Jpn. J. Cancer Res. **90**, 432–438, April 1999

p53 Status Predicts the Efficacy of Postoperative Oral Administration of Tegafur for Completely Resected Non-small Cell Lung Cancer†

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Although postoperative adjuvant therapy for non-small cell lung cancer (NSCLC) had not been reported to be effective, it has been reported recently that oral administration of tegafur (1-[2-tetrahydrofuryl]-5-fluorouracil, FT) may improve the postoperative prognosis. In the present paper, to examine whether p53 status affects the efficacy of FT as postoperative adjuvant chemotherapy for NSCLC, a total of 236 consecutive patients with completely resected pathologic stage I–IIIa NSCLC were retrospectively reviewed. p53 status was determined by immunohistochemical staining. For all patients, the 5-year survival rate of patients with FT administration (FT group) was 78.1%, being significantly higher than that (69.1%) of patients without FT administration (control group) (P=0.046). For patients without immunohistochemical evidence of p53 overexpression, the 5-year survival rate in the FT group was 87.1%, being significantly higher than that (74.0%) in the control group (P=0.036). This demonstrates an improvement of postoperative prognosis by FT administration. On the other hand, for patients with p53 overexpression, there was no significant difference in the postoperative prognosis between the FT group and the control group (5-year survival rate 63.2% and 60.1%, respectively; P=0.514), demonstrating that FT administration was not effective for these patients. In conclusion, p53 status may be useful for predicting the efficacy of postoperative adjuvant chemotherapy using FT. A prospective randomized study stratified by p53 status is needed to clarify the effect of postoperative FT administration.

Key words: p53 — Tegafur — Non-small cell lung cancer

Non-small cell lung cancer (NSCLC) is a common malignancy with a poor prognosis, and the 5-year survival rate of patients with NSCLC who have undergone surgery has remained at only 20–35%.[1)] Although adjuvant therapy has been introduced to improve the postoperative prognosis, it has been concluded that radiation therapy does not improve the prognosis of these patients, even if it may reduce the rate of local recurrence. Moreover, the efficacy of adjuvant chemotherapy has not been established, even following the introduction of a variety of novel chemotherapeutic agents with potent anti-tumor effects (3rd IASLC Workshop, Bruges, 1993).[2)]

It has recently been reported, however, in a retrospective study conducted by Kyoto University that oral administration of tegafur (1-[2-tetrahydrofuryl]-5-fluorouracil, FT; a prodrug that persistently releases 5-fluorouracil (5-FU)[3, 4)]) may be effective as postoperative adjuvant chemotherapy for NSCLC.[5)] Furthermore, the efficacy of postoperative administration of UFT[6–8)] (a mixture of FT and uracil (U), an inhibitor of 5-FU hydrolysis) for patients with completely resected NSCLC has been proven by a prospective randomized study by the West Japan Study Group for lung cancer surgery.[9)] The 5-year survival rate was 64.1% in a group postoperatively treated with UFT (400 mg/body/day for 1 year), 60.6% in a group postoperatively treated with UFT following combination chemotherapy using cisplatin (CDDP)+vindesine, and 49.0% in a surgery alone group, with a significant difference among the three groups as well as between the UFT group and the surgery alone group.[9)] Moreover, a prospective randomized study by The Study Group of Adjuvant Chemotherapy for Lung Cancer (Chubu, Japan) also demonstrated the efficacy of UFT as postoperative adjuvant chemotherapy.[10)]

5-FU, an anti-tumor agent efficacious against a variety of solid tumors, has not been reported to be effective against NSCLC. However, in all such previous studies, 5-FU was administered as an intravenous bolus injection. Experimental and clinical studies have revealed that potent anti-tumor effects of 5-FU are achieved at low doses if 5-FU is in contact with tumors for a long period of time, whereas the anti-tumor effects of 5-FU are extremely small even at high doses if 5-FU is in contact with tumors for only a short period of time.[11, 12)] Considering the time-dependent pharmacokinetics of 5-FU, oral administration, which can continuously maintain a required 5-FU concentration, may be more advantageous than intravenous bolus injection, which is in agreement

† Part of this article appeared in *Proc. ASCO*, **16**, 485a (1997).

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with clinical results demonstrating the efficacy of oral administration of FT or UFT for NSCLC.[5, 9, 10]

Although these findings suggest the efficacy of FT as postoperative adjuvant chemotherapy for completely resected NSCLC, in some cases, postoperative recurrence was observed even with postoperative administration of FT, and FT administration was ineffective. Therefore, if patients who respond well to FT can be selected, their postoperative prognosis can be improved with FT administration, and both medical and economic loss can be prevented since chemotherapeutic agents then need not be administered to patients who would not respond to them. Although some prognostic factors related to postoperative survival have been documented, no factor that can predict the efficacy of postoperative adjuvant chemotherapy for NSCLC has been reported.

The *p53* tumor suppressor gene protects the genome against DNA damage.[13] If mutated, it loses its normal functions and may allow malignant transformation.[14] p53 mutations have commonly been found in primary lung cancer as well as in many other malignant tumors.[13, 15] p53 mutations can be identified by immunohistochemical staining (IHS),[16–18] single-stranded conformation polymorphism (SSCP) analysis[19] and other techniques. p53 mutations have been reported to be an important factor in predicting a poor prognosis.[16, 17, 19] Moreover, it has recently been reported that p53 status may predict the efficacy of chemotherapy, and that it has potential to play a major role in decision-making concerning therapy.[20, 21] Bergh and coworkers reported that complete sequencing of the *p53* gene provided useful information concerning the efficacy of postoperative adjuvant therapy for breast cancer, and that adjuvant systemic therapy along with radiotherapy appeared to be of less value for tumors with p53 mutations than for those without p53 mutations.[20] Rusch and coworkers reported that aberrant p53 expression as determined by IHS predicted clinical resistance to CDDP-based chemotherapy for locally advanced NSCLC.[21] The purpose of the present study is to determine whether the efficacy of FT administration as an adjuvant therapy for completely resected NSCLC can be predicted by p53 status.

PATIENTS AND METHODS

A total of 237 consecutive patients with pathologic stage I–IIIa NSCLC who underwent complete tumor resection and mediastinal lymph node dissection without preoperative chemotherapy or radiation therapy at the Department of Thoracic Surgery, Kyoto University between January 1, 1985 and December 31, 1990, were reviewed. Complete tumor resection was considered to have been achieved when no microscopic cancer was identified in either the margin of resection of the tumor or the highest mediastinal lymph nodes.[22] Pathologic stage was determined by the TNM classification as revised in 1986,[23] and the pathologic stage of patients treated before 1986 was reevaluated using the same criteria. Histological type was determined using the classification by the World Health Organization.[24] One patient was excluded from the study due to operation-related death, and thus a final total of 236 patients (170 males and 66 females, mean age: 62.4 years) were evaluated. For all these patients, the inpatient medical records, chest X-ray films, whole-body CT films, bone and gallium scanning data, and records of surgery were reviewed. Follow-up of the postoperative clinical course was conducted by examination of outpatient medical records and by inquiries by telephone or letter, and the follow-up survey was successfully completed for 100% of patients for 5 years after surgery. The day of thoracotomy was considered the starting day for counting postoperative survival days.

Clinical characteristics of patients (Table I) Of the 236 patients, FT was administered to 58 patients (FT group), and not administered to 178 patients (control group). In the FT group, FT was administered as Futrafur (Taiho Pharmaceutical Co., Tokyo) to 19 patients, and as UFT (a mixture of FT and uracil, Taiho Pharmaceutical Co.) to 39 patients. Doses of Futrafur and UFT were 600–800 mg/day/body and 300–400 mg/day/body, respectively. Oral administration of FT was initiated within 1 month after surgery. FT was administered for at least 1 year if the patients remained alive; for patients who died, FT was administered until oral administration became impossible. The average period of FT administration was 16.2 months, ranging from 3 months to 6 years. There were no significant differences in clinical characteristics of patients between the FT group and the control group. CDDP-based adjuvant chemotherapy was performed for 55 patients (15 patients in the FT group and 40 in the control group). Postoperative radiation therapy was performed for 35 patients (8 patients in the FT group and 27 in the control group). There was no significant difference in the percentage of patients who underwent postoperative CDDP-based chemotherapy or radiation therapy between the two study groups.

IHS IHS against p53 was performed using the streptoavidin-biotinylated horseradish peroxidase complex method (LSAB kit; DAKO Japan, Kyoto), as described in the previous paper.[25] Briefly, deparaffinized 4 μm sections were heated in a microwave oven for 10 min, and incubated with mouse anti-human p53 monoclonal antibody (MoAb) DO-7 (mouse IgG2b, kappa, DAKO Japan) diluted at 1:50. After incubation with biotinylated sheep anti-mouse IgG antibody, the sections were incubated with horseradish peroxidase-labeled streptoavidin. As a chromogen, 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co., St. Louis, MO) was used. Stained sections

Table I. Characteristics of Patients (Comparison of Patients with and without Postoperative Administration of FT)

	All patients	Patients with FT administration	Patients without FT administration	*P* value
Gender (male/female)	170/66	37/21	133/45	0.107
Age (mean, years)	62.4	60.5	63.0	0.364
Performance status				
0	206	52	154	
1	28	6	22	0.654
2	2	0	2	
Histology				
Squamous cell	85	20	65	0.779
Adenocarcinoma	130	35	95	0.354
Large cell	13	1	12	0.118
Others	8	2	6	0.972
Pathologic stage				
I	138	40	98	
II	26	3	23	0.112
IIIa	72	15	57	
Postoperative chemotherapy (CDDP-based)				
(+)	55	15	40	0.596
(−)	181	43	138	
Postoperative radiation therapy				
(+)	35	8	27	0.798
(−)	201	50	151	
Percentage of p53-positive cells				
≤10%	143	36	107	0.791
>10%	93	22	71	

were evaluated by two of the authors [F.T. and Y.O.] independently without knowledge of clinical data. A total of 1,000 tumor cells were counted, and the percentages of positive cells were determined. When the percentage of the cells with nuclear positive staining exceeded 10%, the slide was judged to exhibit overexpression of p53 protein.[16)]

Statistical methods Counts were compared by using the χ^2 test, and trends in counts were analyzed by using the χ^2 test for trends. Continuous data were compared using Student's *t* test if the distribution of samples was normal, or using the Mann-Whitney *U* test if the sample distribution was asymmetrical. Postoperative survival rate was analyzed by the Kaplan-Meier method, while differences in survival rates were assessed by applying the log-rank test. Multivariate analysis of prognostic factors was performed using Cox's regression model. Differences were considered significant when the *P* value was less than 0.05. All statistical manipulations were performed using the SPSS for Windows software system (SPSS Inc., Chicago, IL).

RESULTS

IHS Overexpression of p53 was observed in 93 (39.4%) of the 236 patients. Overexpression of p53 was observed in 22 (37.9%) of the 58 patients in the FT group, and in 71 (39.9%) of the 178 patients in the control group. There was no significant difference in the percentage of patients exhibiting p53 overexpression between the two groups (Table I). The 5-year survival rate of patients who exhibited overexpression of p53 was 62.1%, being significantly lower than that (77.3%) of patients who did not exhibit p53 overexpression ($P=0.037$, Fig. 1).

Efficacy of postoperative FT administration The overall 5-year survival rate of the FT group was 78.1%, which was significantly higher than that (69.1%) of the control group ($P=0.047$, Fig 2). Next, patients were subdivided on the basis of p53 status as determined with IHC, and the efficacy of FT administration was analyzed. For patients who did not exhibit p53 overexpression, the 5-year survival rate of patients who received FT administration was 87.1%, while that of patients who did not receive FT administration was only 74.0%; this difference was statistically significant ($P=0.036$, Fig. 3), demonstrating that FT administration improved the postoperative prognosis of patients without p53 overexpression. On the other hand, for patients who did exhibit p53 overexpression, the 5-year survival rate of patients with FT administration was 63.2%, and was the same as that (60.1%) of patients without FT administration ($P=0.514$, Fig. 4), demonstrating that FT administration did not improve the postoperative prognosis.

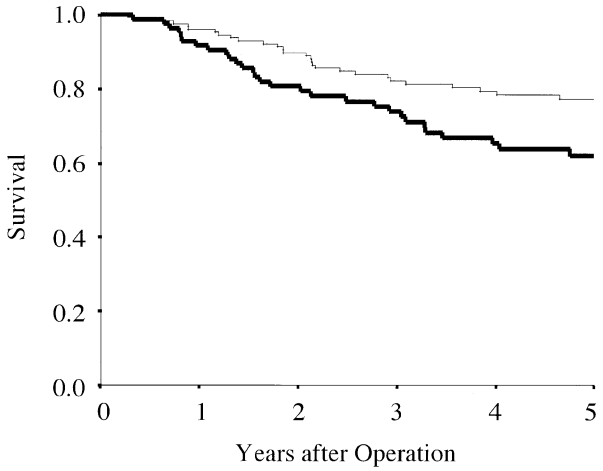

Fig. 1. Survival after complete tumor resection with lymph node dissection for non-small cell lung cancer (NSCLC) during 1985–1990 at Kyoto University. Comparison of patients with and without p53 overexpression. ── p53-positive cells≤10% (n=143), 5-year survival rate: 77.3%. ━━ p53-positive cells >10% (n=93), 5-year survival rate: 62.1%. Log-rank test: P=0.037.

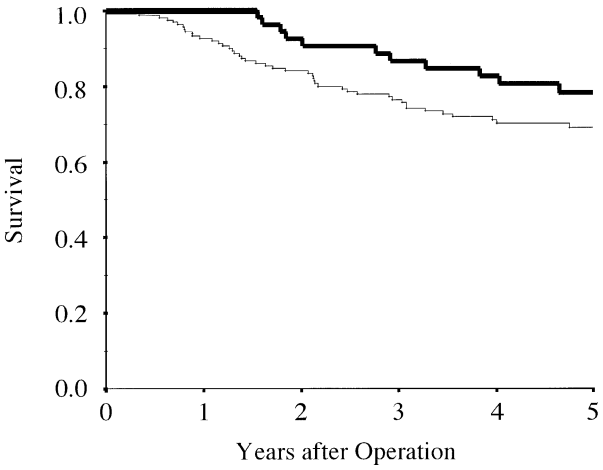

Fig. 2. Survival after complete tumor resection with lymph node dissection for non-small cell lung cancer (NSCLC) during 1985–1990 at Kyoto University. Comparison of patients with and without postoperative oral administration of tegafur (FT). ━━ FT administration (+) (n=58), 5-year survival rate: 78.1%. ── FT administration (–) (n=178), 5-year survival rate: 69.1%. Log-rank test: P=0.047.

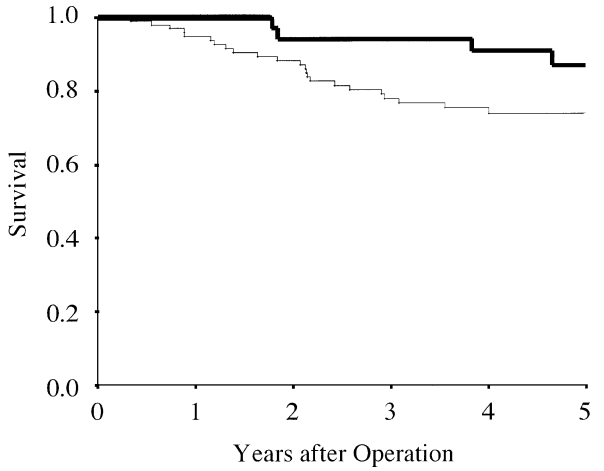

Fig. 3. Postoperative survival of patients without p53 overexpression. Comparison of patients with and without oral administration of tegafur (FT). ━━ FT administration (+) (n=36), 5-year survival rate: 87.1%. ── FT administration (–) (n=107), 5-year survival rate: 74.0%. Log-rank test: P=0.036.

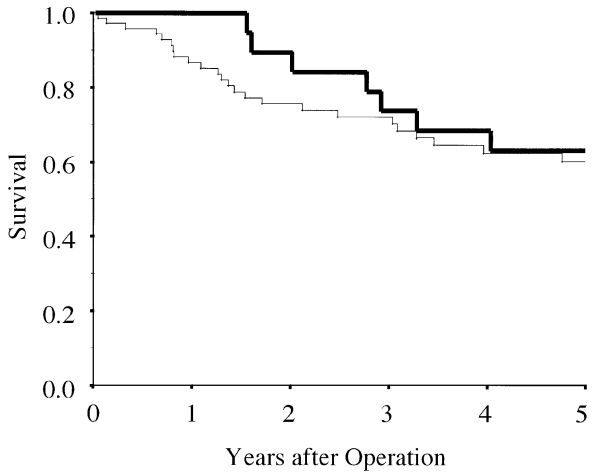

Fig. 4. Postoperative survival of patients with p53 overexpression. Comparison of patients with and without oral administration of tegafur (FT). ━━ FT administration (+) (n=22), 5-year survival rate: 63.2%. ── FT administration (–) (n=71), 5-year survival rate: 60.1%. Log-rank test: P=0.514.

Postoperative CDDP-based chemotherapy, and radiation therapy The 5-year survival rate of patients who underwent CDDP-based chemotherapy as postoperative adjuvant therapy was 77.0%, and that of patients who did not undergo CDDP-based chemotherapy was 69.3%, with no significant difference between these two groups (P=0.300). Postoperative prognosis was determined after subgrouping by p53 status. CDDP-based chemotherapy did not improve the postoperative prognosis of patients with p53 overexpression (P=0.397), nor did it improve

Table II. Multivariate Analysis of Prognostic Factors

Prognostic factors	Difference measured	β	*P* value	Relative hazard (95% confidence interval)
Gender (male, female)		−0.655	0.078	0.52 (0.251–1.077)
Age	10-years interval	0.402	0.014	1.495 (1.086–2.058)
Performance status	0, 1, 2	0.098	0.765	1.103 (0.580–2.095)
Histologic type (non-adenocarcinoma, adenocarcinoma)		0.424	0.167	1.528 (0.837–2.790)
Pathologic stage	I, II, IIIa	0.369	0.029	1.447 (1.038–2.016)
p53 overexpression (no, yes)		0.609	0.024	1.838 (1.086–3.113)
Postoperative adjuvant therapy				
Oral administration of FT (no, yes)		−1.212	0.036	0.298 (0.069–0.925)
CDDP-based chemotherapy (no, yes)		−0.177	0.379	0.838 (0.564–1.243)
Radiation therapy (no, yes)		0.001	0.272	1.008 (0.994–1.023)

the prognosis of those without p53 overexpression (P=0.744).

Our findings showed that patients who underwent postoperative radiation therapy had a significantly poorer prognosis than those who did not (P=0.004). However, with regard to patients' background factors, the percentage of patients with advanced disease in the radiation therapy group was significantly higher than that in the non-radiation therapy group (the percentages of patients with pathologic stage IIIa diseases were 74.3% (26 out of 35 patients) in the radiation group and 22.9% (46 out of 201 patients) in the non-radiation group, P<0.001).

Multivariate analysis of prognostic factors (Table II) Multivariate analysis of prognostic factors for all patients revealed that overexpression of p53 (relative hazard (RH): 1.838, 95% confidence interval (CI)=1.086–3.113) and postoperative administration of FT (RH: 0.298, 95%CI= 0.069–0.925), as well as age and pathologic stage, were significant prognostic factors. Sex, performance status, histological type, postoperative CDDP-based chemotherapy, and radiation therapy were not significant prognostic factors.

Next, to examine and confirm the efficacy of FT administration in the patient-group without p53 overexpression or in the patient-group with p53 overexpression, multivariate analysis of prognostic factors was performed in each patient-group. In patients without p53 overexpression, postoperative administration of FT (RH: 0.219, 95%CI=0.059–0.905), as well as pathologic stage, was a significant prognostic factor. In contrast, postoperative administration of FT (RH: 1.056, 95%CI=0.435–2.567) was not a significant prognostic factor in the patients with p53 overexpression. Postoperative CDDP-based chemotherapy or postoperative radiation therapy was not a significant prognostic factor in either patient-group.

DISCUSSION

In patients with completely resected NSCLC, the major factor associated with poor prognosis is distant metastasis, which frequently occurs following surgery. A recent study revealed that tumor growth in micrometastatic lesions was suppressed by apoptosis.[26] Since a variety of anticancer drugs, including 5-FU, can induce apoptosis of malignant cells even at concentrations insufficient to cause general metabolic dysfunction,[27] it can be speculated that oral administration of FT promotes apoptosis of tumor cells in micrometastatic lesions, thereby improving the postoperative survival of patients with NSCLC.

Wild-type p53 is a cell cycle checkpoint determinant, since it induces G1 cell cycle arrest in response to DNA damage.[28] Wild-type p53 also induces apoptosis of a variety of cell types.[29] Since apoptosis plays important roles in suppressing tumor growth,[26, 30] if the *p53* gene is mutated and its functions are impaired, cells will not undergo apoptosis in a normal fashion and tumor growth will not be suppressed.[29] Moreover, anti-tumor effects of chemotherapeutic agents, in particular, those of 5-FU and other drugs that inhibit proliferation of tumor cells by inducing apoptosis at relatively low concentrations, will be markedly reduced if the *p53* gene is mutated.[31] It has recently been reported that systemic adjuvant therapy after surgery for mammary cancer was effective for patients without p53 mutations, but not for those with p53 mutations.[20] In our present study as well, it was demonstrated that the efficacy of postoperative oral administration of FT may vary depending on the p53 status of tumor cells, and that FT administration is effective in patients without p53 overexpression, but not in patients with p53 overexpression. These results seem reasonable, when the function of p53 is taken into consideration. In patients having normal p53 function, apoptosis is easily induced by chemotherapeutic agents such as FT and postoperative adjuvant therapy is effective; in patients without normal p53 function, apoptosis is not easily induced and postoperative adjuvant therapy may not be effective.

In the present study, p53 status was determined with IHS. The presence of p53 mutations can be accurately detected only after complete sequencing of the *p53*

gene.[20] However, IHS,[16–18] and polymerase chain reaction (PCR)-SSCP[19] have been widely used as indirect methods for detection of mutations in the *p53* gene. Whereas both wild-type p53 protein and mutant-type p53 protein can be recognized by anti-p53 antibodies, only mutant-type p53 protein, which can accumulate to abnormally high levels because of a long half-life, can be detected with IHS.[32] Thus, aberrantly accumulated p53 protein can be detected as overexpression of p53.[33] Compared with PCR-SSCP, IHS has poor sensitivity. p53 mutations cover many types, such as missense, nonsense (truncations), splicing abnormalities, or deletions. Among these mutations, only missense mutants have been reported to be associated with p53 overexpression, which means that p53 mutation does not always cause p53 overexpression that can be detected with IHS. Moreover, false positives may occur with IHS if wild-type p53 accumulates excessively because of altered regulation of p53 expression or the presence of p53-interacting proteins.[34] In such cases, p53 overexpression does not imply p53 mutation. The accuracy of IHS to predict p53 mutation has been reported to be around 70%.[34, 35] Nevertheless, IHS is clinically valuable, because it is extremely easy to perform and inexpensive, and because it is sufficiently reliable even when applied to paraffin-embedded sections.

Rusch and coworkers reported that aberrant p53 expression as determined by IHS was useful as a predictor of clinical resistance to CDDP-based chemotherapy of NSCLC.[21] We could not, however, demonstrate in the present study the value of p53 status for prediction of efficacy of CDDP-based postoperative adjuvant chemotherapy, which may suggest that the usefulness of intensive CDDP-based chemotherapeutic regimens for patients whose lesions have already been resected completely during surgery is different from that for patients having tumor lesions. This may present a problem in applying intensive chemotherapeutic regimens, which have been reported to be effective for non-resectable NSCLC from the viewpoint of response rate, to patients whose tumor has already been resected completely during surgery. Moreover, as patients' immunity might have been reduced by the operation, postoperative intensive chemotherapy may worsen the patients' natural resistance to tumor cells, and may even activate tumor cells. Furthermore, compliance with drug administration may become very poor due to excessive side effects. Therefore, the efficacy of therapy for advanced-stage NSCLC and that for completely resected NSCLC may be considerably different.

The present study is retrospective, and a prospective randomized study with stratification by p53 status should be conducted, in order to clarify the significance of p53 overexpression and the efficacy of postoperative oral administration of FT.

ACKNOWLEDGMENTS

We thank Miss Tomoko Yamada for the preparation of histological sections. We also thank Nobuyuki Hamajima M.D., M.P.H.(Division of Epidemiology, Aichi Cancer Research Institute, Aichi, Japan) for helpful comments and a critical reading of the statistical section of the manuscript.

(Received November 24, 1998/Revised February 2, 1999/ Accepted February 10, 1999)

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