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# P-glycoprotein as an Intermediate End Point of Drug Resistance to Neoadjuvant Chemotherapy in Locally Advanced Gastric Cancer

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*The expression of p-glycoprotein (p-gp) was evaluated in pre- and post-chemotherapy states after the administration of adriamycin-based chemotherapy in 24 gastric cancer patients. Among them, group A was composed of twelve patients who relapsed after surgery plus adjuvant chemotherapy and group B was composed of another twelve patients who received neoadjuvant chemotherapy plus surgery. Pre-chemotherapy p-gp was evaluated in 18 out of 24 patients (6 patients had no pre-chemotherapy paraffin blocks) and post-chemotherapy p-gp was evaluated from all 24 patients. Pre- and post-chemotherapy p-gp was expressed in 5 of 18 patients (27.8%), and 9 of 24 patients (37.5%), respectively, with immunohistochemical stain using monoclonal antibody JSB-1. No differences of disease-free survivals were observed in Group A based on post-chemotherapy p-gp expression from relapsed lesions. In Group B, there was a higher relapse rate (p=0.04) and a lower one-year disease-free survival rate (p=0.04) in post-chemotherapy p-gp positive patients when adjuvant treatment was done with the same regimen as neoadjuvant chemotherapy. In all patients studied, post-chemotherapy p-gp expression correlated with a higher systemic recurrence (p=0.04). These data suggest that p-gp can be induced by an adriamycin-based chemotherapy in gastric cancer. Thus, we suggest that the prognosis of gastric cancer may be poor if a multidrug resistance(MDR)-related regimen is used in the presence of p-gp after neoadjuvant chemotherapy with an adriamycin-based regimen, even if the initial response is good.*

**Key Words:** Gastric cancer, Neoadjuvant chemotherapy, p-glycoprotein

Gastric cancer is the most common malignant disease in Korea. However, the overall benefit from chemotherapy is still unsatisfactory. Treatment response in advanced cases is only 30~40% with a FAM (McDonald *et al.* 1980), FAMTX (Wils *et al.* 1991) and EAP regimen (Kelsen *et al.* 1990), even though some chemotherapeutic effects have been found in neoadjuvant chemotherapy (Wilke *et al.* 1989).

Resistance to anti-cancer drugs is one of the major factors accounting for the poor clinical response to chemotherapy. Multidrug resistance (MDR) mediated by p-glycoprotein (p-gp) is the most frequently described mechanism of chemo-resistance in human tumor cell lines (Gros *et al.* 1986). The MDR1 gene expression can be detected by measuring mRNA with cDNA probes or by detecting p-gp using mono-

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clonal antibodies from tissue samples. In untreated gastric cancer, expression of MDR1 mRNA was found in 46% (Mizoguchi *et al.* 1990) and p-gp was found in 22~67% (Sugimoto *et al.* 1989; Chung *et al.* 1991). As the immunohistochemical analysis using monoclonal antibodies described by Dalton *et al.* (1989) is a readily applicable method to clinical study, current efforts are directed to define the role of the MDR1 gene product, p-gp, in tumor tissues.

In this study, we hypothesized that the prognosis of gastric cancer may be poor if a MDR-related regimen was used when the p-gp is expressed after neoadjuvant chemotherapy, even if the initial response was good. Therefore, the study was designed to detect pre- and post-chemotherapy p-gp expression in patients with relapse after adjuvant chemotherapy (Group A) and in patients with neoadjuvant chemotherapy plus surgery (Group B). Patients in Group A and B received adriamycin-based regimens both in adjuvant and neoadjuvant treatment. Patients in Group B received the same regimen after surgery because the tumor response before surgery was good with an adriamycin-based regimen. Therefore, the purposes of this trial were to study the incidence of p-gp expression after chemotherapy in gastric cancer, and to define the rationale of chemotherapy with MDR-related drugs, especially in neoadjuvant trials.

## MATERIALS AND METHODS

### Subjects

Two groups of patients were included in this study. The first group of 12 patients had advanced resectable gastric cancer (Group A). Initially, these patients had received curative gastric resection ($D_2$ or $D_3$ resection) and adjuvant chemotherapy. Main gastric masses were examined for pre-chemotherapy p-gp expression in 10 patients from paraffin sections. In two patients, paraffin tissues were unavailable. In all 12 patients, recurrent lesions which developed during or after adjuvant chemotherapy were examined for p-gp expression from the paraffin sections. Eleven patients with pathological stage III received adjuvant chemotherapy with a FA regimen (5-fluorouracil 500 mg/m$^2$ weekly for 18 months, adriamycin 40 mg/m$^2$ every 3 weeks for 12 courses) and one patient with stage IV had received an infusional FAM regimen (5-fluorouracil 1,000 mg/m$^2$ 24 hour infusion day 1~3, adriamycin 40 mg/m$^2$ day 1, mitomycin 10 mg/m$^2$ day 1).

The second group of 12 patients had locally advanced, unresectable gastric cancer (Group B) as determined by UGI, ultrasonography and abdominal CT scanning. Patients in Group B received 3~6 courses of neoadjuvant chemotherapy followed by gastric resection because they showed good tumor response with chemotherapy. Paraffin sections from gastrofiberscopic biopsy specimens prior to chemotherapy from eight patients were used in evaluating pre-chemotherapy p-gp expression. Due to the lack of paraffin embedded tissues, we could not perform the pre-chemotherapy p-gp study in the remaining four patients. Paraffin sections from the main gastric masses after surgery from all 12 patients were studied in detecting post-chemotherapy p-gp expression. In 10 patients, the infusional FAM regimen was administered every three weeks. In 2 patients, FADE regimen (5-flurouracil 1,000 mg/m$^2$ 24 hour infusion day 1~3, adriamycin 30 mg/m$^2$ day 1 & 8, cisplatin 40 mg/m$^2$ day 1 & 8, etoposide 100 mg/m$^2$ day 1~3) was administered every three weeks. After surgery, the same regimen was administered as an adjuvant chemotherapy in each patient (Table 1)(Fig. 1).

### Immunohistochemical assay

Sections cut from paraffin-embedded tissues were studied. The sections were deparaffinized in 100% xylene and rehydrated through graded alcohols. The sections were pre-incubated with 30% hydrogen peroxide (Junsei Pure Chemical Co., Tokyo, Japan) for 10 minutes to reduce the endogenous peroxidase activity. After washing for 5 minutes in phosphate-buffered-saline (Gibco Laboratories, Grand Island, NY, USA), the sections were labeled by a triple-layer immunoperoxidase technique (Vectastain ABC kit, Burlingame, CA, USA) using mouse monoclonal antibodies to

**Table 1. Patient characteristics**

| | Group A (n=12) | Group B (n=12) |
|---|---|---|
| Male:Female | 6:6 | 5:7 |
| Age (year) | | |
| Median | 53 | 54 |
| Range | 33~67 | 30~64 |
| T-stage | | |
| T1 | 0 | 1 |
| T2 | 0 | 1 |
| T3 | 12 | 10 |
| N-stage | | |
| N0 | 2 | 3 |
| N1 | 3 | 4 |
| N2 | 6 | 3 |
| M1(N3) | 1 | 2 |
| Stage | | |
| III | 11 | 10 |
| IV | 1 | 2 |
| Differentiation | | |
| Well | 1 | 1 |
| Moderate | 1 | 4 |
| Poor | 6 | 4 |
| Signet ring type | 4 | 3 |

human p-gp (JSB-1, Sanbio, Am Uden, Holland).

**Controls:** Drug sensitive clone KB-3-1 cells and multidrug-resistant clone KB-8-5 cells were used as a negative and a positive control, respectively.

**Assessment of staining:** All tumor sections were examined by an experienced pathologist without prior clinical information. In each cancer cell, the p-gp positivity was defined when the staining intensity was the same or stronger than that of simultaneously stained KB-8-5 cells or normal mucosa. Because the immunostaining in tumors are seldom uniform (Fig. 2), the results were scored in a semi-quantitative way from grade I to grade IV as follows after counting the fraction of p-gp positive tumor cells (Robertson *et al.*, 1989). We did not attempt to grade staining intensity in each intracellular level.

Grade I: ≤ 25% positive
Grade II: 26~50% positive
Grade III: 51~75% positive
Grade IV: ≥ 76% positive

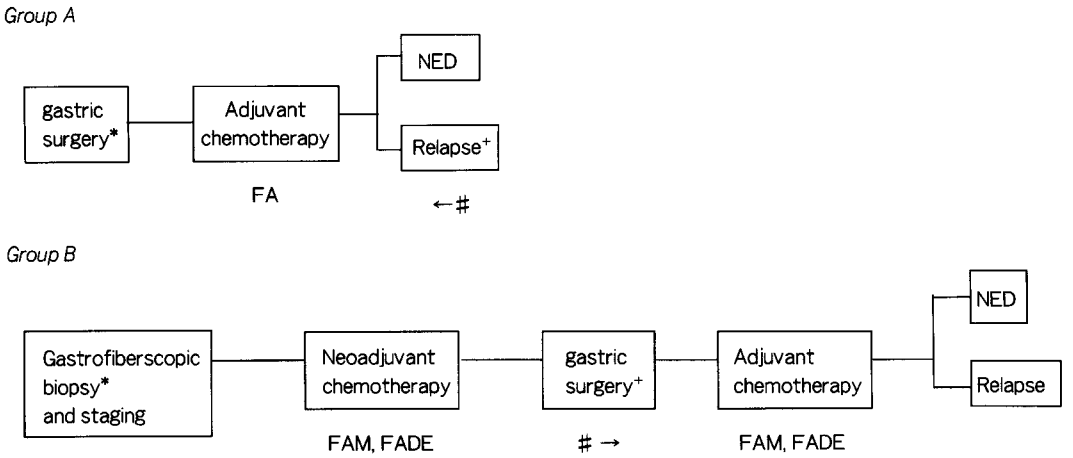


***Fig. 1.*** *Study scheme of the p-gp expression. *: study point of pre-chemotherapy p-gp expression, +: study point of post-chemotherapy p-gp expression, #: starting point of survival comparison according to post-chemotherapy p-gp expression (←: retrograde analysis, →: prospective analysis), NED: no evidence of disease, FAM: 5-fluorouracil+adriamycin+mitomycin, FADE: 5-fluorouracil+adriamycin+cisplatin+etoposide, FA: 5-fluorouracil+adriamycin.*

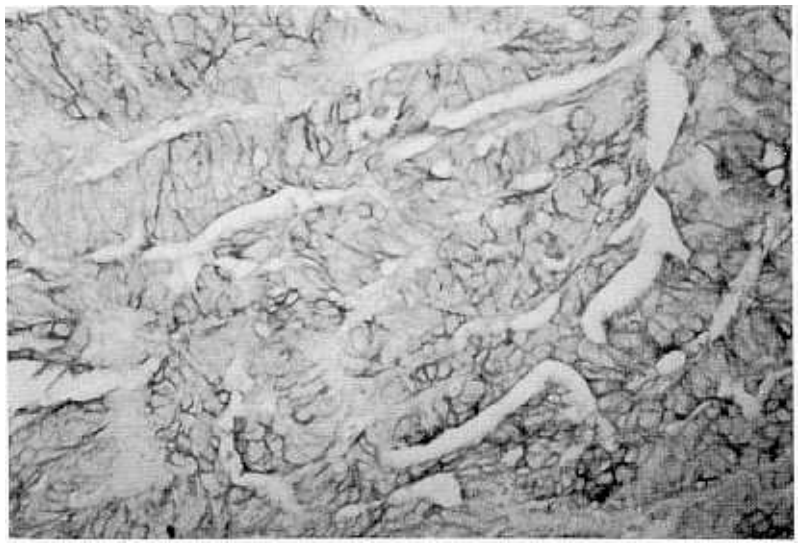

*Fig. 2. p-gp expression with JSB-1 monoclonal antibody (original magnification ×200).*

**Definition of p-gp types:** Pre-chemotherapy p-gp was defined positive when tissue samples expressed p-gp before chemotherapy; main stomach masses after gastrectomy in Group A and gastrofiberscopic biopsy specimens in Group B. Post-chemotherapy p-gp were defined positive when tissue samples (relapsed lesions in Group A and main stomach masses after gastric resection in Group B ) expressed p-gp after chemotherapy.

### Pathological evaluation

Pathological types and differentiation of the cancers were classified according to WHO criteria. Pathological staging was done using the TNM staging system (Beahrs and Myers, 1992).

### Statistical analysis

The data was analysed using the chi-square test. Disease-free survival was measured from the date of surgery to the endpoints defined as follows; last examination date in patients with no evidence of disease, or date of recurrence. Survival rate was calculated using the Kaplan-Meier method and compared by the Cox regression model.

**Table 2. Incidence of p-gp expression in gastric cancer**

| | pre-chemotherapy | post-chemotherapy |
|---|---|---|
| Group A | | |
| Group B | | |

## RESULTS

### Incidence of p-gp expression

The p-gp expression rate was 27.8% (5/18) in the pre-chemotherapy state and 37.5% (9/24) in the post-chemotherapy state (Table 2). In 18 patients for whom metachronous assay was possible, the p-gp expression rates in pre- and post-chemotherapy states were similar to those of the total patients (27.8% versus 33.3 %). In five pre-chemotherapy p-gp positive patients, one patient in group A expressed more p-gp after chemotherapy, whereas two patients in group A did not express p-gp from the relapsed lesions even after chemotherapy.

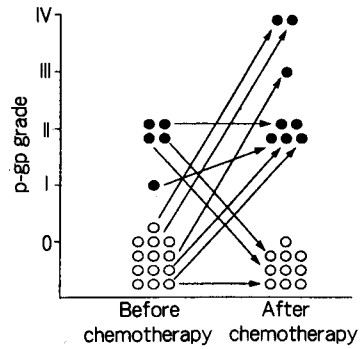


*Fig. 3. Metachronous changes of p-gp expression between pre- and post-chemotherapy states in 18 patients. ○: negative, ●: positive*

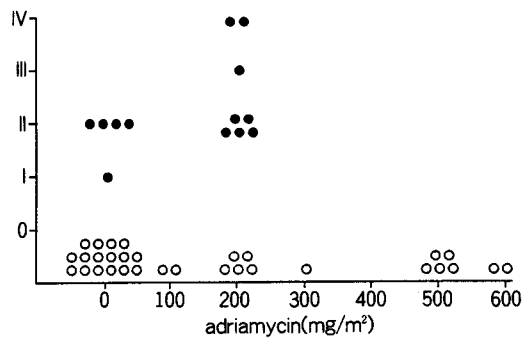


*Fig. 4. Comparison of incidences of pre- and post-chemotherapy p-gp expressions by cumulative adriamycin dosages. ○: negative, ●: positive*

And the two patients from Group B showed the same grades p-gp in pre- as well as post-chemotherapy states (Fig. 3).

**Expression of p-gp according to adriamycin dosage**

No differences in the expression rate or grades of p-gp, between before and after chemotherapy, were observed from zero to 600 mg/m² cumulative dosage of adriamycin (Fig. 4).

**Comparison of disease-free survival and recurrence rates in Group B according to p-gp expression after neoadjuvant chemotherapy**

Five of twelve patients (41.7%) expressed p-gp after chemotherapy. In these five p-gp positive patients after neoadjuvant chemotherapy, four relapsed, while only one patient from seven p-gp negative patients, relapsed during or after the adjuvant chemotherapy with the same regimen as neoadjuvant chemotherapy

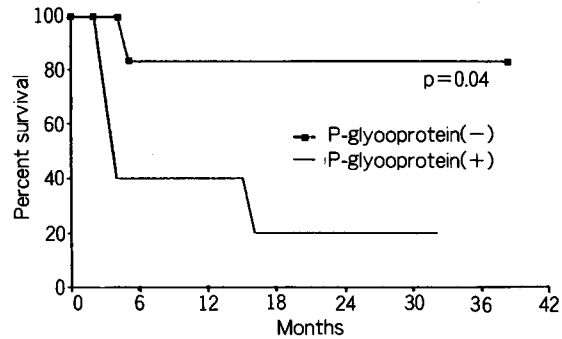


*Fig. 5. Disease-free survival curves in group B patients based on post-chemotherapy p-gp expression.*

**Table 3. Treatment outcome of the patients according to the post-chemotherapy p-gp expression in group B**

| | p-gp | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Recurrence | 4 | 1 | 5 |
| NED | 1 | 6 | 7 |

p=0.04
NED: no evidence of disease

**Table 4. Recurrence pattern according to post-chemotherapy p-gp expression**

| | p-gp | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Loco-regional recurrence | 2 | 7 | 9 |
| Systemic recurrence | 6 | 2 | 8 |
| Total | 8 | 9 | 17 |

p=0.04

(p=0.04)(Table 3). During the median follow-up duration of 12 months after surgery, one-year disease-free survival rate was 83.3% in p-gp negative patients and 40.0% in p-gp positive patients (p=0.04)(Fig. 5).

**Comparison of relapse pattern based on post-chemotherapy p-gp expression**

We compared the recurrence pattern based on p-gp expression after chemotherapy in 17 relapsed patients. Seventy-five percent (6/8) of the p-gp positive patients after chemotherapy showed distant recurrences, while 22.2% (2/9) of the p-gp negative patients showed systemic recurrence (p=0.04)(Table 4).

## DISCUSSION

The association of MDR1 gene expression in intrinsically resistant cancers to chemotherapy and the increased expression of MDR1 gene in tumors with acquired drug resistance indicate the contribution of the MDR1 gene to multidrug resistance in many human cancers (Fojo *et al.* 1987). The significance of p-gp as a prognostic factor or as a response predictor has been suggested mainly in hematologic malignancies such as lymphoma, multiple myeloma and acute leukemia (Dalton *et al.* 1989; Salmon *et al.* 1989; Pirker *et al.* 1991), whereas no association has been suggested in solid tumors such as breast cancer or sarcoma (Merkel *et al.* 1989; Tawa *et al.* 1990).

In gastric cancer, the p-gp expression rate has been reported sporadically (Sugimoto *et al.* 1989; Mizoguchi *et al.* 1990; Chung *et al.* 1991). Sugimoto *et al.* (1989) suggested that the intrinsic insensitivity of human gastric cancer to chemotherapy could be partly explained by p-gp expression. Considering the fact that p-gp is useful in predicting the sensitivity to adriamycin in gastric tumor cell lines (Yamauchi *et al.* 1989), we designed a study to evaluate p-gp expression after adriamycin-based chemotherapy and its clinical significance in gastric cancer treatment.

The expression rate of p-gp in the pre-chemotherapy state was 27.8% which was similar to the result we have reported previously (Chung *et al.* 1991). After chemotherapy, there was an increased tendency of p-gp expression (37.5%), which was similar to those of locally advanced breast cancer after chemotherapy (Koh *et al.* 1991). In contrast to cell line studies, staining heterogeneity was found in almost all specimens after chemotherapy suggesting the heterogenous response to chemotherapy in vivo. Among three pre-chemotherapy p-gp positive patients, two cases maintained the same grade of p-gp expression between pre- and post-chemotherapy, while one case increased the p-gp expression after chemotherapy in comparison to the level of pre-chemotherapy state. In contradistinction, two pre-chemotherapy p-gp positive patients did not express p-gp in relapsed lesions even after chemotherapy. This finding suggested that the relapsed cancer cells might originate from initially p-gp negative clones. Despite the small sample size, we suggest here that the p-gp can be induced or maintained with an adriamycin-based chemotherapeutic regimen in gastric cancer even though p-gp expression varies cell-to-cell.

The cumulative dosage and the exposure duration of adriamycin which induce p-gp are unknown. In vitro cell line studies, longterm exposure to low doses of adriamycin induced p-gp (Rogan *et al.* 1984), and the expressed p-gp amount correlated to adriamycin resistance in myeloma cells (Dalton *et al.* 1989). Ma *et al.* (1987) also suggested that 3~4 courses of chemotherapy is required to induce MDR1 in acute leukemia patients. In our data, there were similar incidences of pre- and post-chemotherapy p-gp expression in each cumulative dosage of adriamycin as we compared up to 600 $mg/m^2$ (Fig. 4). We have not yet been able to ascertain the cumulative dosage or exposure duration of adriamycin which is required for the p-gp induction in gastric cancer.

To investigate the significance of p-gp expression after chemotherapy from a clinical viewpoint, we evaluated the effect of p-gp on relapse in Group A and on MDR-related chemotherapy in Group B. In Group A, we were unable to study the effect of post-chemotherapy p-gp expression on further chemo-

therapy, because none of the patients received any further chemotherapy after relapse. Therefore, in Group A, we compared the survival rate retrospectively based on the post-chemotherapy p-gp expression in relapsed tissues. No survival difference was found (data not shown). This may be the result of combination chemotherapy with MDR-non-related drugs (5-fluorouracil in our trial), different tumor cell growth, or other drug resistance mechanisms rather than MDR in p-gp negative patients. However, as we hypothesized, there was a increased recurrence rate in post-chemotherapy p-gp positive patients (80.0%) than in negative patients (14.3%) ($p=0.04$), despite the small number of patients in group B. Also, there was a marginal survival benefit of one-year disease-free survival rate in p-gp negative patients (83.3%) than in that of p-gp positive patients (40.0%) ($p=0.04$) (Fig. 5). These results suggest that if the p-gp is expressed after chemotherapy, there might be an increased chance of relapse when further adjuvant treatment is continued further with MDR-related drugs. Similar findings have been reported by Chan *et al.* (1990) in sarcoma, and in breast cancer based on pre-chemotherapy p-gp expression (Verrelle *et al.* 1991).

Morimoto and Tanigawa (1991) have suggested that chemotherapy against metastasis is important to prevent recurrence even when the primary tumor and regional lymph nodes are resected. If the drug-resistant cells survive despite systemic chemotherapy, distant recurrences are more likely. To evaluate this issue, we compared the relapse pattern in 17 relapsed patients based on post-chemotherapy p-gp expression. Accordingly, there was an increased distant recurrence in patients with p-gp expression despite early adjuvant chemotherapy combined with MDR-non-related drugs (Table 4). These data support the fact that drug resistance in human gastric cancers to MDR-related chemotherapy may be partially explained by p-gp expression.

In conclusion, clinically significant levels of p-gp can be induced in gastric cancer after exposure to MDR-related drugs. Once p-gp is activated by adriamycin-based neoadjuvant chemotherapy, further chemotherapy with MDR-related drugs may not be effective even though some MDR-non-related drugs are combined simultaneously.

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