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Role of multidrug resistance protein 2 (MRP2) in chemoresistance and clinical outcome in oesophageal squamous cell carcinoma

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BACKGROUND: Although multidrug resistance protein 2 (MRP2) confers chemoresistance in some cancer types, its implication on oesophageal squamous cell carcinoma (ESCC) remains unclear.

METHODS: We evaluated MRP2 expression by immunohistochemistry and RT–PCR using 81 resected specimens from ESCC patients who did or did not receive neo-adjuvant chemotherapy (NACT), including 5-fluorouracil, doxorubicin, and cisplatin (CDDP). Correlation between MRP2 expression and response to chemotherapy was also examined in 42 pre-therapeutic biopsy samples and eight ESCC cell lines.

RESULTS: MRP2-positive immunostaining was more frequently observed in ESCCs with NACT than in those without NACT (27.3 *vs* 5.4%). The MRP2-positive patients showed poorer prognosis than MRP2-negative patients (5-year survival rate, 25.6 *vs* 55.7%). Concordantly, ESCC with NACT showed 2.1-fold higher mRNA expression of MRP2 than those without NACT ($P=0.0350$). In pre-therapeutic biopsy samples of patients with NACT, non-responders showed 2.9-fold higher mRNA expression of MRP2 than responders ($P=0.0035$). Among the panel of ESCC cell lines, TE14 showed the highest MRP2 mRNA expression along with the strongest resistance to CDDP. Inhibition of MRP2 expression by small-interfering RNA reduced chemoresistance to CDDP.

CONCLUSION: Our data suggested that MRP2 is one of molecules, which regulate the sensitivity to chemotherapy including CDDP in advanced ESCC patients.

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Oesophageal squamous cell carcinoma (ESCC) is the major histological form of oesophageal cancer in East Asian countries. It is one of the most lethal malignancies of the digestive tract and in most cases the initial diagnosis is established only once the malignancy is in the advanced stage (Shimada *et al*, 2003). Multimodal therapies are therefore necessary to prolong the survival of ESCC patients. Chemotherapy has become the standard first-line therapy for advanced ESCC patients, especially neo-adjuvant chemotherapy (NACT) (Tamoto *et al*, 2004). However, the initial response rate for NACT remains at 35–66% (Ajani *et al*, 1992; Iizuka *et al*, 1992; Hilgenberg *et al*, 1988; Ilson *et al*, 1998, 1999; Millar *et al*, 2005) and non-responders risk serious adverse effects without achieving any survival benefit.

The effectiveness of chemotherapy is often limited by drug-resistance factors in the tumours themselves. In fact, some tumours are intrinsically resistant to many kinds of chemotherapeutic agents, whereas other tumours, initially sensitive, often recur or become resistant not only to the initial agents used but also to those used subsequently. These two types of chemoresistance, intrinsic and acquired, are clinically serious problems in many types of cancer including ESCC; however, the molecular mechanisms underlying this resistance are not fully understood. More investigation into the mechanisms of chemoresistance in ESCC is needed with the goal of identifying novel predictive markers that can accurately identify non-responders before the administration of chemotherapy, thus enabling personalised therapies in ESCC patients.

Several members of the ATP-binding cassette (ABC) transporter superfamily have an important role in drug resistance in tumour cell models as well as in the clinic (Lage, 2003). These transporters mediate the ATP-dependent cellular efflux of chemotherapeutic drugs. Of the 48 human ABC transporters, multidrug resistance protein 2 (MRP2; also designated as ABCC2 or cMOAT) is expressed in the hepatocyte canalicular membrane (Kool *et al*, 1997), in which it functions as the major exporter of organic anions from the liver into the bile (Wada *et al*, 1998). Multidrug resistance protein 2 is also expressed in the kidney, gall bladder, small intestine, colon, and lung (Surowiak *et al*, 2006). Interestingly, several cisplatin (CDDP)-resistant human cancer cell lines overexpress MRP2, including ovarian cancer, hepatocellular carcinoma, bladder cancer, and colon cancer (Taniguchi *et al*, 1996; Kool *et al*, 1997; Liedert *et al*, 2003; Materna *et al*, 2005). *In vitro* data also implicated MRP2 in multidrug resistance (MDR) mechanisms during chemotherapy in some cancer cell lines

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(Koike *et al*, 1997; Materna *et al*, 2006; Ma *et al*, 2009). However, few studies have investigated MRP2 expression in ESCC (Gan *et al*, 2010; Tanaka *et al*, 2010), and thus the relationship between MRP2 expression and chemoresistance in ESCC remains unclear. The present study examined the clinical significance of MRP2 expression and its role in intrinsic and acquired resistance to chemotherapy in ESCC patients.

PATIENTS AND METHODS

Patients and treatments

The present study examined samples from 81 patients with histopathologically confirmed primary thoracic oesophageal cancer who underwent surgical resection at our hospital from 1988 to 2007. Table 1 details the patient characteristics. The cohort comprised 9 female and 72 male patients, aged from 42 to 80 years (median 62 years). Sub-total oesophagectomy by right thoracotomy with two or three-field lymphadenectomy was performed in all patients. Curative resection (R0) was achieved in 75 patients (92.6%), whereas the remaining 6 (7.4%) patients underwent a non-curative resection (R1, 2). None of the patients died of post-operative complications. A total of 44 patients (54.3%) with lymph node metastasis at initial diagnosis received NACT comprising two courses of 5-fluorouracil (5-FU), CDDP, and doxorubicin (DXR) (Akita *et al*, 2006; Yano *et al*, 2006; Matsuyama *et al*, 2007; Makino *et al*, 2008, 2010). Only a few patients who showed multiple metastatic lymph nodes in the surgical specimen received a regimen of docetaxel or CDDP plus 5-FU after operation (Ando *et al*, 2003).

Table 1 Correlation between MRP2 expression by immunohistochemistry and various clinico-pathological parameters

	MRP2 expression			
Parameter	**Positive**	**Negative**	**Total**	***P*-value**
Age (years)				
<65	8 (16.3)	41 (83.7)	49	0.7731
⩾65	6 (18.8)	26 (81.2)	32	
Gender				
Male	10 (13.9)	62 (86.1)	72	0.0435
Female	4 (44.4)	5 (55.6)	9	
Histopathology				
Well-, moderately differentiated	10 (16.7)	50 (83.3)	60	0.7500
Poorly differentiated	4 (19.0)	17 (81.0)	21	
Location				
Upper, middle thoracic oesophagus	6 (11.5)	46 (88.5)	52	0.1227
Lower thoracic oesophagus	8 (27.6)	21 (72.4)	29	
Neo-adjuvant chemotherapy				
Yes	12 (27.3)	32 (72.7)	44	0.0161
No	2 (5.4)	35 (94.6)	37	
pT				
T0–2	4 (16.7)	20 (83.3)	24	>0.9999
T3–4	10 (17.5)	47 (82.5)	57	
Number of pN				
<4	6 (11.1)	48 (88.9)	54	0.0594
⩾4	8 (29.6)	19 (70.4)	27	
pStage				
Stages 0–2	4 (12.1)	29 (87.9)	33	0.3800
Stages 3–4	10 (20.8)	38 (79.2)	48	

pT, pN, pStage (pathological classification) according to TNM classification.

After surgery, the patients were surveyed every 3 months by physical examination and measurement of serum tumour markers, every 6 months by CT scan and abdominal ultrasonography, and every year by endoscopy until tumour recurrence was evident. Patients with tumour recurrence received chemotherapy or chemoradiotherapy as long as their systemic condition permitted. The mean overall survival (OS) was 31.6 months and mean disease-free survival was 28.3 months. The mean follow-up period after surgery was 42.9 months.

Immunohistochemical analysis

MRP2 protein accumulation was examined by immunohistochemical (IHC) staining of formalin-fixed and paraffin-embedded ESCC tissue sections (Makino *et al*, 2009). Briefly, after deparaffinization in xylenes and dehydration through graded ethanol solutions; endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 20 min. The tissue sections were then heated at 95°C for 40 min in citrate buffer (0.05 mol l^{-1}, pH 6.0) for antigen retrieval. The sections were then incubated with mouse monoclonal antibody to MRP2 (Clone: M_2III-6, ALEXIS Biochemicals, dilution 1 : 10) for 2 h at room temperature, and antibody binding was visualised using the labeled-streptavidin biotin method. Negative controls for the IHC included omission of the primary antibody. Normal human liver tissue was used as a positive control. MRP2 staining for each ESCC sample was judged 'positive' when more than 10% of the cancer cells in the section were immunoreactive to MRP2, and 'negative' when 10% or less of the cells were positive. All slides were assessed by two observers, independently and then in conference; both were blinded to the clinico-pathological parameters.

Quantitative RT–PCR analysis

Total RNA was extracted from fresh frozen resected tumours or endoscopic biopsy samples from ESCCs patients, and from cancer cell lines using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was generated from 1 μg RNA in a final volume of 20 μl containing oligo-(dT)-15 primer and avian myeloblastosis virus transcriptase, using the Reverse Transcription System (Promega, Madison, WI, USA). Analysis by PCR was performed using a LightCycler, real-time monitoring thermal cycler. Reaction mixture for PCR was prepared containing 2 μl of cDNA template, 3 mmol l^{-1} $MgCl_2$, and 250 nmol l^{-1} of primer pairs, using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany). The amount of each transcript was normalised against the expression of the housekeeping gene porphobilinogen deaminase (PBGD). Standard curves were constructed with 10-fold serial dilutions of cDNA obtained from non-cancerous oesophageal mucosal cell layers of tissue samples from 10 cases as a standard mixture. The sequences of PCR primers for PBGD, MRP2 were as follows: forward primer 5′-TGTCTGGTAACGGCAATGCGGCTGCAAC-3′, reverse primer 5′-TCAATGTTGCCACCACACTGTCCGTCT-3′ used for amplification of PBGD, forward primer 5′-TAATGGTCCTAGACAACGGG-3′, reverse primer 5′-GGGCCTTCTGCTAGAATTT-3′ for MRP2. The PCR cycling condition was set as follows: an initial denaturing step at 95°C for 10 min and 40 cycles at 95°C for 15 s, 58°C for 10 s, and 72°C for 25 s. The relative amount of cDNA in each sample was measured by interpolation on the standard curve, and then the relative ratio of MRP2/PBGD mRNA expression in log2 scale was calculated for each ESCC sample.

Knockdown analysis using MRP2-siRNAs

Two small-interfering RNA (siRNA-1, -2) of MRP2 (HSS102057, HSS174719) and negative control (NC) (Medium GC duplex of stealth RNAi NC duplexes) were purchased from Invitrogen.

Among the eight ESCC cell lines supplied by RIKEN cell bank (Tsukuba, Japan), TE14 cells showed the highest MRP2 mRNA expression and were subsequently transfected with 15 nmol l^{-1} siRNA using Lipofectamine RNAiMAX (Invitrogen) in Opti-MEM I Reduced Serum Medium (Invitrogen). After 24 h, the medium was replaced by standard medium, and then 96 h from the siRNA administration, cells were collected for the following growth inhibitory assay as described below.

Growth inhibitory assay

Cells (TE14, 1×10^4 cells per well) were added in triplicate to a 96-well microplate, and after overnight incubation, the medium was replaced with 100 μl of fresh medium containing various concentrations of DXR and CDDP, both of which chemoagents have been reported to be transported by MRP2 in some types of cell lines. The TE14 cells suspended in complete medium were used as a control for cell viability. After 4 h (DXR and CDDP) treatment, the cells were washed with fresh medium. The number of viable cells was assessed by the 3-(4-, 5-dimethylthiazol-2-yl)-2, 5-dyphenyl tetrazolium bromide (MTT) (Sigma, St Louis, MO, USA) assay. Briefly, 10 μl (50 μg) of MTT were added to each well after 48 h (DXR and CDDP) from the chemoadministration. The plate was incubated for 4 h at 37°C, followed by removal of medium and the addition of 100 μl of 2-propanol to each well to dissolve the resultant formazan crystals. Plate absorbance was measured in a microplate reader at a wavelength of 650 nm. After a pulsed exposure, the IC_{50} was calculated as percentage of control cultures that were not exposed to chemoagents using an interpolated logarithmic concentration curve. Results were derived from three independent sets of triplicate experiments.

Statistical analysis

Data are expressed as mean ± s.d. Correlations between MRP2 expression and various clinico-pathological parameters were each evaluated by the χ^2 test and Fisher's exact probability test. Differences in continuous parameters between two groups were evaluated by the Mann–Whitney's *U*-test. Prognostic variables were assessed by log-lank test, and OS was analysed by Kaplan–Meier method. These analyses were carried out using SPSS for Windows v10 (SPSS, Chicago, IL, USA). A *P*-value of less than 0.05 denoted the presence of statistical significance.

RESULTS

MRP2 protein expression by immunohistochemistry in ESCC and its correlation with clinico-pathological parameters

A total of 81 samples that contained both cancerous and non-cancerous lesions were evaluated for MRP2 protein expression by IHC analysis. As a positive control, liver tissue showed strong MRP2 immunostaining mainly in the hepatocyte plasma membrane (Figure 1A). No normal squamous epithelium showed significant levels of immunostaining (Figure 1B). Of all samples, 14 (17.3%) showed positive MRP2 expression, mainly in the cell membrane and cytoplasm of tumour cells (Figure 1C), whereas the remaining 67 (82.7%) were negative for MRP2 expression (Figure 1D). The positive staining was almost homogeneous in single-cancer nests and among different areas (surface, central, and deepest areas) of the cancer lesion.

Table 1 lists the correlations between MRP2 expression and various clinico-pathological parameters. Of note, MRP2 expression was exceptional in the ESCC patients without NACT (2 out of 37, 5.4%), but was significantly more frequent in patients after NACT (12 out of 44, 27.3%). Women tended to have a higher rate of MRP2 expression than men (44.4 *vs* 13.9%, respectively), although the difference was small. Other clinico-pathological parameters including age, histological type, tumour location, pT, pN, and pStage were not associated with MRP2 expression.

Disease recurrence after curative resection was diagnosed in 35 (46.7%) of 75 patients with curative resection (R0) and the mean

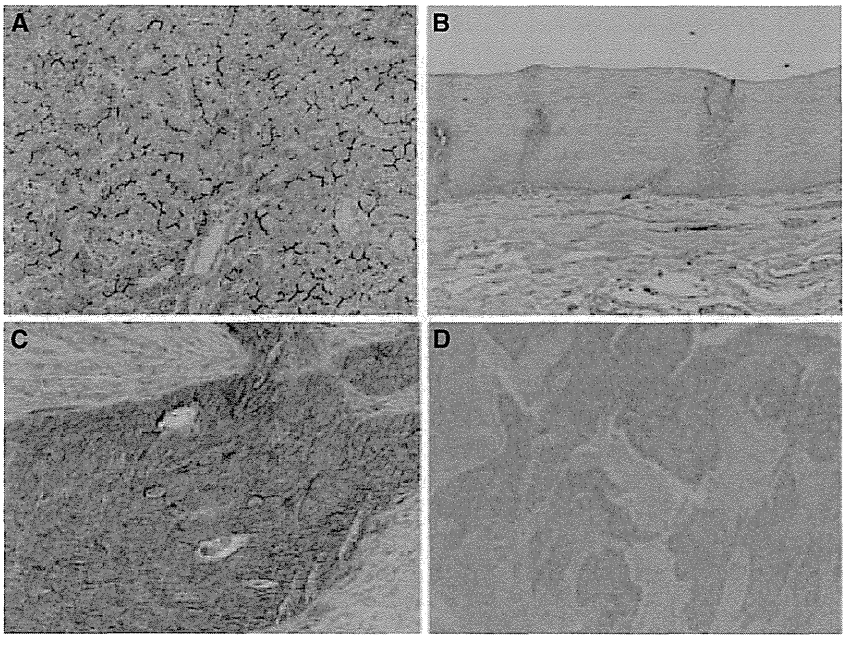

Figure 1 MRP2 expression by immunohistochemistry. (**A**) Strong MRP2 expression in liver tissue as a positive control (magnification, × 400). (**B**) Representative normal squamous epithelium negative for MRP2 expression (magnification, × 200). (**C**) Representative MRP2-positive ESCC showing staining mainly in the membrane and cytoplasm of tumour cells (magnification, × 200). (**D**) Representative MRP2-negative oesophageal squamous cell carcinoma with no appreciable staining of tumour cells (magnification, × 200).

time to recurrence was 10.5 months. A total of 35 (43.2%) patients died and their average survival time from diagnosis to death was 1.4 years (range 0.2–4.2 years). The total 5-year OS rate was 50.9% and MRP2-positive patients showed a significantly poorer prognosis than MRP2-negative patients (5-year OS 55.7 *vs* 25.6%) (Figure 2).

MRP2 mRNA expressions in resected specimens and endoscopy biopsy samples

RT–PCR analysis was performed to quantify the expression of MRP2 mRNA in surgically removed specimens from 26 representative cases, including 16 with NACT and 10 without NACT. MRP2 mRNA expression in tumours with NACT was 2.1-fold higher than in those without NACT, although there was no significant difference in TNM stage and other clinico-pathological parameters between the groups (data not shown) (Figure 3A).

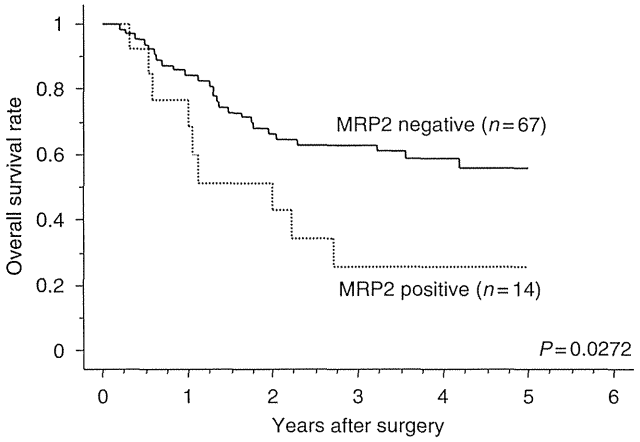

Figure 2 Survival curves according to MRP2 expression. Overall survival curve classified according to MRP2 expression for all patients were plotted by Kaplan–Meier method. Differences between two groups were evaluated by log–rank test. Ordinate: overall survival rate, abscissa: time after surgery (years).

The association between MRP2 mRNA expression and the effect of NACT was investigated in biopsy samples before NACT from 42 patients; the response of these patients to NACT was classified as non-responder in 22 and responder in 20. As shown in Figure 3B, MRP2 mRNA expression in non-responders was 2.9-fold higher than that in responders. Again, although these 42 samples were all advanced tumours with clinically positive lymph node metastases, there was no significant difference in clinical background parameters between the groups (data not shown).

Association between MRP2 mRNA expression and chemoresistance in ESCC cancer lines

To explore whether MRP2 expression functions specifically in chemoresistance to CDDP, we tested for a correlation between MRP2 mRNA expression and CDDP resistance (IC_{50}) in eight ESCC cell lines (Figure 4). Relatively high MRP2 expression was observed in TE14 and TE5 cell lines, both of which displayed strong resistance to CDDP. Regression analysis showed a significant correlation between MRP2 mRNA expression and IC_{50}

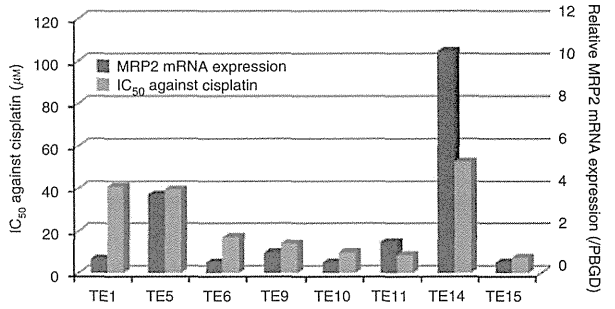

Figure 4 Correlation between MRP2 mRNA expression and CDDP-resistance (IC_{50}) in eight cell lines of ESCC. Relatively high MRP2 expression was observed in TE14 and TE5 cell lines, both of which displayed strong resistance to CDDP. Black bar: the relative ratio of MRP2 mRNA expression, grey bar: IC_{50} values against CDDP.

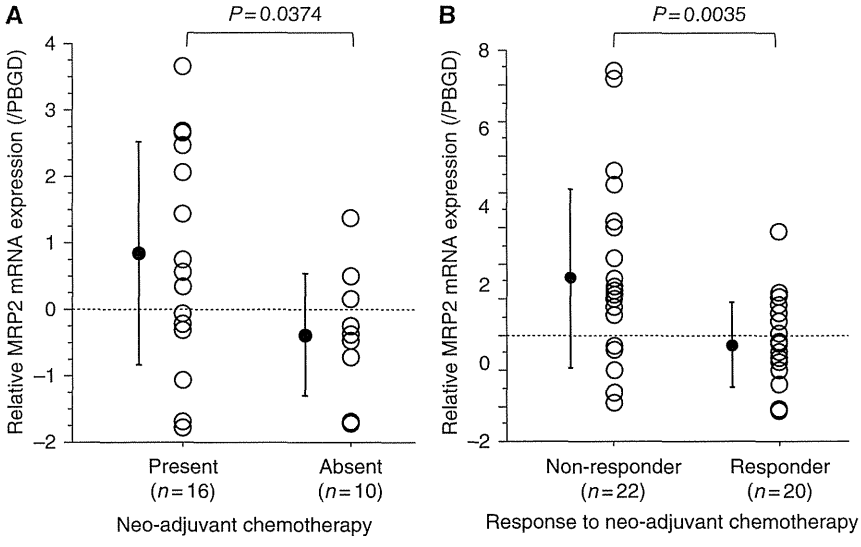

Figure 3 Differences in MRP2 mRNA expression between patients with and without neo-adjuvant chemotherapy in resected specimens (**A**), and between responders and non-responders at biopsy (**B**). (**A**) The relative ratio of MRP2 mRNA expression in resected tumours treated with neo-adjuvant chemotherapy ($n=16$) was significantly higher than in untreated cancers ($n=10$). (**B**) In endoscopy biopsy samples, the relative ratios of MRP2 mRNA expression in responders ($n=22$) were significantly higher than those in non-responders ($n=20$). Data are shown as mean ± s.d. (log2 values).

Table 2 Modulation of resistance against cisplatin and doxorubicin by MRP2 siRNA

	IC_{50}	
	Cisplatin (μM)	**Doxorubicin (μM)**
TE14 NC	32.4 (± 1.2)	6.2 (± 0.16)
TE14 siRNA-1	20.5 (± 1.4)[a]	5.8 (± 0.47)[b]
TE14 siRNA-2	17.8 (± 1.2)[c]	5.4 (± 0.54)[d]

Abbreviations: NC = negative control; IC_{50} = half maximal inhibitory concentration; siRNA = small-interfering RNA. [a]$P = 0.0003$, compared with NC. [b]$P = 0.2869$, compared with NC. [c]$P = 0.0005$, compared with NC. [d]$P = 0.2285$, compared with NC. Data are shown as mean ± s.d.

against CDDP ($R = 0.741$, $R2 = 0.549$), suggesting that ESCC cell lines with higher MRP2 mRNA expression were more resistant to CDDP compared with those showing lower MRP2 expression.

To confirm these findings by an alternative approach, we transfected MPR2 siRNAs into the TE14 line, which had the highest cellular MRP2 expression. The specific gene silencing started 48 h after the administration of siRNA (two siRNAs for MRP2 with different sequences were used: siRNA-1 and siRNA-2) and continued for 144 h, which was examined by quantitative PCR, resulting in 63.8% (siRNA-1) and 65.9% (siRNA-2) of peak MRP2 downregulation compared with NCs. The knockdown effect was stable during this period. As shown in Table 2, downregulation of MRP2 conferred increased sensitivity to CDDP, but not to DXR. IC_{50} values against CDDP were significantly lower in TE14 cell lines transfected with siRNA-1 and siRNA-2 compared with those transfected with NC (20.5 ± 1.4, 17.8 ± 1.2 *vs* 32.4 ± 1.2 μM, (siRNA-1 *vs* NC); $P = 0.0003$, (siRNA-2 *vs* NC); $P = 0.0005$). On the other hand, IC_{50} values of DXR were almost similar among TE14 cells transfected with siRNA-1, siRNA-2, and NC (5.8 ± 0.47, 5.4 ± 0.54 *vs* 6.2 ± 0.16 μM, (siRNA-1 *vs* NC); $P = 0.2869$, (siRNA-2 *vs* NC); $P = 0.2285$).

DISCUSSION

To our knowledge, this study is the first to identify the clinical significance of MRP2 expression in chemoresistance in ESCC. Such a relationship was strongly suggested by the findings that (1) MRP2 expression in the clinical biopsy samples before NACT was significantly negatively correlated with the effect of NACT, and (2) in the cultured cell line, artificial MRP2 downregulation resulted in increased resistance to the chemotherapy. Furthermore, the clinical samples of patients treated with NACT showed significantly higher expression of MRP2 at both the protein and mRNA levels than those without NACT, and the increased MRP2 expression was associated with poor prognosis. Although complicated, these clinical observations implicated MRP2 in the acquired resistance to chemotherapy commonly encountered in ESCC patients.

Intrinsic or acquired drug resistance is a major factor limiting the effectiveness of chemotherapy in various cancers including ESCC. Drug resistance by tumours occurs not only to a single cytotoxic agent but also in the form of cross-resistance to many agents called MDR. One of the major mechanisms of MDR is an increased ability of tumour cells to actively efflux drugs, decreasing the intracellular drug accumulation. This mechanism is mediated by ATP-dependent drug efflux pumps known as ABC transporters (Leonard *et al*, 2003; Ozben, 2006). To date, at least 48 human ABC transporters have been identified, and they have been divided into seven sub-families, ABC-A through ABC-G. The first ABC transporter identified in this context was P-glycoprotein (PgP, MDR1, ABCB1) (Kartner *et al*, 1983), and in the absence of overexpressed MDR1, the protein MRP1, ABCC1 was discovered because of the MDR phenotype (Cole *et al*, 1992).

Cisplatin resistance is not a feature of MDR phenotypes conferred by either MDR1 or MRP1 (Borst *et al*, 2000). The finding that ABC transporter MRP2 could mediate active efflux of CDDP conjugated to glutathione (Taniguchi *et al*, 1996), supported by evidence that intracellular glutathione levels were related to CDDP toxicity (Ozols, 1985), suggested a possible role for active efflux as a resistance mechanism. In addition, human carcinoma cell line studies showed increased levels of MRP2 mRNA associated with relative CDDP resistance, decreased intracellular accumulation of CDDP, and decreased DNA adduct formation (Kool *et al*, 1997; Liedert *et al*, 2003). In ESCC cell lines (TE2, TE13) Tanaka *et al* (2010), in their analysis of the intracellular localisation of CDDP by using in-air micro-particle induced X-ray emission, recently reported that TE2 cells, which express lower MRP2 than TE13, had higher intracellular CDDP concentrations and sensitivity than TE13 cells. This is also in agreement with our present *in vitro* data regarding CDDP. In human tissue samples, accumulating evidence indicates that MRP2 expression is also associated with intrinsic CDDP resistance in the clinical setting, using tissues obtained from patients with colorectal cancer (Hinoshita *et al*, 2000), small-cell lung carcinoma (Ushijima *et al*, 2007), and ovarian cancer (Surowiak *et al*, 2006). These results are also consistent with our data from cancer tissue samples, although our *in vitro* data involving each single agent could not necessarily be translated directly to a clinical response to combination chemotherapy because of possible synergistic effects. However, in contrast with these data, other studies failed to show a significant association between MRP2 expression and chemosensitivity in patients with ovarian cancer (Arts *et al*, 1999; Materna *et al*, 2004) or lung cancer (Filipits *et al*, 2007; Kim *et al*, 2009). It therefore seems likely that multiple factors such as drug accumulation, DNA repair capacity, and apoptotic sensitivity contribute to clinical tumour chemosensitivity, and a mechanistic relationship could be difficult to detect amongst an unselected patient cohort in which a number of other factors also affect clinical outcome. As clinical significance of MRP2 other than chemosensitivity, Gan *et al* (2010) reported that MRP2 expression was significantly higher in poorly differentiated ESCC tumours compared with moderate or well differentiated ones, which was not observed in our study.

In terms of the contribution to acquired chemoresistance, the present IHC and qRT-PCR data showed higher MRP2 expression in resected tumours with NACT compared with those without NACT, implying residual tumours after NACT acquired the feature of chemoresistance. Unfortunately, we could not compare MRP2 expression levels in cancer tissues from the same patient before and after NACT because no samples were available. Nooter *et al* (1998) reported significantly higher MRP expression, although not specific MRP2 expression, in ESCC tumours from non-responders to CDDP-based chemotherapy when comparing MRP levels in paired tumour samples before and after chemotherapy, suggesting that chemotherapy was selected for drug-resistant cell clones. Furthermore, other *in vitro* analyses by Noma *et al* (2008) established two CDDP-resistant pancreatic cancer cell lines (SUIT-2-CD3 and SUIT-2-CD4) by continuously administering 10 nM CDDP for 3 and 4 months, respectively. Results of RT-PCR indicated that induction of MRP2 mRNA expression was significantly increased by 1.5- and 2.5-fold in SUIT-2-CD3 and SUIT-2-CD4 cells, respectively, compared with parent cells, whereas MRP1 and MRP3 expression remained unchanged, implying a contribution of MRP2 to acquired resistance for CDDP in pancreatic cancer.

An important observation regarding the functional significance of MRP2 expressed in tumour cells could be the sub-cellular localisation. In normal tissues, MRP2 is expressed in functionally

polarised cells in which it specifically localises to the apical membrane of these cells. Apical localisation has also been described in tumours arising from these sites, a feature attributed to a targeting signal in the C-terminus of the MRP2 molecule (Harris *et al*, 2001). Single-nucleotide polymorphisms in MRP2 have been described that result in cytoplasmic localisation of the protein and that may reduce *in vivo* function (Hirouchi *et al*, 2004). Reduced CDDP sensitivity has also been reported in polarised mammalian kidney cells transfected with appropriately localised MRP2 (Cui *et al*, 1999). Furthermore, data of Surowiak *et al* (2006) indicated that MRP2 could confer resistance to CDDP in ovarian carcinoma only when expressed at the nuclear membrane, and this was supported by *in vitro* data (Materna *et al*, 2006). Although our IHC results showed MRP2-positive staining of both cytoplasm and membrane in tumour cells, MRP2 protein located in the cell cytoplasm might not function as an efflux pump (Evers *et al*, 1998). Further analysis focusing on the sub-cellular localisation of MRP2, and on the functional and clinical significance of such cellular location, is needed to elucidate the specific mechanism of chemoresistance induced by MRP2 in ESCC.

In conclusion, MRP2 expression seems to be associated with intrinsic resistance to chemotherapy in patients with ESCC, and is likely to also have a role in acquired chemoresistance. Further studies with larger cohorts are warranted to verify these results prospectively. The findings of this study open the door for exploration of efficacious treatment strategies and development of new therapeutic approaches for ESCC.

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Gastric Cancer
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ORIGINAL ARTICLE

Long-term quality-of-life comparison of total gastrectomy and proximal gastrectomy by Postgastrectomy Syndrome Assessment Scale (PGSAS-45): a nationwide multi-institutional study

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Abstract
Background Although proximal gastrectomy (PG) is widely accepted as a function-preserving operation for early upper-third gastric cancer, postoperative disorders, such as reflux or gastric stasis, have often been pointed out. From the perspective of postoperative disorder, the choice of total gastrectomy (TG) or PG for such cancers is still controversial. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS)-45, the quality of life after TG and PG was compared.
Methods The PGSAS-45 consists of 45 items composed of the SF-8 and GSRS scales and 22 new items. The main outcomes are measured by seven subscales (SS) covering symptoms, physical and mental component summary (SF-8), meals (amount and quality), ability to work, dissatisfaction for daily life, and change in body weight. A total of 2,368 eligible questionnaires were acquired from 52 institutions. From these, 393 patients with TG and 193 patients with PG were selected and compared.
Results The PG was better than TG in terms of body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.003$), necessity for additional meals (2.4 vs. 2.0; $p < 0.001$), diarrhea SS (2.3 vs. 2.0; $p = 0.048$), and dumping SS (2.3 vs. 2.0; $p = 0.043$). There were no differences in the other main outcome measures.
Conclusions Proximal gastrectomy appears to be valuable as a function-preserving procedure for early upper-third gastric cancer.

Keywords Proximal gastrectomy · Total gastrectomy · Postgastrectomy syndrome · Quality of life · Stomach cancer

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Introduction

Gastric cancer remains the second leading cause of cancer death in the world and is the most frequent malignancy in Japan, South America, and Eastern Europe [1, 2]. Long-term survivors after radical gastrectomy have been increasing as the result of better early detection and improved surgical techniques [3–5]. The better surgical outcome has led to greater interest in the quality of life (QOL) of gastrectomized patients. For prevalence of postgastrectomy disorder, the procedures used in gastrectomy for early gastric cancer are designed as function-preserving operations or various reconstructions to restore postoperative QOL [6]. Although the postgastrectomy disorders greatly influence the living condition (QOL) of gastrectomized patients, there are limits to evaluation of outpatients because of the difficulty in measuring subjective and physical symptoms. In recent years, questionnaires have been developed to create objective rating systems for QOL [7–11]. The Japan Postgastrectomy Syndrome Working Party was founded in order to investigate symptoms and lifestyle changes among patients who have undergone gastrectomy. This Working Party collaboratively developed a questionnaire to evaluate the symptoms, i.e., living status and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed.

The frequency of cancers in the upper third of the stomach and gastroesophageal junction has been increasing in both Western and Asian countries [12–15]. Total gastrectomy (TG) and proximal gastrectomy (PG) are operative options for proximal gastric cancer. In PG, the gastric fundic gland region is kept, and gastric-acid secretion and Castle intrinsic factor are maintained, but patients often suffer from reflux or gastric stasis. The choice of TG or PG has been discussed from the viewpoint of postoperative disorders, especially reflux esophagitis and nutrition. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS-45), QOL after TG and PG for gastric cancer was compared.

Methods

Patients

Fifty-two institutions participated in this study. The PGSAS-45 questionnaire was distributed to 2,922 patients between July 2009 and December 2010. Of these forms, 2,520 (86.2 %) were retrieved, of which 152 were deemed ineligible because of patient age >75 years ($n = 90$), postoperative period <1 year ($n = 29$), co-resection of other organs ($n = 8$), and other factors ($n = 25$). As a result, 2,368 questionnaires (81 %) were decided as eligible for inclusion in various analyses related to the PGSAS-45. Of these, 393 patients who had undergone TG and 193 who had undergone PG were identified and retrieved for the current study (Fig. 1).

Patient eligibility criteria

Patient eligibility criteria were: (1) pathologically confirmed stage IA or IB gastric cancer; (2) first-time gastrectomy; (3) age ≥ 20 and ≤ 75 years; (4) no history of chemotherapy; (5) no known recurrence or distant metastasis; (6) gastrectomy conducted one or more years prior to the enrollment date; (7) performance status (PS) ≤ 1 on the Eastern Cooperative Oncology Group (ECOG) scale; (8) full capacity to understand and respond to the questionnaire; (9) no history of other diseases or operations that might influence the responses to the questionnaire; (10) no organ failure or mental illness; and (11) provision of written informed consent. Patients with dual malignancy or concomitant resection of other organs (with co-resection equivalent to cholecystectomy being the exception) were excluded.

QOL assessment

The PGSAS-45 is a newly developed, multidimensional QOL questionnaire (QLQ) based on the Short-Form Health Survey (SF-8) [16] and the Gastrointestinal Symptom Rating Scale (GSRS) [17–20]. The PGSAS-45 questionnaire consists of 45 questions, with eight items from the SF-8, 15 from the GSRS, and 22 clinically important items selected by the Japan Postgastrectomy Syndrome Working Party (Table 1). The PGSAS-45 questionnaire includes 23 items pertaining to postoperative symptoms (items 9–33), including 15 items from the GSRS and eight newly selected items. In addition, 12 questionnaire items pertaining to dietary intake, work, and level of satisfaction for daily life are included. Dietary intake items include five about the amount of food ingested (items 34–37 and 41) and three about the quality of ingestion (items 38–40). One questionnaire item pertains to work (item 42), while three address the level of satisfaction for daily life (items 42–45). For the 23 symptom items, a seven-grade (1–7) Likert scale is used. A five-grade (1–5) Likert scale is used for all other items except 1, 4, 29, 32, and 34–37. For items 1–8, 34, 35 and 38–40, higher scores indicate better conditions. For items 9–28, 30, 31, 33, and 41–45, higher scores indicate worse conditions. The main outcome measures were refined through consolidation and selection. Twenty-three symptom items were consolidated into seven symptom subscales by factor analysis, as listed in Tables 1 and 2. Assessment data include total symptom score, quality of ingestion subscale, level of satisfaction for daily life, physical component summary (PCS), and mental component

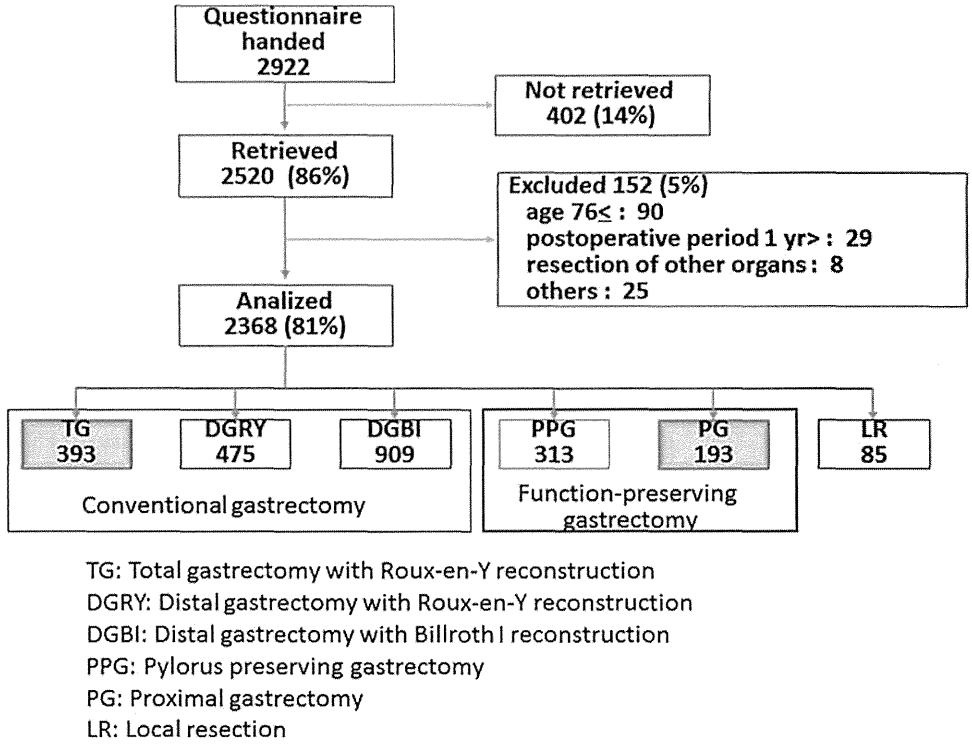

Fig. 1 Outline of the study

summary (MCS) of the SF-8 as main outcome measures. In addition, the following results were selected as main outcome measures: changes in body weight, amount of food ingested per meal, necessity for additional meals, ability to work, dissatisfaction with symptoms, dissatisfaction at the meal, and dissatisfaction at working. Each subscale score is calculated as the mean of composed items, and the total symptom score is calculated as the mean of seven symptom subscales (Table 2).

Study methods

This study utilized continuous sampling from a central registration system for participant enrollment. The questionnaire was distributed to all eligible patients as they presented to participating clinics. Patients were instructed to return completed forms to the data center. All QOL data from questionnaires were matched with individual patient data collected via case report forms.

This study was registered with the University Hospital Medical Information Network's Clinical Trials Registry (UMIN-CTR; registration number 000002116). It was approved by the ethics committees at all institutions. Written informed consent was obtained from all enrolled patients.

Statistics

In comparing patient QOLs after TG and PG, statistical methods included the t test and Chi square test. All outcome measures that exhibited significant difference in univariate analysis were further analyzed using multiple regression analysis. $p < 0.05$ was considered statistically significant. In the case of $p < 0.1$ by univariate analysis, Cohen's d was calculated. In the case of $p < 0.1$ in multiple regression analysis, standardization coefficient of regression (β), a decision coefficient (R^2), and the p value were calculated and shown in a table. Cohen's d, β, and R^2 measure effect sizes. Interpretation of effect sizes were 0.2 $\leq$ small, 0.5 $\leq$ medium, and 0.8 $\leq$ large in Cohen's d; 0.1 $\leq$ small, 0.3 $\leq$ medium, and 0.5 $\leq$ large in β; and 0.02 $\leq$ small, 0.13 $\leq$ medium, and 0.26 $\leq$ large in R^2.

StatView software for Windows Ver. 5.0 (SAS Institute Inc.) was used for all statistical analyses.

Results

Patient characteristics

Background data of both groups of patients are shown in Table 3. Reconstruction procedures were not regulated by the protocol, and depended on the principle of the institution or discretion of each surgeon. Consequently, whereas all patients treated by TG (393 patients) underwent Roux en Y reconstruction, the reconstruction after PG (193 patients) was varied and consisted of gastro-esophagostomy (115 patients), jejunal interposition (34 patients), and jejunal pouch interposition (44 patients).

Table 1 Structure of PGSAS-45

Domains	Subdomains	Items		Subscales
QOL	SF-8 (QOL)	1 Physical functioning*	Five-point or six-point Likert scale	Physical component summary*
		2 Role physical*		Mental component summary*
		3 Bodily pain*		
		4 General health*		
		5 Vitality*		
		6 Social functioning*		
		7 Role emotional*		
		8 Mental health*		
Symptoms	GSRS (symptoms)	9 Abdominal pains	Seven-point Likert scale except items 29 and 32	Esophageal reflux subscale (items 10, 11, 13, 24)
		10 Heartburn		Abdominal pain subscale (items 9, 12, 28)
		11 Acid regurgitation		Meal-related distress subscale (items 25–27)
		12 Sucking sensations in the epigastrium		Indigestion subscale (items 14–17)
		13 Nausea and vomiting		Diarrhea subscale (items 19, 20, 22)
		14 Borborygmus		Constipation subscale (items 18, 21, 23)
		15 Abdominal distension		Dumping subscale (items 30, 31, 33)
		16 Eructation		
		17 Increased flatus		Total symptom scale (above seven subscales)
		18 Decreased passage of stools		
		19 Increased passage of stools		
		20 Loose stools		
		21 Hard stools		
		22 Urgent need for defecation		
		23 Feeling of incomplete evacuation		
	Symptoms	24 Bile regurgitation		
		25 Sense of foods sticking		
		26 Postprandial fullness		
		27 Early satiation		
		28 Lower abdominal pains		
		29 Number and type of early dumping symptoms		
		30 Early dumping general symptoms		
		31 Early dumping abdominal symptoms		
		32 Number and type of late dumping symptoms		
		33 Late dumping symptoms		

Table 1 continued

Domains	Subdomains	Items	Subscales
Living status	Meals (amount) 1	34 Ingested amount of food per meal*	–
		35 Ingested amount of food per day*	
		36 Frequency of main meals	
		37 Frequency of additional meals	
	Meals (quality)	38 Appetite*	Quality of ingestion subscale* (items 38–40)
		39 Hunger feeling*	
		40 Satiety feeling*	
	Meals (amount) 2	41 Necessity for additional meals	–
	Social activity	42 Ability for working	–
QOL	Dissatisfaction (QOL)	43 Dissatisfaction with symptoms	Dissatisfaction for daily life subscale (items 43–45)
		44 Dissatisfaction at the meal	
		45 Dissatisfaction at working	

In items or subscales with * higher score indicates better condition. In items or subscales without * higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales (except PCS and MCS of SF-8). Items 29 and 32 do not have score. Therefore, they were analyzed separately

Table 2 Domains and main outcome measures

Domains/subdomains		Main outcome measures
Symptoms	Subscales	Seven symptom subscales
		Esophageal reflux (10, 11, 13, 24), *abdominal pain* (9, 12, 28), *meal-related distress* (25–27), *indigestion* (14–17), *diarrhea* (19, 20, 22), *constipation* (18, 21, 23), *dumping* (30, 31, 33)
	Total	*Total symptom score*
Living status	Body weight	Change in body weight (%)*
	Meals (amount)	Ingested amount of food per meal* (34)
		Necessity for additional meals (41)
	Meals (quality)	*Quality of ingestion subscale** (38–40)
	Work	Ability for working (42)
QOL	Dissatisfaction	Dissatisfaction with symptoms (43), at the meal (44), at working (45)
		Dissatisfaction for daily life subscale (43–45)
	SF-8	*Physical component summary** (1–5)
		*Mental component summary** (4–8)

Main outcome measures that are italicized are composed of more than two items. In items or subscales with *, higher score indicates better condition; in items or subscales without *, higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales

In the PG group, the mean postoperative period was significantly longer (TG 35.0 ± 24.6 months vs. PG 40.5 ± 28.1 months; $p = 0.0163$), and the rates of celiac and pyloric branch preservation were significantly higher, while the rates of laparoscopic approaches, D2 lymph node dissection, and combined resections were significantly lower than in the TG group.

QOL assessments

The results of the main outcome measures by univariate analysis are shown in Table 4. The body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.0001$; Cohen's $d = 0.35$), diarrhea subscale (TG 2.3 vs. PG 2.0; $p = 0.0016$; Cohen's $d = 0.29$), and dumping subscale (TG 2.3 vs. PG 2.0; $p = 0.0118$; Cohen's $d = 0.24$) in the PG group were significantly lower than those in the TG group.

The necessity for additional meals was significantly lower in the PG group than in the TG group (TG 2.4 vs. PG 2.0; $p < 0.001$; Cohen's $d = 0.40$), which indicates a better status in the PG group. However, the constipation subscale value of the PG group was significantly higher than that of the TG group (TG 2.1 vs. PG 2.3; $p = 0.0145$; Cohen's $d = 0.21$), and the quality of ingestion subscale value of the PG group was significantly lower than that of

Table 3 Patient background and operative features

Type of gastrectomy	TG Mean (SD)	PG Mean (SD)	*p* value
Number of patients	393	193	
Postoperative period (months)	35.0 (24.6)	40.5 (28.1)	0.0163
Age	63.4 (9.2)	63.7 (7.7)	>0.1
Sex (male/female)	276/113	139/53	>0.1
BMI (preoperative)	23.0 (3.3)	23.1 (3.0)	>0.1
Operation background			
Approach (laparoscopic/open)	97/293	33/159	0.0364
Celiac branch of vagus (preserved/divided)	12/371	83/105	<0.0001
Pyloric branch of vagus (preserved/divided)	4/379	120/62	<0.0001
Extent of lymph node dissection			<0.0001
D2	164	7	
D1b	192	93	
D1a	28	72	
D1	4	7	
D1>	0	6	
None	0	0	
Combined resection			<0.0001
Cholecystectomy	83	14	
Splenectomy	52	2	
Others	2	1	
None	246	162	

TG Roux en Y reconstruction (n = 393); *PG* Gastro-esophagostomy (n = 115), Jejunal interposition (n = 34), Jejunal pouch interposition (n = 44)

Table 4 Main outcome measures by univariate analysis

Measure	TG		PG		Cohen's *d*	*p* value
	Mean	SD	Mean	SD		
Change in body weight*	−13.80 %	7.90 %	−10.90 %	8.20 %	0.35	0.0001
Esophageal reflux subscale	2.0	1.0	2.0	1.0		>0.1
Abdominal pain subscale	1.8	0.8	1.7	0.7		>0.1
Meal-related distress subscale	2.6	1.1	2.6	1.1		>0.1
Indigestion subscale	2.3	0.9	2.2	0.8		>0.1
Diarrhea subscale	2.3	1.2	2.0	1.0	0.29	0.0016
Constipation subscale	2.1	0.9	2.3	1.1	0.21	0.0145
Dumping subscale	2.3	1.1	2.0	1.0	0.24	0.0118
Total symptom score	2.2	0.7	2.1	0.7		>0.1
Ingested amount of food per meal*	6.4	1.9	6.5	1.9		>0.1
Necessity for additional meals	2.4	0.8	2.0	0.8	0.40	<0.0001
*Quality of ingestion subscale**	3.8	0.9	3.6	1.0	0.20	0.0281
Ability for working	2.0	0.9	2.0	0.9		>0.1
Dissatisfaction with symptoms	2.1	1.0	2.0	0.9		>0.1
Dissatisfaction at the meal	2.8	1.1	2.7	1.1		>0.1
Dissatisfaction at working	2.1	1.1	2.0	1.1		>0.1
Dissatisfaction for daily life subscale	2.3	0.9	2.2	0.9		>0.1
*Physical component summary**	49.6	5.6	49.5	6.1		>0.1
*Mental component summary**	49.2	6.0	49.0	6.0		>0.1

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

the TG group (TG 3.8 vs. PG 3.6; $p = 0.0281$; Cohen's $d = 0.20$), both of which indicate worse status of the PG group.

The physical and mental component summaries were not different in the two groups.

To eliminate confounding factors, multiple regression analysis was performed by adding postoperative period, age, sex, surgical approach, and celiac branch of vagal nerve preservation as explanatory variables (Table 5). Although the effect size of the advantages in PG over TG is relatively small, comparing the type of gastrectomy, the PG group was better than the TG group in body weight loss ($\beta = 0.148$; $p = 0.003$), diarrhea ($\beta = 0.097$; $p = 0.048$), dumping ($\beta = 0.106$; $p = 0.043$), and necessity for additional meals ($\beta = 0.192$; $p < 0.001$). Constipation and quality of ingestion, which were worse in the PG group by univariate analysis, showed no difference by multivariate analysis.

Multiple regression analysis revealed that the postoperative period influenced the extent of body weight loss ($\beta = 0.097$; $p = 0.030$), diarrhea ($\beta = -0.076$; $p = 0.078$), and quality of ingestion ($\beta = 0.092$; $p = 0.0365$). This means that as the postoperative period lengthens, body weight loss and diarrhea improve.

The age influenced the constipation subscale ($\beta = 0.147$; $p = 0.001$), dumping ($\beta = -0.114$; $p = 0.010$), and the quality of ingestion ($\beta = -0.126$; $p = 0.034$). At older ages, although dumping decreased, constipation increased.

Diarrhea was often found in men ($\beta = 0.137$; $p = 0.001$), and surgical approach and celiac branch preservation had little influence on any of the main outcome measures by multiple regression analysis.

Discussion

Optimal evaluation methods for postgastrectomy disorders are important for selecting and improving the operative procedures and maintaining the high QOL for gastric cancer patients [21–23]. The Japan Postgastrectomy Syndrome Working Party developed a questionnaire to evaluate general features; i.e., symptoms, living status, and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed. This was the first nationwide survey of its type and involved 52 medical institutions throughout Japan. The necessary QOL data were collected from 2,520 patients, and the final sample size, following exclusion and participant selection, was sufficient for statistical validity of this type of study.

In recent years, a tendency to increasing numbers of proximal gastric cancers has been reported, and early detection and potentially curative operations by PG for upper-third gastric cancers have been increasing [24, 25].

Table 5 Main outcome measures by multivariate analysis

Measure	Type of gastrectomy (TG)		Postoperative period		Age		Gender (male)		Approach (laparoscopic)		Celiac branch of vagus (preserved)		R^2	p value
	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value		
Change in body weight	−0.148	0.003	0.097	0.030		>0.1		>0.1		>0.1		>0.1	0.037	0.0024
Diarrhea subscale	0.097	0.048	−0.076	0.078		>0.1	0.137	0.001		>0.1		>0.1	0.045	0.0002
Constipation subscale	−0.086	0.081		> 0.1	0.147	0.001		>0.1		>0.1		>0.1	0.030	0.0108
Dumping subscale	0.106	0.043		> 0.1	−0.114	0.010		>0.1		>0.1		>0.1	0.039	0.0027
Necessity for additional meals	0.192	0.0001		> 0.1	0.085	0.045		>0.1	0.083	0.058		>0.1	0.052	< 0.0001
*Quality of ingestion subscale**		>0.1	0.092	0.037	−0.126	0.003		>0.1		>0.1		>0.1	0.033	0.0056

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

If β is positive, the score of the outcome measure of the patients belonging to the category in brackets is higher in cases when the factor is a nominal scale, and the score of outcome measure of the patients with larger values is higher in cases when the factor is a numeral scale

In this study, the effect of tumor progression was removed by constraining patient selection to those with pathologic Stage IA/IB disease, and it is thought that accurate QOL comparison between operative procedures is possible under these circumstances. Although QOL scores usually depend on the time after surgery, Kobayashi et al. [11] reported that the QOL after gastrectomy was impaired during a few months after surgery, but more or less stabilized at around 6 months after surgery. This is the reason that, in this nationwide survey, we chose to evaluate patients who had lived for 12 months or more after surgery. In addition, we used multiple regression analysis with time relapse after surgery as one of variables so as to adjust this problem.

Whereas the reconstruction for TG was only by the Roux-en-Y method, the reconstructions of PG could be by esophagogastrostomy, jejunal interposition, and jejunal pouch interposition [6]. Because the best reconstruction for PG has not yet been established, various procedures are performed. However, as the gastric fundic gland region is preserved in PG, gastric-acid secretion and production of Castle intrinsic factor and ghrelin, a gut hormone known increase to appetite, are maintained [26, 27].

In the PG group, the rates of celiac and pyloric branch vagal nerve preservation were significantly higher, and the rates of laparoscopic approaches, D2 lymph node dissection, and combined resection were significantly lower than in the TG group. Standard TG is composed of more D1b dissection and sacrifice of the vagal nerve, often with combined resection, such as of the spleen and gallbladder [6, 28]. On the other hand, PG, which is a function-preserving operation, usually consists of less than D1b dissection and preservation of the vagal nerve [6]. The differences in the surgical background are caused by the procedure itself. Therefore, there seems to be no problem in comparing the QOL scores of these two groups.

From the results of the main outcome measures by univariate and multivariate analysis, body weight loss, diarrhea, dumping, and necessity for additional meals were significantly lower in the PG than in the TG group. Although esophageal reflux is common after PG [29, 30], various reconstruction methods have recently been described that reduce this problem [31, 32]. In this study, there was no difference in the esophageal reflux subscale values between the groups. This result suggests that PG is not necessarily disadvantageous with regard to reflux.

As three types of reconstruction with various modifications were performed with PG reconstruction, it is necessary to compare the three procedures in future studies. Dumping symptoms, such as early dumping with systemic symptoms, early dumping with abdominal symptoms, and late dumping, were examined in detail. Late dumping was significantly less common in the PG than in the TG group. Also, a tendency toward less early dumping with abdominal symptoms was seen in the PG group (data not shown). As a result, PG performed well on the dumping subscale. Although PG reflected the storage capacity and pylorus-preserving function, in TG, solid food is passed rapidly to the jejunum because of no storage ability [33].

Although the constipation subscale results and quality of ingestion subscale values were worse with PG than with TG by univariate analysis, multivariable regression analysis revealed that there were no statistical differences in these subscales as the result of the type of gastrectomy. Body weight loss and quality of ingestion subscale improved if the postoperative period was long. This means that gastrectomized patients adapt in some ways to the anatomic changes over time, even after more than 1 year following gastrectomy.

Multivariable regression analysis showed that dumping decreased and constipation increased with advancing age. This result may reflect the known intestinal peristaltic decrease in older patients [34–37].

By multivariable regression analysis, men were more likely to have diarrhea than women. This may be a consequence of the fact that the intestinal transit time is longer in women than in men at equivalent ages [37–39]. As for the effect of the surgical approaches and celiac branch preservation, no differences were found by multivariable regression analysis.

There were no statistical differences between the groups with regard to ability to work, dissatisfaction with symptoms, dissatisfaction at working, dissatisfaction for daily life subscale, PCS, or MCS. It is suggested that daily life is largely unchanged and that statistically different post-gastrectomy disorders do not have a major effect on adaptation.

In conclusion, although the effect size of the advantages of PG over TG is relatively small, our results indicate that PG is useful as a function-preserving procedure for early upper-gastric cancer. Although this study is limited in that it is retrospective and examines a single time point, it suggests the value of PG, use of which should be encouraged. To confirm this conclusion, a randomized study to determine the most desirable reconstruction for PG to achieve a good long-term QOL will have to be conducted using the PGSAS-45 questionnaire and successive endoscopic examinations.

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Conflict of interest The authors declare no conflicts of interest with regard to this manuscript.

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Effects of rikkunshito, a kampo medicine, on quality of life after proximal gastrectomy

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ABSTRACT

Background: The loss of the gastroesophageal junction after proximal gastrectomy (PG) induces various gastrointestinal symptoms, such as regurgitation, anorexia, and body weight loss, leading to impairment of the postoperative quality of life. In the present study, we investigated the long-term quality of life and the effects of rikkunshito, a traditional Japanese medicine (kampo), on the gastrointestinal symptoms and plasma ghrelin levels in patients with gastric cancer who had undergone PG.

Methods: Nineteen patients who had undergone PG> 6 mo before entry into the present study were enrolled. The plasma ghrelin levels, body weight, appetite, and Gastrointestinal Symptom Rating Scale (GSRS) scores were examined before and after the 4-wk administration of rikkunshito. A subgroup analysis was performed of patients showing a GSRS score of ≥ 2 before treatment, indicating the presence of gastrointestinal symptoms.

Results: The patients' body weight increased significantly after the administration of rikkunshito. Neither their appetite nor plasma acylated and deacylated ghrelin levels were significantly affected. In the subgroup analysis, the mean total GSRS score improved significantly from 2.6 ± 0.6 before the administration of rikkunshito to 1.9 ± 0.7 after administration because of the significant improvement in the subscale scores for abdominal pain, acid reflux, diarrhea, and constipation.

Conclusions: The long-term quality of life was well preserved in the patients who had undergone PG at our hospital. In the patients with a baseline GSRS score of ≥ 2, rikkunshito significantly improved the symptoms of postgastrectomy syndrome, and its effect was possibly independent of the plasma ghrelin levels.

1. Introduction

With the progress of endoscopic technology, a greater number of gastric cancer cases are being diagnosed at an early stage [1]. Total gastrectomy (TG) has been established as the standard surgery for advanced gastric cancer located in the cardia or the upper body of the stomach. However, proximal gastrectomy (PG) to maintain the reservoir function of the stomach is an optional surgical procedure for early stage gastric cancer or gastrointestinal stromal tumor in a proximal location, because metastasis in the distal perigastric lymph node is rare [2]. After PG, the loss of the lower esophageal

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sphincter and the acute angle of His will lead to acid reflux, regurgitation, and anorexia, inducing weight loss and impairing postoperative quality of life (QOL) [3]. At Kitano Hospital, when the remnant stomach is large enough, an esophagogastric anastomosis with cardioplasty has been the standard procedure for reconstruction after PG; it maintains cardia function by forming a pseudo-fornix and pseudo-angle of His and thus improving postoperative QOL (Fig. 1) [4].

Operations on the stomach affect the secretion of various digestive hormones such as gastrin, motilin, vasoactive intestinal polypeptide, leptin, somatostatin, and ghrelin, resulting in impaired digestive function [5,6]. Ghrelin acts on the pituitary gland to enhance secretion of growth hormone [7] and on the hypothalamus to increase appetite [8]. Ghrelin is secreted mainly from the gastric endocrine X/A-like cells and, in part, from the duodenum, jejunum, and lung [8,9]. Among the gastric endocrine cells, X/A-like cells represent the second largest cell population (the largest being histamine-secreting enterochromaffin-like cells) [8]. The plasma ghrelin concentration decreases immediately after gastrectomy and recovers gradually thereafter; however, recovery has been poor after both TG and PG [6], because ghrelin-secreting X/A-like cells are abundant in the gastric fundus [8].

Rikkunshito (Tsumura, Tokyo, Japan), a traditional Japanese medicine (kampo) and a granular preparation of an herbal extract, was reported to improve gastric emptying in patients with functional dyspepsia [10,11] and in patients who underwent pylorus-preserving gastrectomy [12]. Recently, rikkunshito was reported to increase food intake and body weight in a rat anorexia model induced by administration of cisplatin. One of rikkunshito's mechanisms of action is said to enhance the secretion of acyl-ghrelin (i.e., the activated form of ghrelin) [13]; rikkunshito was also reported to improve symptoms in patients with functional dyspepsia, and the plasma ghrelin levels increased in these patients [14].

Although a previous study showed that plasma ghrelin levels decreased immediately after PG [6], it remains to be clarified whether rikkunshito affects ghrelin levels in the long term in postgastrectomy patients, because they lack the gastric fundus containing most of the ghrelin-secreting X/A cells. In the present study, we investigated the postoperative QOL of patients during long-term follow-up after PG and the effects of rikkunshito on postgastrectomy symptoms and plasma ghrelin levels in these patients.

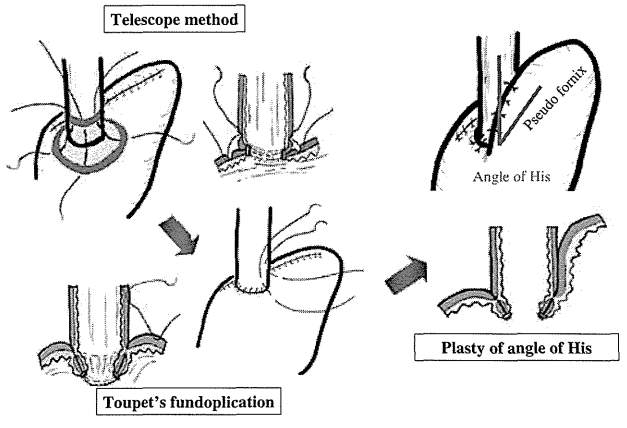

Fig. 1 – Illustration of cardioplasty after PG and esophagogastric anastomosis, using a telescope method combined with a Toupet fundoplication, forming a pseudo-fornix and pseudo-angle of His to maintain cardia function. (Color version of figure is available online.)

2. Methods

2.1. Patients

Of 90 patients with gastric cancer, including gastrointestinal stromal tumor, who had undergone PG at Tazuke Kofukai Medical Research Institute, Kitano Hospital from January 1992 to March 2009, 69 patients survived and could be followed up. Of these patients, 19, who met the following inclusion criteria, were enrolled in the present study: (1) age ≥20 but <80 y; (2) stage IA, IB, or II gastric cancer according to the International Union Against Cancer, 6th edition, TNM classification; (3) interval after surgery of ≥24 wk; and (4) grade 1 or greater anorexia according to the Common Terminology Criteria for Adverse Events, version 3.0. The exclusion criteria were (1) the presence of recurrent or metastatic cancer; (2) the presence of major organ damage, cardiac failure, or acute inflammatory disease; (3) difficulty in taking oral drugs; (4) female patients who were pregnant, desiring pregnancy, or breastfeeding; (5) a history of hypersensitivity to any kampo preparation; and (6) ineligibility judgment by the investigator. A QOL questionnaire survey on digestive symptoms, using the Gastrointestinal Symptom Rating Scale (GSRS), was administered to all the participants.

2.2. Study design

The present study was an open-label, prospective, single-arm study performed to investigate the long-term QOL of patients who underwent PG for early-stage gastric cancer >6 mo before the present study and to determine the pharmacologic effects, efficacy, and safety of rikkunshito in those patients.

Rikkunshito (Tsumura) is a granular preparation extracted with hot water of a mixture of eight crude drugs: *Atractylodis lanceae rhizoma, Ginseng radix, Pinelliae tuber, Hoelen, Zizyphi fructus, Aurantii nobilis pericarpium, Glycyrrhizae radix, and Zingiberis rhizoma* [13]. The patients were administered 2.5 g of rikkunshito 3 times daily before each meal for 4 wk. During treatment with rikkunshito, the patients were prohibited from using antiulcer agents such as proton pump inhibitors or gastrointestinal motility-enhancing agents such as metoclopramide or mosapride.

The primary endpoint was to evaluate whether rikkunshito improved the QOL and appetite of those patients; the method of evaluation has been described in the next section. The secondary endpoints were to evaluate the plasma concentration of both the active acylated and the inactive deacylated form of ghrelin, the ratio of acylated to deacylated ghrelin (A/D ratio), and the body weight. These parameters were measured before and after 4 wk of rikkunshito administration on the same day as the blood sampling.

The study was conducted according to the ethical guidelines for clinical studies in consideration of the patients' human rights and privacy. The institutional review board of