) and median overall survival was 26 months (95% CI: 22.81–29.19 months). Serum HSP70 concentration increased and maintained a significantly high level compared with the baseline before hyperthermia administration.

Conclusions: The novel therapy of gefitinib combined with radiofrequency hyperthermia is safe and effective for advanced NSCLC patients. Whether an improvement in therapeutic efficacy is associated with the elevation of serum HSP70 concentration requires further study.

Introduction

Gefitinib is a tyrosine kinase inhibitor (TKI) that targets the epidermal growth factor receptor (EGFR). Patients with non-small-cell lung cancer (NSCLC) with EGFR gene mutation (e.g. exon 19 deletion, 21 L858R) are hypersensitive to gefitinib treatment. Compared with the standard platinum-based doublet chemotherapy, advanced NSCLC patients can achieve longer progression-free survival (PFS) with gefitinib alone as first-line therapy.¹ However, acquired drug resistance of gefitinib after effective

treatment is one of the most important reasons contributing to the failure of gefitinib treatment. Hyperthermia has been confirmed to be effective in the treatment of many solid tumors, while with chemotherapy and/or radiotherapy in combination, its anti-tumor effect can even be enhanced.^{2,3} Heat shock protein (HSP)70 is a significant member of the HSP family, highly conserved in structure with a role of maintaining cell stability, and induced in the body by heat.⁴ Local radiofrequency hyperthermia in vitro has been widely applied in clinical settings. Previous studies have suggested that local radiofrequency hyperthermia

can significantly improve the tumor control rate and the quality of life of advanced NSCLC patients, with good safety and tolerance.⁵ However, there are no relevant research findings for local radiofrequency hyperthermia combined with EGFR-TKI for the treatment of advanced NSCLC.

In this study, we selected patients with advanced NSCLC who responded to gefitinib as a first line treatment, and evaluated the feasibility of the novel therapy of gefitinib combined with radiofrequency hyperthermia.

Methods

Patients

Criteria for enrollment in the study were: adenocarcinoma of the lung confirmed by pathologic diagnosis; advanced NSCLC; patients treated with gefitinib for one month, which was confirmed by imaging to be effective; presence of at least one measurable lesion in the thoracic cavity; Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2; no contraindication of local radiofrequency hyperthermia; and feasible long-term follow-up with good patient compliance.

The exclusion criteria were: pregnancy or breast-feeding; severe infection; significant cardiovascular disease; administration of other therapy (radiotherapy, chemotherapy, biological therapy) or participation in a clinical trial within one month of the study; presence of hepatic and renal dysfunction; and unmanageable psychosis.

All patients volunteered to participate and signed informed consent after fully communicating with researchers before entry into the study. The ethics committee of West China Hospital approved the study.

Treatments

Patients who had not received anti-tumor treatment after diagnosis with NSCLC and were identified with sensitive mutations (e.g. 19Del, 21 L858R) detected in tumor tissues received 250 mg gefitinib (IRESSA, AstraZeneca, London, UK) orally once daily as first line treatment for up to a month. Patients evaluated as achieving complete remission (CR) and partial response (PR) continued gefitinib treatment combined with local radiofrequency hyperthermia (LRFH). Treatment was suspended if patients developed unacceptable adverse reactions. When an adverse reaction became tolerable, patients resumed treatment until intolerable toxicity returned. As the disease progressed, hyperthermia was discontinued, and the physicians in charge determined whether gefitinib treatment should be continued.

The Thermotron RF-8 local radiofrequency hyperthermia system (Vinita Co. Ltd., Kyoto, Japan) was used with an operating frequency of 13.5 MHz, an adjustable endogenous temperature of 38°C to 45°C, and a cold water circulation system to control epidermal overheating. The hyperthermia target lesion was the main lesion in the thoracic cavity, while the heating target area was the surface at which the lesion was projected. Our standard protocol was to administer hyperthermia 40–60 minutes twice a week, at intervals of at least 72 hours, with a target temperature of 42°C. Thermotherapy should be discontinued at disease progression (Fig 1).

Detection of heat shock protein (HSP)70

Intravenous blood samples were taken to detect HSP70 before and 30, 60, and 90 days after heating therapy (advanced or postponed no more than 7 days). Enzyme-

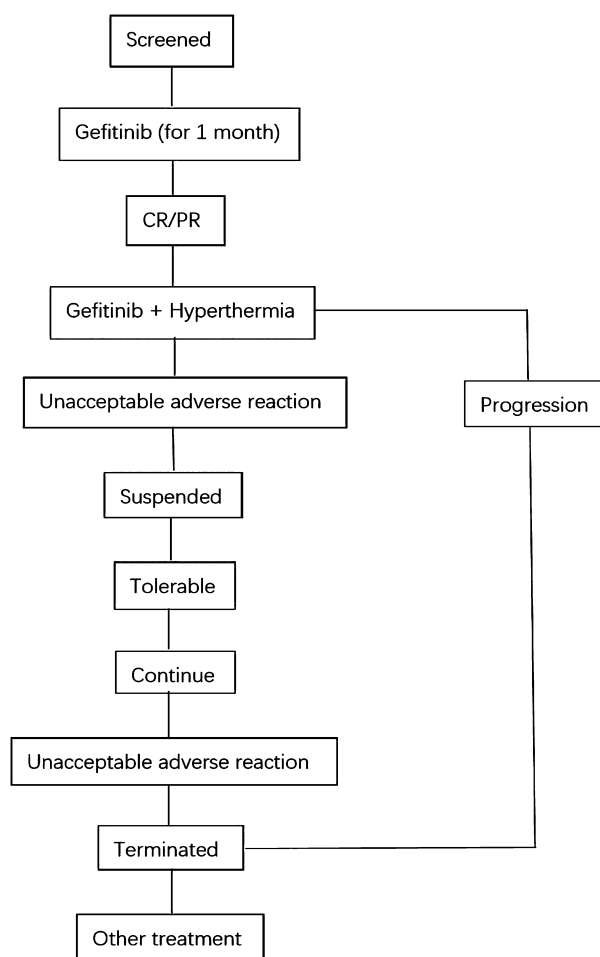


Figure 1 Treatment protocol. CR, complete response; PR, partial response.

linked immunosorbent assay kits were used, following the manufacturer’s instructions.

Follow-up

Enrolled patients were required to visit the doctor bi-weekly, at which time they would undergo physical and auxiliary examinations and imaging efficacy evaluation and revision of the hyperthermia target area. A patient could visit their doctor at any time when there was any change in their condition or any urgent need. Once the first progression occurred, patients were advised to visit their doctor every two to three months or participate in follow-up by telephone until the conclusion of the study (Fig 2).

Assessments

The study was designed as a prospective, small sample, non-randomized controlled study. The primary endpoint included grade 3 ~ 4 adverse events and PFS, while the explorative experimental measurement was the variation of serum HSP70. PFS was defined as the time period from gefitinib therapy to the first confirmed progression; overall survival (OS) was defined from gefitinib therapy to death. Responses were assessed based on Response Evaluation Criteria in Solid Tumors.⁶

Statistical analysis

We used a *t*-test to compare measurement data and the Kaplan–Meier method to determine survival analysis. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was employed to analyze all statistical data.

Results

Patients

Eleven patients with advanced NSCLC were enrolled in the study from March 2012 to March 2013. All patients were pathologically reviewed as positive for EGFR mutation and confirmed as responsive to gefitinib after one-month of treatment via imaging (CR or PR). Patient characteristics are listed in Table 1. There were three men (27.3%) and eight women (72.7%), with a median age of 56 years (44–69 years). There were 10 cases of adenocarcinoma and one case of adenosquamous carcinoma. All enrolled patients were evaluated for tumor response as PR with one-month of gefitinib treatment, and were administered hyperthermia from the second month of gefitinib treatment. Hyperthermia treatment ranged from 64 to 206 times for each patient, up to 1655 times in total.

Toxicity

No therapy-related death or serious adverse events occurred. Major adverse events experienced prior to hyperthermia treatment included: nine (81.8%) cases of skin rash, five (45.5%) of abnormal liver function, four (36.4%) of diarrhea, and two (18.2%) of fatigue, all of which varied from 1 to 2 degrees according to Common Terminology Criteria for Adverse Events. New adverse effects when gefitinib was combined with hyperthermia included four (36.4%) cases of fatty scleroma and one (9.1%) case of fever. There was also a case of third-degree skin rash, which abated to one degree after a one-week suspension of gefitinib and hyperthermia, symptomatically treated. Adverse events did not require suspension of therapy in any other patients. After the completion of our study, two

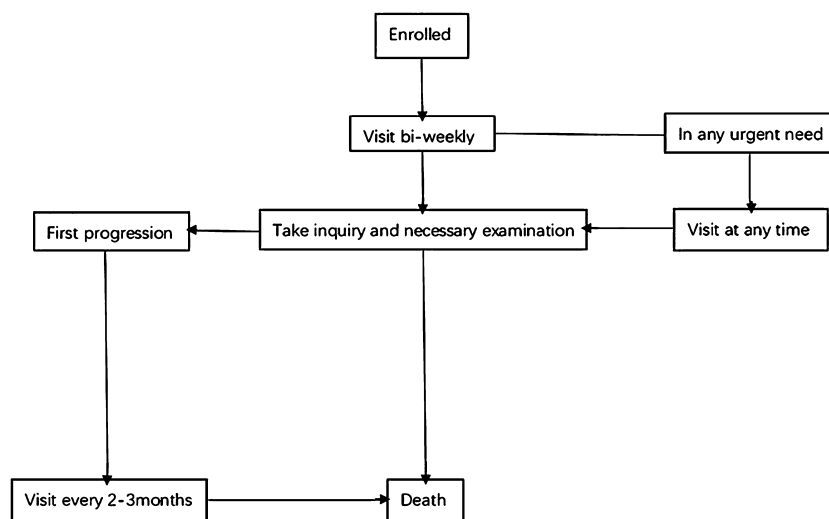


Figure 2 Follow- up process.

patients were left with second-degree skin rash, while adverse events experienced by other patients disappeared or alleviated to one degree (Table 2).

Serum HSP70 before and after hyperthermia

The baseline level of serum HSP70 was 4.366 ± 1.18 ng/mL before hyperthermia, increasing to 5.901 ± 1.399 ng/mL 30 days after hyperthermia, 5.692 ± 0.974 ng/mL 60 days after, and 6.362 ± 1.326 ng/mL 90 days after, with an average increase of 1.619 ± 1.149 ng/mL. HSP70 maintained a high level after hyperthermia, which had statistical significance, compared with the baseline (Fig 3).

Survival analysis

The longest follow-up period was 27 months (median 24 months). By the conclusion of the study, definite disease progression had occurred in seven patients. The median PFS was 22 months (95% confidence interval [CI]: 12.95–31.05 months). Once tumor progression was detected, five of the seven patients were administered at least one cycle

of systematic chemotherapy as subsequent anticancer treatment. Five patients had died once data was collected; the median OS was 26 months (95% CI: 22.81–29.19 months) according to the Kaplan–Meier method (Figs 4–5).

Discussion

Gefitinib is a tyrosine kinase inhibitor that targets the epidermal growth factor receptor, which is effective in tumor control for NSCLC patients with positive EGFR mutations.⁷ A previous study reported a response rate of over 70%, with a median PFS of nearly 10 months and a median OS of 24 months.¹ Gefitinib has already been approved for first line use in patients with advanced NSCLC harboring EGFR mutations. However, acquired drug resistance has led directly to treatment failure and disease progression, counteracting the therapeutical benefit. Prior study has shown that the duration of response lasts for 10–12 months in gefitinib responsive patients following chemotherapy or other anti-cancer treatment, with a short survival duration after progression.⁸ Recent findings have demonstrated that the leading cause of acquired drug resistance of EGFR-TKI is closely associated with a secondary mutation in cancer cells, mainly T790M.⁹ Determining

Table 1 Patient characteristics

Parameter	N = 11(%)
Gender	—
Male	3 (27.3%)
Female	8 (72.7%)
Median age (range)	56 (44 ~ 69)
Performance status (ECOG)	11 (100%)
0	—
1	—
2	—
Pathological type	10 (90.1%)
Adenocarcinoma	1 (9.1%)
Adenosquamous	—
Response to gefitinib in 1 month	—
CR	11 (100%)
PR	—

CR, complete response; ECOG, Eastern Cooperative Oncology Group; PR, partial response.

Table 2 Safety and toxicity evaluation

Adverse events	Common Terminology Criteria for Adverse Events			
	Grade 1	Grade 2	Grade 3	Grade 4
Skin rash	4 (36.4%)	4 (36.4%)	1 (9.1%)	0
Abnormal liver function	4 (36.4%)	1 (9.1%)	0	0
Diarrhea	4 (36.4%)	0	0	0
Fatigue	2 (18.2%)	0	0	0
Fatty scleroma	4 (36.4%)	0	0	0
Fever	0	1 (9.1%)	0	0

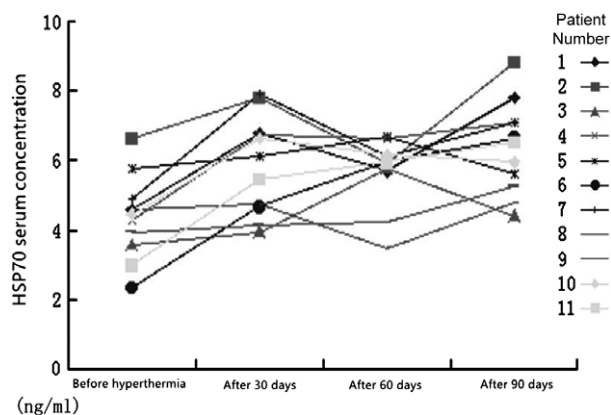


Figure 3 Heat shock protein 70 (HSP70) serum concentration before and after hyperthermia.

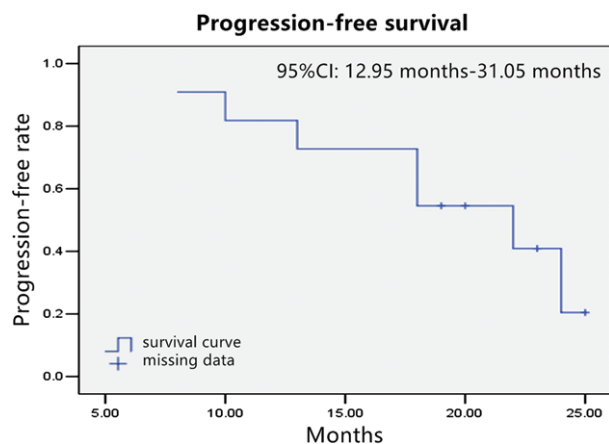


Figure 4 Progression-free survival (PFS) of patients treated with gefitinib combined with hyperthermia. CI, confidence interval.

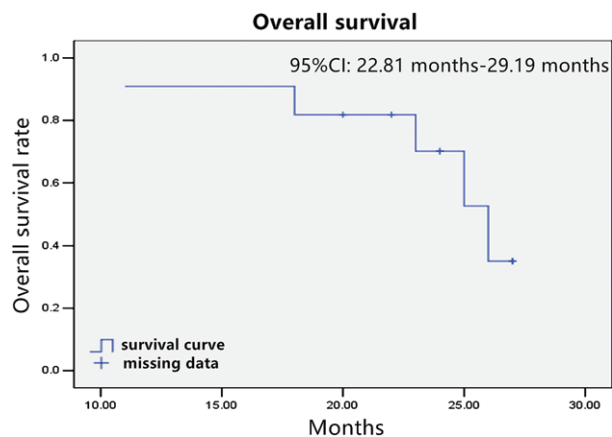


Figure 5 Overall survival (OS) of patients treated with gefitinib combined with hyperthermia.

a method to avoid or delay the secondary mutation may prolong the duration of response.

Hyperthermia is a kind of therapy that kills cancer cells and enhances anti-tumor efficacy by elevating the local or whole body temperature and maintaining it for a period of time.² LRFH *in vitro*, widely clinically applied, has the advantage of strong penetrability, endogenic heating field, acceptable side effects, and good tolerance.¹⁰ Many clinical studies have been performed to evaluate the comprehensive therapy model combining hyperthermia with chemotherapy and/or radiotherapy in patients with advanced NSCLC, and have reported improved treatment efficiency and prognoses.^{3,5,11,12} However, no relevant research determining whether LRFH combined with EGFR-TKI is feasible and beneficial to prognosis exists.

Heat shock proteins are a set of proteins induced under stress, particularly in high temperatures. HSP70 is a significant member of HSP family, highly conserved in structure,

which maintains the stability of cells and the conformation of proteins.¹³ Induced by heat, it is amply expressed in lung cancer cells.¹⁴ According to a preclinical study in human ovarian cancer cells, cisplatin resistance is associated with a higher expression of HSP70.¹⁵ Therefore, downregulation of HSP70 seems to selectively sensitize malignant cells to chemotherapeutic agents.¹⁶ Unlike the negative effects the HSP family causes to cytotoxic treatment, HSP70 may play a different role in EGFR-TKI and hyperthermia combination therapy. We assumed that TKI acquired resistance caused by secondary mutation could be delayed by stabilizing the conformation of the EGFR intracellular structure by increasing HSP70. Our results determined that combined hyperthermia extends the response duration of gefitinib, while the HSP serum level was significantly elevated (in only 1 case the HSP70 level was lower than the baseline, the 30 and 90 day levels were higher, while the 60 day measurement lowered the average data; the cause is unclear). According to our results, the combined treatment of gefitinib and hyperthermia can result in a PFS of 22 months, which is superior to the PFS for gefitinib alone.

Further exploration of this innovative therapeutic model of EGFR-TKI treatment combined with hyperthermia is necessary. Because of restrictions in the study design, we cannot draw a definite conclusion from this preliminary study whether the improved therapeutic effect resulted from the combined treatment or was merely a coincidence. Further exploration is merited by the median PFS result in order to definitely prove the superiority of this combination therapy.

Our analysis indicated that the combination therapy model of EGFR-TKI with hyperthermia is safe and effective for the treatment of advanced NSCLC. The addition of hyperthermia may prolong the period of treatment efficacy in patients with advanced NSCLC who were treated effectively with gefitinib. Further study is required to determine whether the improvement is associated with an elevation of serum HSP70 concentration.

Acknowledgments

This study obtained financial support from the Wu Jieping Medical Foundation (320.6799.1136).

Disclosure

No authors report any conflict of interest.

References

- 1 Maemondo M, Inoue I, Kobayashi K *et al.* Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; **362**: 2380–8.

- 2 Wust P, Hildebrandt B, Sreenivasa G *et al.* Hyperthermia in combined treatment of cancer. *Lancet Oncol* 2002; **3**: 487–97.
- 3 Mitsumori M, Zeng ZF, Oliynychenko P *et al.* Regional hyperthermia combined with radiotherapy for locally advanced non-small cell lung cancers: A multi-institutional prospective randomized trial of the International Atomic Energy Agency. *Int J Clin Oncol* 2007; **12**: 192–8.
- 4 Kiang JG, Tsokos GC. Heat shock protein 70 kDa: Molecular biology, biochemistry, and physiology. *Pharmacol Ther* 1998; **80**: 183–201.
- 5 Jiang Z, Yan W, Ming J, Yu Y. Docetaxel weekly regimen in conjunction with RF hyperthermia for pretreated locally advanced non-small cell lung cancer: A preliminary study. *BMC Cancer* 2007; **7**: 189.
- 6 Eisenhauer EA, Therasse P, Bogaerts J *et al.* New response evaluation criteria in solid TUMORS: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228–47.
- 7 Paez JG, Jänne PA, Lee JC *et al.* EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 2004; **304**: 1497–500.
- 8 Kim H, Yun T, Lee YJ, Han JY, Kim HT, Lee GK. Post-progression survival in patients with non-small cell lung cancer with clinically acquired resistance to gefitinib. *J Korean Med Sci* 2013; **28**: 1595–602.
- 9 Yu H, Arcila ME, Rekhtman N *et al.* Analysis of mechanisms of acquired resistance to EGFR TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* 2013; **19**: 2240–7.
- 10 Wust P, Stahl H, Löffel J, Seebass M, Riess H, Felix R. Clinical, physiological and anatomical determinants for radiofrequency hyperthermia. *Int J Hyperthermia* 1995; **11**: 151–67.
- 11 Guo J, Liu W, Zhang H. Clinical study of hyperthermia combined with chemotherapy in advanced non small cell lung cancer (NSCLC). *J Mod Oncol* 2013; **11**: 151–67.
- 12 Shen H, Li XD, Wu CP, Yin YM, Wang RS, Shu YQ. The regimen of gemcitabine and cisplatin combined with radio frequency hyperthermia for advanced non-small cell lung cancer: A phase II study. *Int J Hyperthermia* 2011; **27**: 27–32.
- 13 Mayer MP, Bukau B. Hsp70 chaperones: Cellular functions and molecular mechanism. *Cell Mol Life Sci* 2005; **62**: 670–84.
- 14 Gao S, Wang L, Wu W, Zhou F. [Synergistic effect of thermotherapy in combination with chemotherapy on lung tumor A549 cells growth through activation of c-Jun N-terminal kinase and inhibition of heat shock protein70 expression.]. *J Hyg Res* 2008; **37**: 529–32. (In Chinese.)
- 15 Yang X, Wang J, Zhou Y, Wang Y, Wang S, Zhang W. Hsp70 promotes chemoresistance by blocking Bax mitochondrial translocation in ovarian cancer cells. *Cancer Lett* 2012; **321**: 137–43.
- 16 Goloudina AR, Demidov ON, Garrido C. Inhibition of HSP70: A challenging anti-cancer strategy. *Cancer Lett* 2012; **325**: 117–24.