Aspirin reduces adverse effects of gefitinib

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We measured serum levels of soluble (s) P-selectin and thromboxane B_2 (TxB_2) in patients with lung cancer treated with gefitinib, and investigated the effect of low-dose aspirin on some adverse effects of gefitinib. The serum levels of sP-selectin and TxB_2 increased significantly in all patients who received gefitinib for 2 weeks. Forty patients were recruited, and 28 received gefitinib without low-dose aspirin (Group 1) and 12 were co-administered low-dose aspirin (Group 2). In Group 2, the frequency of adverse events, skin rash and diarrhea was evidently reduced by the low-dose aspirin therapy, despite having shown no remarkable change in gefitinib responsiveness between both groups. In one of the 12 patients in Group 2, aspirin therapy was suspended due to the occurrence of nasal bleeding. Four days after treatment suspension, she developed a skin lesion in her finger. However, the skin lesion improved after re-administration of aspirin without any other medications. After treatment, TxB_2 significantly decreased, but not sP-selectin. These results suggest that one of the mechanisms causing gefitinib-related adverse effects depends on platelet activation. Administration of gefitinib with low-dose aspirin to lung cancer patients may prevent the development of gefitinib-related complications. *Anti-Cancer Drugs* 17:423–427 © 2006 Lippincott Williams & Wilkins.

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Introduction

Recently, the application range of anti-platelet therapy has been expanding as platelets have been found to participate in various diseases. Although one of the representative drugs for anti-platelet therapy is aspirin, some problems have been pointed out [1]. Epidermal growth factor receptor (EGFR) is considered to be an important target for cancer therapy. Gefitinib selectively inhibits the tyrosine kinase of EGFR, and blocks the signal transduction pathways for the proliferation and survival of cancer cells and other host-dependent processes promoting cancer growth [2,3]. Although the response rate was higher in Japanese patients than in those from other countries in the Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 2 trial, acute lung injury, including interstitial pneumonia and alveolar hemorrhage, is a severe adverse effect in patients receiving gefitinib in Japan [4,5]. However, in the Iressa Survival Evaluation in Lung Cancer (ISEL) study, including 28 countries across Europe, Asia, Central and South America, Australia, and Canada, the most common side-effects of this drug as well as other agents targeting the EGFR were skin rash and diarrhea [6]. These effects may be severe and are frequently found intolerable for patients, leading to dose reduction or withdrawal from therapy. The mechanisms underlying these adverse events are poorly understood. For the management of skin rash, topical anti-inflammatory drugs are commonly employed, albeit with only moderate success. Studies addressing the management of anti-EGFR therapy-related side-effects are therefore of great interest. We have recently found that some platelet-related factors such as thromboxane B_2 (TxB_2), soluble (s) P-selectin and RANTES (regulated on activation normally T cell expressed and secreted) are being elevated in Japanese patients receiving gefitinib treatment [7,8], and we postulated that some mechanisms of action of gefitinib were related to platelet activation. Therefore, we investigated the effect of low-dose aspirin on skin eruption, which is a common adverse effect of gefitinib. The purpose of the present study was to elucidate the relationship between gefitinib-related adverse effects and platelet activation. This is the first report describing the effectiveness of low-dose aspirin for complications experienced in patients receiving gefitinib treatment.

Materials and methods

Patients with histologically or cytologically confirmed non-small cell lung cancer (NSCLC), which was not curable by surgery, were considered for enrolment in the study. Although patients were not randomized, they were recruited prospectively. In brief, patients were recruited who were admitted to our hospital between October 2000 and December 2004 for assessment of lung cancer (Fig. 1). For the first 2 years, all patients received

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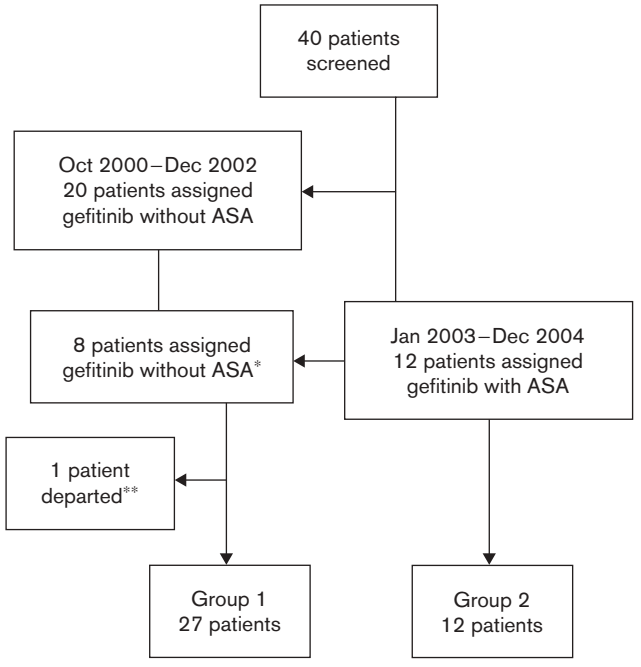
gefitinib without aspirin. After January 2003, patients were recommended treatment with gefitinib plus aspirin. However, there were some patients who were excluded due to being unacceptable or having a bleeding tendency, and these patients were included in the non-aspirin group. The protocol for this study was approved by the institutional review board at the medical institution and written informed consent was also obtained from each study subject prior to the trial. Patients received gefitinib at a dose of 250 mg/day. Patients who could not receive gefitinib over 2 weeks were ineligible. As 10–14 days of administration were required to achieve a stable concentration of gefitinib, we examined the patients before and after 2 weeks of gefitinib administration. Aspirin and gefitinib treatments were started simultaneously. Nonsteroidal anti-inflammatory drugs (NSAIDs) and steroid were continued at the same concentrations. In total, 40 patients were entered into the study (Fig. 1).

Group 1 (non-aspirin cases)

Twenty-eight patients tolerated gefitinib treatment over 2 weeks without any severe adverse effects such as acute lung diseases or thromboembolic diseases. However, one patient was excluded due to discontinuation of chemotherapy for an unknown reason. Thus, a total of 27 patients were recruited into the study. Nineteen patients had previously received several courses of chemotherapies, two patients received concurrent chemotherapies and four patients were treatment-naive cases (Table 1). The characteristics of this patient group were: age 42–82 years (median: 64 years); gender (male versus female): 14 versus 13; tumor histology (adenocarcinoma versus squamous versus undifferentiated): 22 versus 4 versus 1. Ten patients were responders (10/27; 37.0%), although two of them received concurrent chemotherapies.

Fig. 1



Trial design. *Eight patients were excluded due to being unacceptable or having a bleeding tendency, and these patients were included in the non-aspirin group. **This patient was transferred to another hospital.

Group 2 (aspirin cases)

Twelve patients were recruited and all of them received gefitinib with low-dose aspirin (100 mg/day). The characteristics of this patient group were: age 48–80 years (median 69 years); gender (male versus female): 10 versus 2; tumor histology (adenocarcinoma versus squamous cell carcinoma): 10 versus 2; stage (IIIb versus IV): 4 versus 7; response rate: 4/12 (33.3%); skin adverse effect (grade 0 versus 1 versus 2): 7 versus 3 versus 2.

Measurement of TxB_2 and sP-selectin

Blood samples were withdrawn from the patients with NSCLC before and after the administration of gefitinib. The blood was allowed to clot at room temperature for a minimum of 1 h, and then the serum or citrated plasma was separated by centrifugation at 1000 g for 20 min and stored at 4°C until assayed. TxB_2 (the stable metabolite of TxA_2) and sP-selectin were measured by ELISA (TxB_2: Immunotech, Marseille, France; sP-selectin: BioSource International, Camarillo, California, USA). The ELISA kit was used according to the manufacturer's instructions.

Results

In Group 1, the serum level of TxB_2 increased significantly in all patients who received gefitinib for 1 or 2 weeks ($n = 27$, $P < 0.05$, $P < 0.01$, respectively)

Table 1 Patient characteristics

	Group 1 (non-aspirin)	Group 2 (aspirin)
Number	27	12
Median age [years (range)]	64 (42–82)	69 (48–80)
Male/female	14/13	8/4
Smoking [*n* (%)]		
habitual smoker	6 (22.2)	3 (25.0)
ex-smoker	5 (18.5)	3 (25.0)
Tumor histology [*n* (%)]		
adenocarcinoma	22 (81.5)	10 (83.3)
squamous cell	4 (14.5)	2 (16.7)
other	1 (3.7)	0 (0)
Disease stage at diagnosis [*n* (%)]		
I	2 (7.4)	1 (8.3)
II	1 (3.7)	0 (0)
IIIa	4 (14.5)	0 (0)
IIIb	3 (11.1)	4 (33.3)
IV	17 (63.0)	7 (58.3)
Previous therapy [*n* (%)]	19 (70.4)	9 (75.0)
NSAIDs	5 (18.5)	1 (8.3)
steroid	0 (0)	1 (8.3)
responder	10 (37.0)	4 (33.3)

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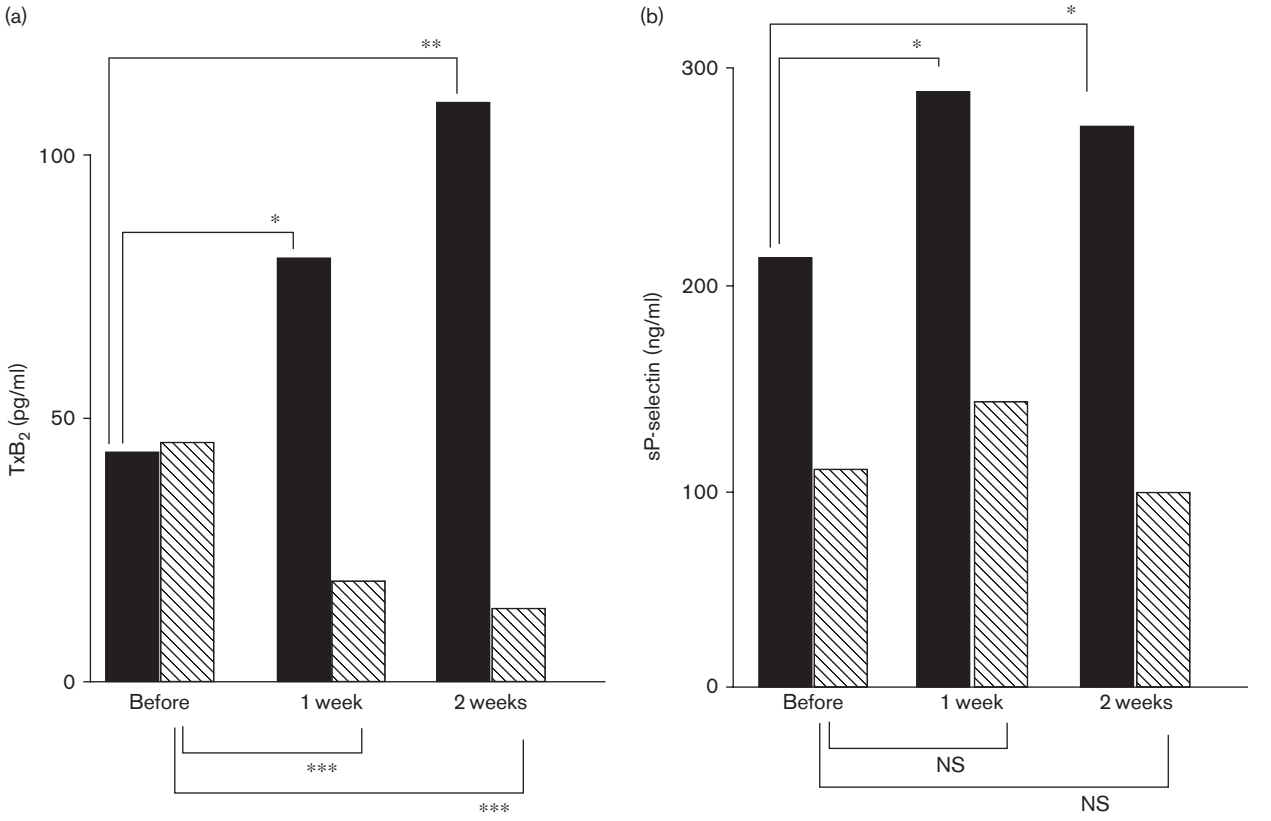
Fig. 2



Comparison of TxB_2 and sP-selectin before and after gefitinib treatment in patients with lung cancer. (a) TxB_2. (b) sP-selectin. Group 1 (solid bars) non-aspirin cases. Group 2 (shaded bars): aspirin cases. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: not significant.

(Fig. 2a) and that of sP-selectin exhibited the same results ($n = 27$, $P < 0.05$, $P < 0.05$, respectively) (Fig. 2b). In Group 2, however, the serum level of TxB_2 decreased significantly in all patients who received gefitinib with low-dose aspirin for 1 or 2 weeks ($n = 12$, $P < 0.001$, $P < 0.005$, respectively) (Fig. 2a), but that of sP-selectin exhibited no significant change (Fig. 2b).

The types and frequencies of adverse events experienced by patients are shown in Table 2. Compared with Group 2 (aspirin), the overall frequency of adverse events was found to be greater in Group 1 (non-aspirin) (77.8 versus 58.3%), and the frequency of rash and diarrhea was also remarkably higher in Group 1 (non-aspirin) (74.1 versus 33.3% for rash; 18.5 versus 0% for diarrhea). In addition, there was no remarkable change in gefitinib responsiveness (Group 1: 37%, Group 2: 33%). In one of the 12 patients in Group 2, aspirin therapy was suspended due to the occurrence of nasal bleeding. Four days after treatment suspension, she developed a skin rash in her finger (Fig. 3a). However, the skin rash improved after re-administration of aspirin without any other medications (Fig. 3b).

Table 2 Adverse events [*n* (%)] occurring after treatment

	Group 1 (non-aspirin) ($n=27$)	Group 2 (aspirin) ($n=12$)
Adverse effects	21 (78.8)	7 (58.3)
Rash	20 (74.1)	4 (33.3)
Diarrhea	5 (18.5)	0 (0)

Discussion

EGFR signaling is not critical for cell proliferation alone, since several studies have demonstrated that EGFR-mediated signals also contribute to other processes that are crucial for cancer progression, including angiogenesis, metastatic spread and inhibition of apoptosis [9,10]. Gefitinib, which is a blocker of EGFR, causes a proliferative reduction of malignant cells. In the present study, serum levels of TxB_2 and sP-selectin were elevated in patients receiving gefitinib, and these results suggest that gefitinib activates platelets. Recently, we reported that accelerated platelet aggregation was observed in patients receiving gefitinib and EGF inhibited secondary platelet aggregation induced by ADP [11]. As EGFR was not detected in platelets previously, EGF could effect

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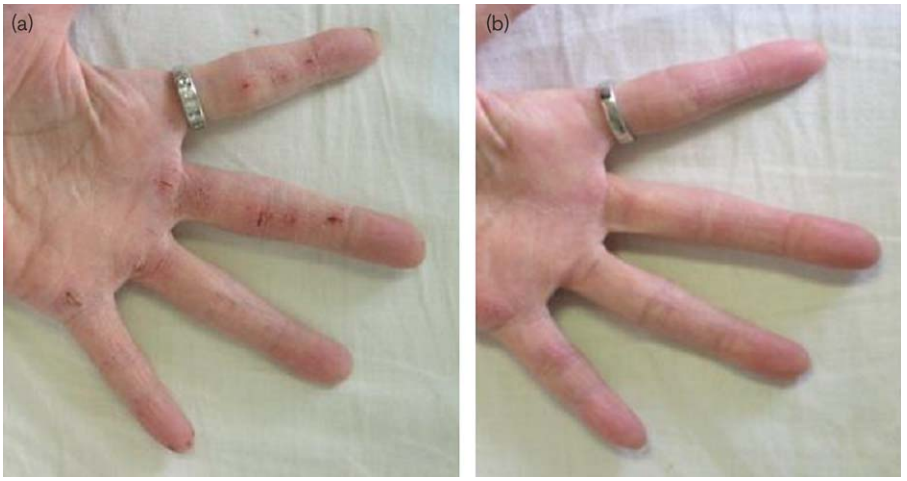
Fig. 3



Change of skin lesion before and after aspirin therapy (a) Treatment with gefitinib 4 days after suspension of aspirin therapy. (b) Treatment with gefitinib 2 weeks after re-administration of aspirin.

ADP-induced secondary platelet aggregation by an unknown mechanism(s).

We also reported that serum levels of RANTES were elevated in patients with NSCLC who received gefitinib [8]. RANTES is a chemokine produced by activated platelets, and causes chemotaxis of T lymphocytes and eosinophils [12]. We postulated that platelet activation by gefitinib would play an important role in both the anti-tumor effect of T lymphocytes and the occurrence of some gefitinib-related complications.

From the above background, we investigated the effect of low-dose aspirin on the Group 2 patients. In this group, serum levels of TxB_2 significantly decreased, but sP-selectin exhibited no significant change. These findings suggested that platelets were activated by gefitinib and the activated platelets resulted in an elevation of TxB_2. Furthermore, gefitinib appeared to activate platelets selectively or insufficiently, since sP-selectin exhibited no significant difference. It is common knowledge that low-dose aspirin inhibits platelet aggregation mainly by inhibiting cyclooxygenase (COX)-1. Thus, some sP-selectins in the gefitinib-treated patients may have been produced by COX-2 dependence. Skin rash and diarrhea are important common adverse effects of gefitinib [6]. In the present study, the frequency of adverse events, skin rash and diarrhea was evidently reduced by low-dose aspirin therapy, despite having shown no remarkable change in gefitinib responsiveness. In addition, one patient developed a skin rash after suspension of the aspirin therapy; the skin rash improved after re-administration of aspirin without any other medications. Thus, inhibition of COX-1 by low-dose aspirin is thought to be very important for the prevention of gefitinib-related complications.

We presume that dissociation tendencies of TxB_2 and sP-selectin may have a very important meaning because immunity induced by RANTES from platelets activated by gefitinib is needed for anti-tumor action in addition to the direct effect of EGFR blocking by gefitinib [8]. Therefore, the opportune platelet activation may be necessary in the region near the tumor cell. The present study suggested low-dose aspirin has the ability to reduce the platelet-dependent immuno-response and adverse effects of gefitinib through inhibiting platelet COX-1. Otherwise, we expect the platelet-dependent immuno-response for the tumor cell in the focal region to be included in lung cancer. In the focal region, ample platelets would be activated by the COX-2 dependence. In other words, in the focal region more immunity induced by RANTES from platelets is being activated. In this sense, low-dose aspirin is thought to be an ideal anti-platelet drug for the therapy of lung cancer.

We conclude that a new strategy, gefitinib plus low-dose aspirin, is reasonable for treatment and prevents adverse effects of gefitinib, since low-dose aspirin mainly inhibits COX-1. Further randomized studies will be needed to determine the usefulness of our new therapy.

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