Table 1 Patient and tumor characteristics

Characteristic	Bursectomy ($n = 104$)	Nonbursectomy ($n = 106$)	p*
Age (years)			0.099
Median	65	63	
Range	31–79	34–78	
Sex			0.761
Male	73	77	
Female	31	29	
Body mass index			0.653
Median	22.3	22.5	
Range	15.7–28.9	15.6–29.4	
Tumor size (cm)			0.311
Median	4.3	4.5	
Range	0.9–11.0	1.5–12.0	
Histological type			0.784
Differentiated	47	50	
Undifferentiated[a]	57	56	
Clinical T stage[b]			0.572
cT2	61	67	
cT3	43	39	
Clinical N stage[b]			1.000
cN0	59	61	
cN1	45	45	
Pathologic T stage[b]			0.902
pT1	17	19	
pT2	62	64	
pT3-4	25	23	
Pathologic N stage[b]			0.119
pN0	49	60	
pN1	37	24	
pN2-3	18	22	
Residual tumor			1.000
R0	101	102	
R1	3	4	

* The p values were calculated by Fisher's exact test for sex, histological type, clinical T stage, clinical N stage, and residual tumor; by the chi-squared test for pathologic T stage and pathologic N stage; and by the Mann–Whitney U-test for age, body mass index, and tumor size

[a] Undifferentiated type included one endocrine cell carcinoma case in the nonbursectomy group

[b] T stage and N stage were according to the 13th edition of the Japanese Classification of Gastric Carcinoma

Table 2 Profile of surgical treatment

Treatment	Bursectomy ($n = 104$)	Nonbursectomy ($n = 106$)	p*
Gastrectomy			0.515
Total	22	27	
Distal subtotal	82	79	
Reconstruction method			0.705
Roux-en-Y	48	55	
Billroth I	54	49	
Other[a]	2	2	
Combined resection of other organs			0.055
Present	44	59	
Gallbladder	41	57	
Spleen	12	14	
Other[b]	1	2	
Absent	60	47	
Operating time (min)			0.368
Median	222	221	
Range	134–488	111–360	
Blood loss (ml)			0.047
Median	475	350	
Range	80–3970	55–2901	
No. of dissected lymph nodes			0.417
Median	38	37	
Range	11–98	7–97	

* p values were calculated by Fisher's exact test for gastrectomy and combined resection of other organs (present or absent); by chi-squared test for the reconstruction method; and by the Mann–Whitney U-test for operating time, blood loss, and the number of dissected lymph nodes

[a] Others included one Billroth II method and one intestinal interposition method in the bursectomy group and two Billroth II methods in the nonbursectomy group

[b] Others included one adrenal gland in the bursectomy group and one pancreas and one diaphragm in the nonbursectomy group

Table 3 Postoperative morbidity

Morbidity	Bursectomy ($n = 104$)	Nonbursectomy ($n = 106$)	p*
Any complication	15	15	1.000
Pancreatic fistula	3	7	0.332
Anastomotic leakage	4	3	0.720
Abdominal abscess	3	8	0.214
Bowel obstruction	2	1	0.620
Hemorrhage	1	0	0.495
Pneumonia	1	1	1.000

* The p values were calculated by Fisher's exact test

hemorrhage, and pneumonia, did not significantly differ between the two groups. Among the 10 patients with a pancreatic fistula, 6 underwent splenectomy, but no patients underwent pancreaticosplenectomy. Ten patients suffered from other complications, including two cases of chylous lymphorrhea, two of delayed gastric emptying without obstruction, and one case of afferent loop syndrome, acute cholecystitis, acute enteritis, arteriosclerosis obliterans of the leg, drug-induced hepatitis, and anastomotic stricture. The incidence of these miscellaneous complications tended

to be more frequent in the bursectomy group than in the nonbursectomy group (7.7 vs. 1.9%, $p = 0.057$). The median amylase levels in the drainage fluid on POD 1 were 282 IU/L in the bursectomy group and 314 IU/L in the nonbursectomy group ($p = 0.543$). Reoperation was required in four patients (1.9%): two for intestinal obstruction, one for afferent loop syndrome in the bursectomy group, and one for anastomotic leakage in the nonbursectomy group. The median hospital stay after surgery was 16 days in the bursectomy group and 15 days in the nonbursectomy group ($p = 0.744$).

There were two hospital deaths (0.95%). One patient in the bursectomy group and one patient in the nonbursectomy group died of sepsis after anastomotic leakage and pancreatic fistula formation, respectively. All other patients recovered from surgery and were discharged from the hospital.

Discussion

Two factors are necessary for bursectomy to be accepted as a standard treatment for advanced gastric cancers: safety and oncologic benefit. Only a randomized clinical trial can scientifically evaluate this proposition, and we are the first worldwide to conduct such a trial. This article is an early report of this trial with respect to operative safety. We found that overall morbidity and mortality were equivalent with and without bursectomy. Although the amount of surgical blood loss was significantly increased with bursectomy, overall we concluded that this procedure is safe and acceptable.

The safety of surgical treatments strongly depends on the surgeon's experience. Specific training is required to perform any surgical procedure, particularly when it is done for cancer treatment. There have been clinical trials studying the extent of lymph node dissection during gastric surgery. Two European randomized trials comparing D1 with D2 lymphadenectomy concluded that D2 was not acceptable as a standard treatment because D2 was associated with higher morbidity and mortality than D1 [8, 9]. On the other hand, two randomized trials comparing D1 with D2 and D2 with D3 lymphadenectomy in eastern Asia demonstrated that both D2 and D3 gastrectomy could be performed with low operative risk [13, 14]. This finding can be explained by the high volume of gastric cancer patients treated at that hospital and the high prevalence of gastric cancer in eastern Asia. In this study, all the patients were enrolled from an institution in which more than 50 gastrectomies were performed each year. In our trial the surgical procedures being performed by experienced surgeons accounted for the low mortality rates (0.95%) and low morbidity rates (14.3%).

Among various adverse events after surgery, we were concerned about the increased incidence of pancreatic fistulas after bursectomy because bursectomy requires resection of the capsule covering the pancreas [15]. However, we did not observe a significant increase in the incidence of pancreatic fistulas or inappropriate amylase levels in the postoperative drainage fluid, a surrogate marker of a pancreas fistula. This suggests that a pancreatic fistula is not caused by removal of a pancreatic capsule but may be caused by lymph node dissection adjacent to the pancreas parenchyma.

The next concern included the possibility of adhesion formation. Intestinal obstruction is the representative symptom of adhesion. In this study, two bursectomy patients and one nonbursectomy patient suffered from postoperative bowel obstruction, but there was no significant difference between the two groups. As 3 months' observation after surgery was not enough to evaluate the incidence of intestinal obstruction, a longer observation is necessary to draw a conclusion. Adhesion to the mesocolon and pancreas may cause specific local symptoms, such as delayed gastric emptying or afferent loop syndrome. It is of note that both delayed gastric emptying (two patients) and afferent loop syndrome (one patient) were observed only in the bursectomy group. Although this also did not reach statistical significance, careful observation is required in a larger cohort study.

In general, omentectomy and bursectomy are simultaneously performed for the same purpose, but their clinical pictures are somehow different. As the great omentum has numerous milky spots, which absorb ascites and actively incorporate cancer cells, peritoneal metastasis is frequently observed [16]. On the other hand, bursa omentalis, which is a semi-closed cavity, allows exfoliated cancer cells to remain. As for the surgical aspects, omentectomy is not difficult and does not increase the operating time or the blood loss. In contrast, the bursectomy technique is complicated and increases the operating time and bleeding. Considering the balance between the risk and benefit of each surgical procedure, we performed an omentectomy for all patients and randomly assigned each case to either with or without bursectomy. If we cannot find a benefit of bursectomy in this trial, we should elucidate the significance of omentectomy in the next step.

Conclusions

This study showed that experienced surgeons could safely perform a D2 gastrectomy with bursectomy. Although bursectomy resulted in more blood loss, the major operative complications and hospital deaths were not increased. Regarding the survival benefit of this procedure, we must

wait for the results of the final analysis when the data have matured sufficiently.

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Conflict of interest The authors declare no conflicts of interest.

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Effect of S-1 Adjuvant Chemotherapy on Survival following Recurrence and Efficacy of First-Line Treatment in Recurrent Gastric Cancer

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Key Words
Adjuvant chemotherapy · S-1 · Recurrent gastric cancer · Overall survival · Efficacy of chemotherapy

Abstract
Background: As S-1 monotherapy has recently become the standard adjuvant regimen for stage II-III gastric cancer patients after curative gastrectomy in Japan, the question whether adjuvant S-1 affects the subsequent clinical course of relapsed patients has attracted great concern. ***Patients and Methods:*** We retrospectively evaluated the effect of adjuvant S-1 on survival following recurrence and efficacy of first-line treatment in patients with recurrent gastric cancer after curative gastrectomy. A total of 89 patients were evaluated. Thirty patients received adjuvant S-1 (cohort A), 10 patients were given adjuvant chemotherapy with other oral 5-FU agents (cohort B) and 49 patients received no adjuvant chemotherapy (cohort C). ***Results:*** Median survival time following recurrence was 287 days in cohort A, 451 days in B and 547 days in C, with a significant difference between A and C ($p = 0.0034$). Response rates of the first-line chemotherapy after recurrence were 6.7, 30.0 and 42.9% in cohorts A, B and C, respectively, with a significant difference between A and C ($p = 0.0007$). On multivariate analysis, S-1 adjuvant chemotherapy was independently associated with poor prognosis after recurrence (hazard ratio 2.64). ***Conclusion:*** S-1 adjuvant chemotherapy significantly reduced survival and response to first-line chemotherapy following recurrence in patients with recurrent gastric cancer.

Introduction

Although several meta-analyses have suggested a survival benefit provided by adjuvant chemotherapy for gastric cancer [1–6], there have been only a few treatments with their efficacy established in large clinical trials. Postoperative radiotherapy with 5-FU plus leucovorin has become a standard adjuvant therapy in the US [7], while peri-operative triplet regimen with epirubicin, cisplatin and 5-FU is standard in the UK [8]. Recently in Japan, the ACTS-GC trial has verified the efficacy of S-1 adjuvant chemotherapy after curative gastrectomy for stage II-III disease [9]. However, around 30% of patients still develop recurrence afterwards despite adjuvant S-1. As the number of patients relapsing after S-1 adjuvant chemotherapy increases, it becomes of great concern whether adjuvant S-1 affects the subsequent clinical behavior of the recurrent disease.

This retrospective study was conducted to evaluate the effect of S-1 adjuvant chemotherapy, in comparison with other 5-FU agents or no adjuvant chemotherapy, on sur-

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vival following recurrence and efficacy of first-line chemotherapy given at the time of relapse in patients with recurrent gastric cancer after curative gastrectomy.

Patients and Methods

Patients

A total of 95 patients with recurrent gastric cancer after curative gastrectomy were found at our institution between April 1999 and October 2008. Among them, 89 patients enrolled in this retrospective study fulfilled the following criteria: (1) histologically proven recurrent gastric adenocarcinoma; (2) stage II, III or IV primary disease without any distant metastasis in accordance with the guidelines of the Japanese Gastric Cancer Association [10]; (3) either adjuvant chemotherapy with S-1 or other oral 5-FU agents (UFT or 5'-FUDR) lasting more than 4 weeks or no adjuvant treatment; (4) performance status of 2 or less on the Eastern Cooperative Oncology Group scale; (5) adequate bone marrow function (white blood cell count 4,000–12,000/mm^3, platelet count ≥100,000/mm^3 and hemoglobin ≥8.0 g/dl), hepatic function (total bilirubin ≤1.5 mg/dl, serum transaminases ≤100 μ/l) and renal function (serum creatinine ≤ the upper institutional limit); (6) no other severe medical conditions; (7) no other concurrent active malignancy.

Overall Survival, Efficacy of First-Line Chemotherapy and Statistics

Overall survival (OS) after recurrence was defined as the time from the date of recurrence to the date of death from any cause or the last follow-up. OS was calculated using the Kaplan-Meier method and compared with the log-rank test. Univariate and multivariate analyses were performed by the Cox proportional hazards model to identify variables independently associated with poor prognosis after recurrence.

During the first-line chemotherapy after recurrence, each patient with a measurable lesion was assessed for an objective response to treatment according to the Response Evaluation Criteria in Solid Tumors [11] with computed tomography scans performed every 2 to 3 months until disease progression. Disease control rate (DCR) represented the percentage of patients with complete response, partial response or stable disease (SD). Patients only with nonmeasurable lesions were evaluated as stable disease if neither complete disappearance (complete response) nor obvious progression of the recurrent disease were observed on computed tomography scans.

Differences in proportion were evaluated with the χ^2 test and the differential significance of age was estimated by the Kruskal-Wallis test. Statistical results were considered to be significant with a p value of less than 0.05.

Results

Patient Characteristics

Eighty-nine patients were categorized into the 3 cohorts shown in table 1. Thirty patients in cohort A, 18 males and 12 females with a median age of 62.5 years (range: 32–83), received S-1 adjuvant chemotherapy. S-1 was given orally using a standard dose and schedule (80 mg/m^2/day, for 28 consecutive days followed by a 14-day rest, repeated for 1 year) [9]. Nine patients completed the planned 1-year administration of adjuvant S-1, while 11 patients discontinued the treatment within the first 6 months and 10 patients in the second 6 months after the initiation of S-1 adjuvant chemotherapy. The reasons for treatment withdrawal were treatment toxicity in 1, and recurrent disease in 20 patients. The median duration of adjuvant S-1 administration was 211 days. In cohort B, 10 patients, 6 males and 4 females with a median age of 63.0 years (range: 35–78), were given adjuvant chemotherapy with oral 5-FU agents other than S-1. UFT (a combination of uracil and tegafur at a molar ratio of 4:1) was administered at a dose of 400 mg/body/day in 6 patients and 5'-DFUR (5'-deoxy-5-fluorouridine) at a dose of 800 mg/body/day in 4 patients. Two patients completed the planned 1-year administration of adjuvant UFT/5'-DFUR, while 3 patients discontinued the treatment within the first 6 months and 5 patients in the second 6 months after the initiation of adjuvant chemotherapy. The rea-

Table 1. Patient characteristics

	Cohort A S-1 adjuvant	Cohort B oral 5-FU	Cohort C no adjuvant	p value
Patients	30	10	49	
Gender				0.5254
Male	18	6	35	
Female	12	4	14	
Age, years				0.8537
Median	62.5	63	59	
Range	32–83	35–78	42–84	
Histology (Lauren's)				0.841
Intestinal	9	4	17	
Diffuse	21	6	32	
Stage				0.0053
II	2	4	11	
III	13	5	32	
IV	15	1	6	
Measurable lesions				0.6584
Present	19	6	26	
Absent	11	4	23	
Metastatic sites				0.2531
1	25	10	45	
≥2	5	0	4	
DFI				0.105
<1 year	19	5	19	
≥1 year	11	5	30	

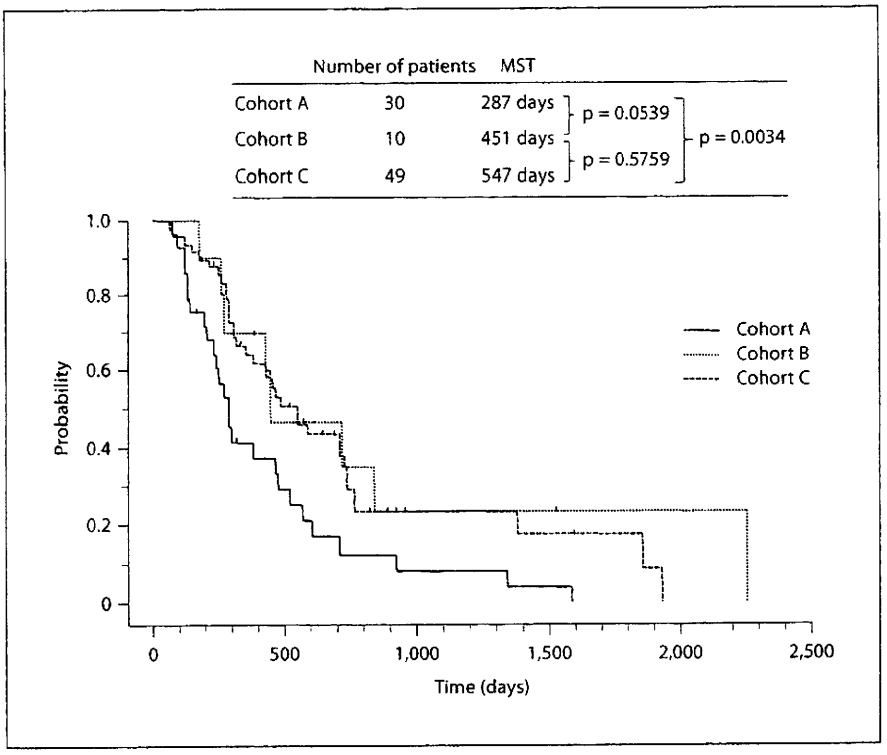

Fig. 1. OS after recurrence.

sons for treatment withdrawal were patient refusal in 1 and recurrent disease in 7 patients. The median duration of adjuvant 5-FU agent administration was 180 days. Forty-nine patients in cohort C, 35 males and 14 females with a median age of 59.0 years (range: 42–84), received no adjuvant chemotherapy. Histologically, around one third of patients had intestinal-type adenocarcinoma and two thirds had diffuse-type adenocarcinoma in each cohort. As for the initial stage of the primary tumor after curative gastrectomy, stage IV disease was significantly more frequent in cohort A than in the other cohorts ($p = 0.0053$). A measurable recurrent lesion was seen in 50–60% of each cohort and multiple metastatic sites were present in 10% of all patients. The disease-free interval (DFI), which was defined as the time from the date of surgery to the date of recurrence, was less than 1 year in approximately 40–60% of patients in either cohort.

Overall Survival

OS after recurrence was compared among the three cohorts. After a median follow-up time of 380 days from the date of recurrence (319 days in 71 dead patients and 560 days in 18 alive patents), the median survival time (MST) was 287 days in cohort A, 451 days in B and 547 days in C. OS was significantly shorter in cohort A than in cohort C ($p = 0.0034$), while there was no significant difference between cohorts B and A or C, as shown in figure 1. In cohort A, the duration of S-1 adjuvant chemotherapy was <6 months in 11 patients, 6 to <12 months in 10 and 12 months in 9. No significant difference in OS (MST, 246 vs. 287 vs. 464 days; $p = 0.4963$) was observed according to the duration of S-1 adjuvant chemotherapy, as shown in figure 2.

Efficacy of First-Line Chemotherapy

Regimens of the first-line chemotherapy delivered after recurrence are shown in table 2. Nine patients (30.0%) in cohort A received S-1-based therapy (S-1 monotherapy [12, 13] in 3, S-1 plus cisplatin [14] in 3, S-1 plus irinotecan [15] in 3, S-1 plus paclitaxel [16] in 0), although 9 patients were treated with paclitaxel monotherapy administered in a weekly fashion [17] and 12 patients with irinotecan-based therapy (irinotecan monotherapy [18] in 5, irinotecan plus cisplatin [19] in 7). In cohort B, 5 patients (50.0%) received S-1-based therapy, with 4 patients being treated with paclitaxel monotherapy and 1 patient with irinotecan plus cisplatin. In cohort C, 42 patients (85.7%) received S-1-based therapy, 4 patients were given paclitaxel monotherapy and 3 patients were given irinotecan plus cisplatin. It seemed inevitable for various regimens to

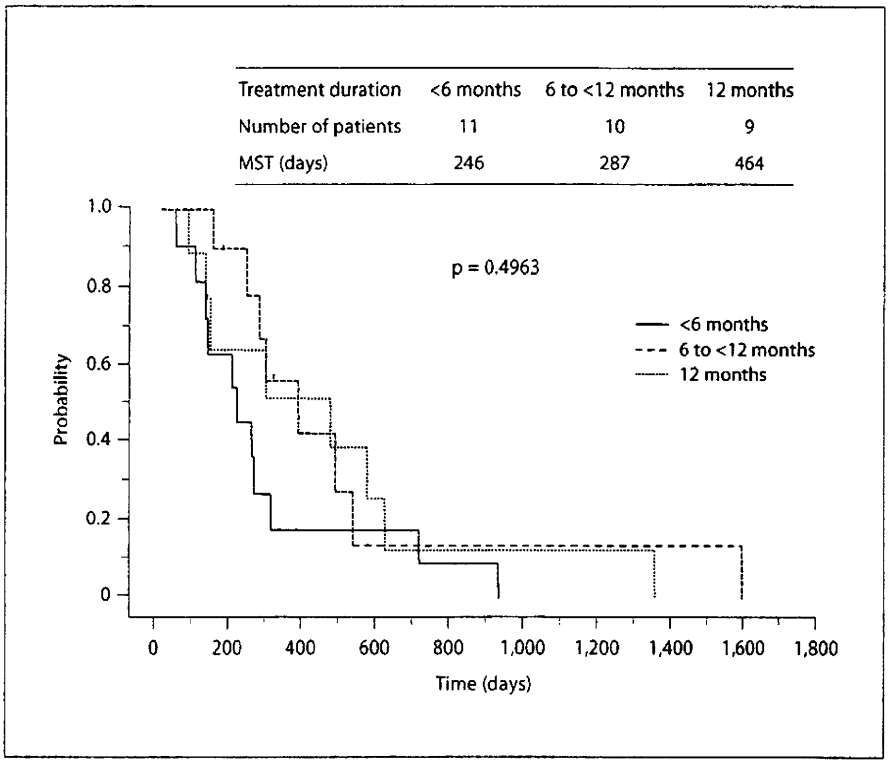

Fig. 2. OS according to the duration of S-1 adjuvant chemotherapy.

Table 2. Regimens of first-line chemotherapy after recurrence

	Cohort A (n = 30)	Cohort B (n = 10)	Cohort C (n = 49)
S-1-based therapy	9	5	42
S-1 monotherapy	3	3	26
S-1 + cisplatin	3	0	4
S-1 + irinotecan	3	1	7
S-1 + paclitaxel	0	1	5
Weekly paclitaxel	9	4	4
Irinotecan-based therapy	12	1	3
Irinotecan monotherapy	5	0	0
Irinotecan + cisplatin	7	1	3

have been given as the first-line treatment because it was obscure whether non-S-1-based therapy was more appropriate for patients relapsed after adjuvant S-1 or which should be chosen as a non-S-1 agent between paclitaxel and irinotecan for patients with recurrent gastric cancer. However, there was a tendency to prefer choosing S-1-based therapy as the first-line treatment after recurrence in cohort C, while non-5-FU regimens were more likely to be chosen in cohorts A and B. The best response to the first-line chemotherapy after recurrence was compared among these 3 cohorts, as shown in table 3. Response rates (RR) were 6.7% [95% confidence interval (CI) 0.8–22.1], 30.0% (95% CI 6.7–65.3), and 42.9% (95% CI 28.8–57.8) in cohorts A, B and C, respectively, with a significant difference between A and C (p = 0.0007). DCR were 50.0% (95% CI 31.3–68.7), 80.0% (95% CI 44.4–97.5) and 89.8% (95% CI 77.8–96.6) in cohorts A, B and C, respectively, with a significant difference between A and C (p = 0.0001).

Prognostic Factors for OS

The results of univariate and multivariate analyses of various factors, such as gender, age, histology, initial stage and presence of measurable lesion, number of metastatic sites, DFI and type of adjuvant chemotherapy for OS following recurrence are summarized in table 4. Among these, absence of a measurable lesion [hazard ratio 2.18 (95% CI 1.28–3.72)], presence of multiple metastatic sites [hazard ratio 2.89 (95% CI 1.28–6.52)] and S-1 adjuvant chemotherapy [hazard ratio 2.64 (95% CI 1.35–4.75)] were identified as significant independent factors for poor prognosis after recurrence.

Table 3. Efficacy of first-line chemotherapy

	CR	PR	SD	PD	Total	RR, %	DCR, %
Cohort A	0	2	13	15	30	6.7 (0.8–22.1)	50 (31.3–68.7)
Cohort B	2	1	5	2	10	30 (6.7–65.3)	80 (44.4–97.5)
Cohort C	4	17	23	5	49	42.9 (28.8–57.8)	89.8 (77.8–96.6)

Figures in parentheses are 95% CI.

Table 4. Prognostic factors for OS

	Univariate analysis			Multivariate analysis		
	patients	MST, days	p	hazard ratio	95% CI	p
Gender						
Male	59	466	0.4212			0.791
Female	30	303		1.082	0.606–1.930	
Age, median 61						
≥61	46	455	0.5071			0.91
<61	43	446		0.969	0.559–1.680	
Histology						
Intestinal	30	455	0.5388			0.948
Diffuse	59	446		0.981	0.556–1.733	
Stage						
II	17	304	0.4825	1.115	0.444–2.800	0.744
III	50	455		1.274	0.667–2.432	
IV	22	479				
Measurable lesion						
Present	51	547	0.0243			0.004
Absent	38	285		2.181	1.279–3.720	
Metastatic sites						
1	80	455	0.0154			0.011
≥2	9	268		2.89	1.281–6.524	
DFI						
<1 year	43	351	0.365	1.349	0.786–2.315	0.277
≥1 year	46	521				
Adjuvant chemotherapy						
None	49	547	0.0061			0.008
S-1	30	287		2.635	1.346–4.747	
Oral 5-FU	10	451		0.98	0.422–2.274	

Discussion

Although adjuvant chemotherapy with S-1 has recently become the standard treatment for stage II-III gastric cancer patients after curative gastrectomy in Japan based on the result of the ACTS-GC trial [9], nearly 30% of patients still relapse, despite the adjuvant S-1 treatment. Since the total number of patients with recurrence after adjuvant S-1 is increasing, it is of great concern to discern whether adjuvant S-1 affects the subsequent clinical course of the patients after recurrence. We, therefore, retrospectively evaluated the effect of adjuvant S-1 on survival following recurrence and the efficacy of first-line chemotherapy given at the time of relapse in patients with recurrent gastric cancer.

As shown in figure 1, patients initially treated with adjuvant S-1 had shorter survival following the recurrence than those receiving no adjuvant treatment (MST 287 vs.

547 days, p = 0.0034). Similarly, adjuvant chemotherapy was reported to have a negative impact on outcome after recurrence in other types of cancer such as colon and breast [20, 21]. As for the results of subset analysis of cohort A shown in figure 2, there may be some controversies. MST of the patients who relapsed after completion of 12 months of S-1 adjuvant chemotherapy was 464 days, equivalent to that of 451 days in cohort B. Although the duration of S-1 adjuvant chemotherapy showed no effect on OS after recurrence, this lack of statistical difference between the subgroups might be due to the small sample size. However, at least, the patients who discontinued S-1 adjuvant chemotherapy within 12 months because of recurrence were very unlikely to be salvaged by the additional chemotherapy given at the time of relapse. Although there was an imbalance of initial stage of the primary tumor between cohorts A and C, as shown in table 1, MSTs at stage II-III and IV in cohort A were 237 and 479 days, respectively, while they were 588 and 290 days in cohort C, respectively, with no significant difference between stage II-III and IV. Furthermore, on multivariate analysis in table 4, S-1 adjuvant chemotherapy but not initial stage was confirmed as an independent prognostic factor for OS after recurrence. Absence of a measurable lesion and presence of multiple metastatic sites also significantly correlated with inferior survival on multivariate analysis. MSTs of patients whose metastatic lesions involved the peritoneum (n = 36), bone/skin (n = 6), lymph nodes (n = 34) and liver (n = 19) were 285, 209, 609 and 426 days, respectively. Prior receipt of S-1 adjuvant chemotherapy as well as absence of a measurable lesion and presence of multiple metastatic sites contributed to the poor prognosis following tumor recurrence. These prognostic factors identified in this study might become useful factors of stratification for future clinical trial design in patients with recurrent gastric cancer.

With respect to the efficacy of first-line chemotherapy given at the time of relapse, patients who had received S-1 adjuvant chemotherapy showed a significantly lower RR than those receiving no adjuvant treatment: 6.7 versus 42.9% (p = 0.0007) as shown in table 3. Likewise, in patients with recurrent breast cancer, adjuvant chemotherapy was demonstrated to be a significant factor in predicting a poor response to first-line chemotherapy after recurrence [21]. As for the choice of first-line regimen given at the time of relapse, about two thirds of patients in cohort A received non-S-1-based therapy after adjuvant S-1. Although 1 retrospective study reported the invalidity of S-1-based chemotherapy as first-line treatment for recurrent disease after adjuvant S-1 in terms of a significantly lower RR, DCR as well as shorter progression-free survival compared to non-S-1-based chemotherapy [22], it still remains a problem to be clarified prospectively whether patients failing S-1 adjuvant chemotherapy should subsequently be treated with non-S-1-based regimens. In fact, in cohort A, patients treated with non-S-1-based chemotherapy showed an MST of 287 days with RR of 9.5% and DCR of 52.4%, while those with S-1-based regimens demonstrated an MST of 268 days with RR of 0% and DCR of 33.3%, with no significant difference among them. These findings suggest that patients who recurred following S-1 adjuvant chemotherapy must have extremely aggressive tumors refractory to any kind of further chemotherapy.

The poor outcome following relapse in patients who had received adjuvant S-1 might be speculatively interpreted as follows. While noncurative adjuvant chemotherapy might eradicate sensitive tumor cells, adjuvant S-1 could screen and select biologically more aggressive cellular clones with intrinsic resistance to cytotoxic agents that progress more quickly once recurrence is identified, or could induce acquired cellular resistance to further chemotherapy, like anthracyclines which induce the development of multidrug resistance [23]. In either case, the tumor mass would be constituted mainly of resistant cells at the time of relapse or, as a consequence, a poor response to first-line chemotherapy and a shorter OS would be expected following recurrence. In a recent report [24], adjuvant S-1, compared to surgery alone, was shown to deteriorate recurrence-free survival as well as OS after curative gastrectomy when confined to patients with high intratumoral mRNA expression of thymidylate synthase (TS). Although high TS expression is well correlated with resistance to 5-FU [25] derived from S-1, these findings suggest that biologically more aggressive cancer cells could be induced by the S-1 administration in a tumor with high TS expression.

Irrespective of types of regimens, adjuvant chemotherapy was reported to be significantly associated with a low probability of response to first-line chemotherapy and shorter survival following recurrence in patients with recurrent breast cancer [21]. However, in the present study, adjuvant treatment with 5-FU agents other than S-1 showed modest effects on OS and RR compared to adjuvant S-1, though adjuvant S-1 adversely affected OS and RR in recurrent gastric cancer patients, as shown in figure 1 and table 3. It is not clear whether this difference in adverse effect between S-1 and other 5-FU agents depends on the ability in inducing chemoresistant cells of the respective agent.

DFI was not significantly prognostic of survival following recurrence on either univariate or multivariate analysis in this study, as shown in table 4. There have been some controversies about the effect of DFI on OS after recurrence. Patients recurred with longer DFI had superior survival to those with shorter DFI in recurrent colon cancer [20]. On the contrary, the MST of metastatic patients pretreated with adjuvant chemotherapy was independent of DFI in recurrent breast cancer [21]. In this study, the numbers of patients with a DFI less than 1 year, from 1 to 2 years, from 2 to 3 years and more than 3 years were 43, 29, 9 and 8, respectively. It remains possible for DFI to become a prognostic factor if the number of patients with a long DFI increases.

Of note, the MST of 287 days following recurrence in patients initially treated with adjuvant S-1 after curative gastrectomy was similar to that of 7 months yielded by the subsequent chemotherapy to S-1 in advanced/recurrent gastric cancer [12, 14]. No matter who received the first-line chemotherapy of S-1 as an adjuvant one or not, the OS after the usage of S-1 might be the same in patients who had recurrent tumor left after S-1 administration.

Although we believe that this is the first report demonstrating that patients initially treated with S-1 adjuvant chemotherapy had significantly inferior survival following recurrence and poorer response to first-line chemotherapy, compared with those without any adjuvant treatment after curative gastrectomy, it should be noted that the present study is a retrospective small-sized analysis performed at a single center. The results shown here warrant further study to elucidate the effect of S-1 adjuvant chemotherapy in patients with recurrent gastric cancer and to investigate an optimal regimen for patients relapsed after adjuvant S-1, though a prospective randomized study seems infeasible because adjuvant S-1 has become the standard treatment for stage II-III gastric cancer patients in Japan.

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Comparison of efficacy of concomitant administration of mitiglinide with voglibose and double dose of mitiglinide in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: When monotherapy with an oral hypoglycemic agent (OHA) is not sufficiently effective for blood glucose control, combination therapy with OHA having different mechanisms of action might be indicated.

Materials and Methods: In the present study, we compared the efficacy of two options in type 2 diabetes mellitus patients whose blood glucose had not been well controlled with mitiglinide (30 mg/day) alone. A total of 20 patients were included in the study and divided into two groups: group A, in which mitiglinide was given concomitantly with the α-glucosidase inhibitor voglibose (0.6 mg/day); and group B, in which a double dose of mitiglinide was given (60 mg/day). Twelve weeks after changing the medication, HbA_{1c}, glycoalbumin and 1,5-anhydroglucitol (1,5-AG) were measured. In addition, at weeks 0 and 12, a meal tolerance test was carried out, and plasma glucose, insulin, glucagon, active glucagon-like peptide-1 (GLP-1) and total glucose-dependent insulinotropic polypeptide levels were measured.

Results: The plasma level of 1,5-AG improved in both groups at week 12. In group A, the plasma insulin level significantly decreased and the plasma active GLP-1 level significantly increased during the meal tolerance test at week 12; thus, bodyweight significantly decreased only in group A.

Conclusions: Our findings suggested that concomitant administration of mitiglinide with voglibose could achieve better glycemic control, particularly in the postprandial period, without bodyweight gain and might have beneficial effects in type 2 diabetic patients at risk of macrovascular complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00082.x, 2010)

KEY WORDS: α-Glucosidase inhibitor, Glucagon-like peptide-1, Mitiglinide

INTRODUCTION

Mitiglinide calcium hydrate (mitiglinide) is a potent, fast-acting, short-duration insulin secretagogue that promotes insulin secretion just after meals and thereby inhibits postprandial hyperglycemia. It is an oral hypoglycemic agent (OHA) that rarely induces hypoglycemia because of its short duration of action. α-Glucosidase inhibitors (α-GI) inhibit glucose absorption in the upper small intestine, improve postprandial hyperglycemia, and regulate delayed and excessive insulin secretion. These OHA are effective for both postprandial hyperglycemia and hyperinsulinemia. Furthermore, α-GI might have other beneficial effects on the secretion of incretins, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which have been reported to possess trophic effects on β-cells. In animal models, native GLP-1 stimulates β-cell proliferation and inhibits apoptosis, which might increase β-cell mass and function[1].

When monotherapy with an OHA is not sufficiently effective in blood glucose control, combination therapy with OHA having different mechanisms of action could be indicated. In case monotherapy with mitiglinide is not sufficient to treat postprandial hyperglycemia, one of the risk factors for macroangiopathy, it remains unclear whether it is better to increase the dose of mitiglinide or to use it concomitantly with an α-GI.

In the present study, we compared these two options in type 2 diabetes mellitus patients, whose plasma glucose levels had not been well controlled by dietary therapy and mitiglinide administration (30 mg/day t.i.d.).

METHODS

Subjects

The subjects included 20 outpatients with type 2 diabetes mellitus (age ≥ 20 years) whose plasma glucose levels had not been

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well controlled with dietary therapy and mitiglinide (10 mg/day t.i.d.) for at least 8 weeks before week 0. The protocol was approved by the Ethics Committee of Hyogo College of Medicine. Each participant gave written informed consent before the start of the study and fulfilled all the following inclusion criteria:

1 A HbA_{1c} level of 6.9–8.9% at week 0;
2 Not treated with OHA other than mitiglinide for 24 weeks (168 days) before week 0;
3 Not treated with insulin for over 8 weeks (56 days) before week 0.

The patient background characteristics (sex, age, duration of diabetes, body mass index [BMI], HbA_{1c} and levels of fasting plasma glucose, insulin, total cholesterol, low-density lipoprotein cholesterol, triglyceride, and blood pressure) at week 0 are summarized in Table 1.

Study Design

The study was carried out at the Division of Diabetes and Metabolism, Department of Internal Medicine, Hyogo College of Medicine and Watanabe Medical Clinic in Nishinomiya, Hyogo, Japan. The subjects were randomly allocated to two groups using a lottery system on an open trial basis: group A (concomitant voglibose group), in which mitiglinide was given concomitantly with voglibose; and group B (double mitiglinide group), in which a double dose of mitiglinide was given.

The study design is shown in Figure 1. During the observation period, 10 mg mitiglinide was given orally three times a day before meals (within 5 min before each meal). During the treatment period, 10 mg mitiglinide and 0.2 mg voglibose were given orally to subjects in group A three times a day before meals, whereas 20 mg mitiglinide was given orally to subjects in group B three times a day before meals. During the observation and treatment periods, neither agent was given after a meal or without a meal. The administration period was 24 weeks, including 12 weeks of observation and 12 weeks of treatment. Dietary therapy was started at least 8 weeks (56 days) before the start of the observation period, and continued without change throughout the study period. If exercise therapy had been used before the study, it was continued without change throughout the study period; however, subjects were not allowed to initiate exercise therapy during the study period. The levels of HbA_{1c}, glycoalbumin (GA) and 1,5-AG were measured at week 12. Considering the relationship between HbA_{1c} value measured by the traditional Japanese standard measurement method (JDS) and the National Glycohemoglobin Standardization Program (NGSP), the HbA_{1c} value (%) in the present study is

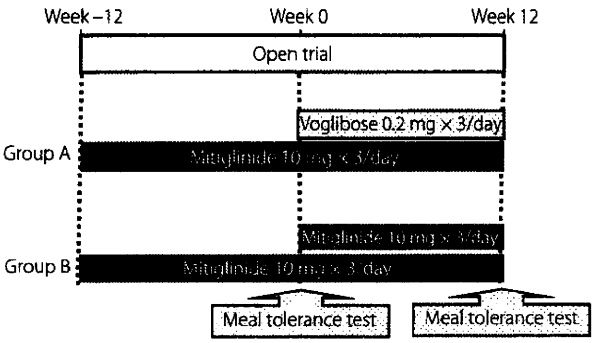

Figure 1 | Study design. Test meal (460 kcal, 56.5 g carbohydrate, 18 g protein, 18 g fat).

Table 1 | Patient characteristics

	All subjects	Group A (concomitant voglibose group)	Group B (double mitiglinide group)	*P*-value (A *vs* B)
No. subjects (male, female)	20 (12, 8)	10 (7, 3)	10 (5, 5)	–
Age (years)	59.9 ± 11.4	57.8 ± 10.9	62.0 ± 12.1	N.S.
Duration of diabetes (years)	9.8 ± 6.0	7.1 ± 3.6	12.4 ± 6.8	0.0460
BMI (kg/m^2)	24.6 ± 4.1	25.9 ± 2.6	23.2 ± 5.0	N.S.
HbA_{1c} (%)	7.8 ± 0.6	8.1 ± 0.6	7.4 ± 0.3	0.0130
Fasting plasma glucose level (mg/dL)	167.9 ± 27.3	179.5 ± 30.8	156.3 ± 18.0	0.0410
SBP (mmHg)	126.9 ± 5.6	125.8 ± 6.4	128.0 ± 4.9	N.S.
DBP (mmHg)	72.6 ± 2.7	73.4 ± 3.4	71.8 ± 1.5	N.S.
TC (mg/dL)	213.0 ± 29.6	210.5 ± 23.6	215.5 ± 35.8	N.S.
LDL-C (mg/dL)	128.7 ± 27.2	129.4 ± 16.9	128.0 ± 35.7	N.S.
TG (μU/mL)	123.0 ± 99.8	143.1 ± 122.2	102.9 ± 72.1	N.S.

BMI, body mass index; DBP, diastolic blood pressure; HbA_{1c}, hemoglobin A_{1c}; LDL-C, low-density lipoprotein cholesterol; N.S., not significant; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

shown as the NGSP equivalent value (%) calculated on the basis of the following formula[2]: HbA_{1c} (NGSP) (%) = HbA_{1c} (JDS) (%) + 0.4 (%). At weeks 0 and 12, a meal tolerance test was carried out using a test meal (JANEF E460F18: 460 kcal, 56.5 g carbohydrate, 18 g protein and 18 g fat). The levels of plasma glucose, insulin, glucagon, active GLP-1 and total GIP were measured at 0, 30, 60 and 120 min during the meal tolerance test.

The plasma level of active GLP-1 was measured by enzyme-linked immunosorbent assay (ELISA) using a GLP-1 Active ELISA Kit (Millipore Corporation, Billerica, MA, USA) according to the manufacturer's instructions. The plasma level of total GIP was also measured by ELISA using a Human GIP (total) ELISA kit (Millipore Corporation). Blood samples for measurement of GLP-1 and GIP were collected in EDTA tubes containing aprotinin and a dipeptidyl peptidase 4 inhibitor (10 μL/mL of blood; Millipore Corporation).

Bodyweight was measured every 4 weeks and the subjects were asked about the occurrence of hypoglycemia throughout the study period. The study was carried out from April 2008 to December 2009.

Statistical Analysis

Data are represented as mean ± standard deviation unless otherwise specified. For intergroup comparisons, the Wilcoxon signed-rank test was used. For multiple comparisons, two-way analysis of variance (ANOVA) was carried out. The results of ANOVA at each time-point of measurement were examined using the Wilcoxon signed-rank test. The area under the curve (AUC) was estimated using the trapezoid method.

RESULTS

Characteristics of Subjects

The characteristics of subjects in groups A and B at week 0 are presented in Table 1. The duration of diabetes was significantly shorter in group A than in group B. HbA_{1c} and fasting plasma glucose levels were significantly higher in group A than in group B (8.1 ± 0.6% *vs* 7.4 ± 0.3% and 179.5 ± 30.8 mg/dL *vs* 156.3 ± 18.0 mg/dL, respectively). BMI was also higher in group A than in group B, though not to a significant degree. Blood pressure and lipid profiles did not differ between the groups.

Changes in HbA_{1c}, GA and 1,5-AG Levels

In group A, 1,5-AG level had improved significantly at week 12 (3.5 ± 2.9 to 6.9 ± 6.6 μg/mL, $P = 0.0039$); GA and HbA_{1c} levels had also improved, though not to a significant degree. In group B, HbA_{1c}, GA and 1,5-AG levels were all improved significantly at week 12 (7.4 ± 0.3 to 7.2 ± 0.4%, $P = 0.0469$; 22.1 ± 2.7 to 20.5 ± 1.9%, $P = 0.0078$; and 4.1 ± 2.0 to 5.9 ± 3.6 μg/mL, $P = 0.0234$, respectively; Table 2).

Changes in Bodyweight and Hypoglycemic Events

In group A, bodyweight significantly decreased (71.9 ± 12.7 to 70.8 ± 12.6 kg, $P = 0.0039$), whereas in group B, there was no significant change (60.0 ± 14.0 to 59.8 ± 16.8 kg, $P = 0.5315$; Table 2). No symptoms of hypoglycemia were noted in either group throughout the study period.

Changes in Plasma Glucose and Insulin Levels in Meal Tolerance Tests at Weeks 0 and 12

Changes in plasma glucose levels after a meal are shown in Figure 2. In group A, plasma glucose levels 30 and 60 min after a meal significantly decreased at week 12 (245.7 ± 38.1 to 202.4 ± 37.5 mg/dL, $P = 0.0039$ and 273.0 ± 51.9 to 229.2 ± 39.2 mg/dL, $P = 0.0059$, respectively). The plasma glucose level peaked 60 min after a meal at week 0 and 120 min after a meal at week 12. In group B, up to 30 min after a meal, the mean plasma glucose level at week 12 remained almost the same as that at week 0. The mean plasma glucose level 120 min after a meal improved at week 12, though not to a significant degree.

Table 2 | Changes in parameters from week 0 to 12 in groups A and B

	Group A (concomitant voglibose group)		*P*-value (*vs* week 0)	Group B (double mitiglinide group)		*P* value (*vs* week 0)
	Week 0	Week 12		Week 0	Week 12	
HbA_{1c} (%)	8.1 ± 0.6	7.9 ± 0.8	N.S.	7.4 ± 0.3	7.2 ± 0.4	0.0469
GA (%)	22.3 ± 2.2	21.8 ± 3.4	N.S.	22.1 ± 2.7	20.5 ± 1.9	0.0078
1,5 AG (μg/mL)	3.5 ± 2.9	6.9 ± 6.6	0.0039	4.1 ± 2.0	5.9 ± 3.6	0.0234
Fasting plasma glucose (mg/dL)	179.5 ± 30.8	168.7 ± 27.6	N.S.	156.3 ± 18.0	150.5 ± 16.2	N.S.
Weight (kg)	71.9 ± 12.7	70.8 ± 12.6	0.0039	60.0 ± 14.0	59.8 ± 16.8	N.S.
Glucose AUC_{0-120} (mg h/dL)	30196.5 ± 5627.4	26044.5 ± 4394.3	0.0098	25363.5 ± 5443.6	24180.0 ± 5366.1	N.S.
Insulin AUC_{0-120} (μU h/mL)	3741.8 ± 2184.6	3229.8 ± 1551.8	N.S.	2878.0 ± 1840.5	3221.1 ± 2365.3	N.S.
GLP-1 AUC_{0-120} (pmol h/L)	648.9 ± 91.6	843.3 ± 336.9	0.0137	604.2 ± 58.8	664.5 ± 103.7	N.S.
GIP AUC_{0-120} (pg h/mL)	24151.1 ± 9506.3	22856.1 ± 10277.1	N.S.	24481.2 ± 8888.7	26751.1 ± 12145.2	N.S.
Glucagon AUC_{0-120} (pg h/mL)	10347.6 ± 2029.6	11090.4 ± 1948.1	N.S.	10373.0 ± 2590.2	9820.5 ± 2151.4	N.S.

1,5-AG, 1,5-anhydroglucitol; AUC, area under the curve; GA, glycoalbumin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, hemoglobin A_{1c}; N.S., not significant.

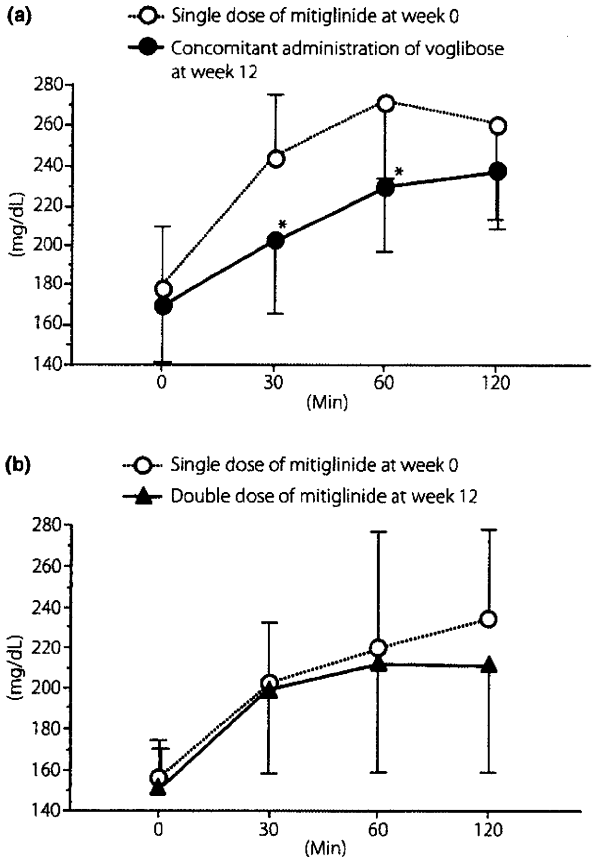

Figure 2 | Changes in plasma glucose level after meal tolerance test from week 0 to 12 in (a) group A and (b) group B. A significant decrease 30 and 60 min after a meal was observed at week 12 in group A. *P < 0.05 *vs* before addition of voglibose (Wilcoxon signed-rank test).

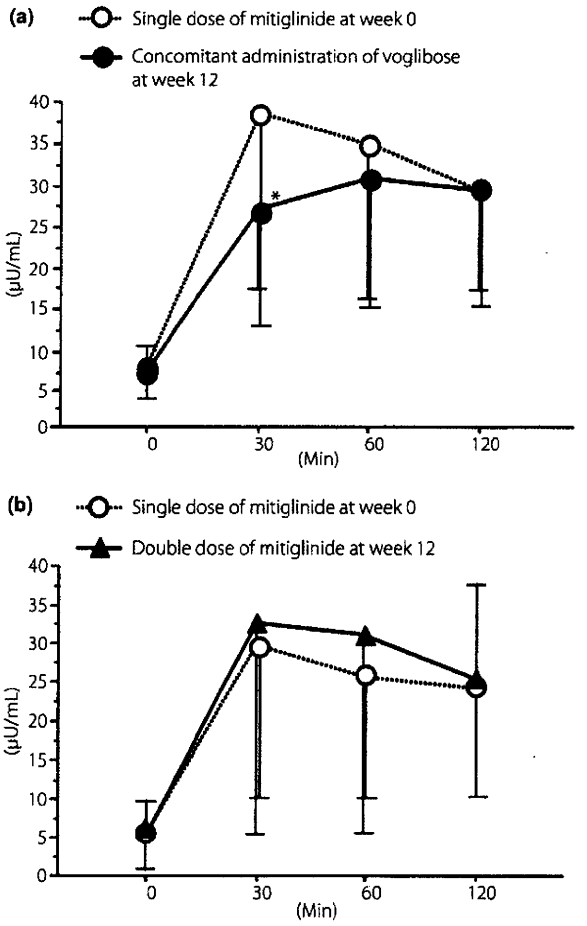

Figure 3 | Change in plasma insulin level after meal tolerance test from week 0 to 12 in (a) group A and (b) group B. A significant decrease 30 min after a meal was observed at week 12 in group A. *P < 0.05 *vs* before addition of voglibose (Wilcoxon signed-rank test).

The plasma glucose level peaked 120 min after a meal at week 0 and 60 min after a meal at week 12.

Changes in plasma insulin level and AUC are presented in Table 2 and Figure 3. In group A, the plasma insulin level 30 min after a meal significantly decreased at week 12 (38.5 ± 27.0 to 27.3 ± 10.4 μU/mL, P = 0.0273). AUC_{0-30} of the plasma insulin level significantly decreased (698.3 ± 227.1 to 521.8 ± 215.6 μU h/mL, P = 0.0273). AUC_{0-120} of the plasma insulin level throughout the meal tolerance test at week 12 in group A was less than that at week 0, though not to a significant degree (3741.8 ± 2184.6 to 3229.8 ± 1551.8 μU h/mL). The plasma insulin level peaked 30 min after a meal at week 0 and 60 min after a meal at week 12. In group B, the plasma insulin levels 30 and 60 min after a meal were increased at week 12, though not to a significant degree. The plasma insulin level peaked 30 min after a meal at both week 0 and 12. AUC_{0-120} of the plasma insulin level at week 12 in group B was higher than that at week 0 (2878.0 ± 1840.5 to 3221.1 ± 2365.3 μU h/mL).

Changes in Plasma Glucagon Level in Meal Tolerance Test at Weeks 0 and 12

There was no significant change in plasma glucagon level or plasma glucagon AUC between week 0 and 12 in either group (Table 2).

Changes in Active GLP-1 and Total GIP Levels in Meal Tolerance Tests at Weeks 0 and 12

Changes in active GLP-1 levels are shown in Figure 4. In group A, the active GLP-1 levels were elevated throughout the experiment at week 12. Among them, active GLP-1 levels 60 and 120 min, and AUC_{0-120} after a meal significantly increased (5.3 ± 0.7 to 7.5 ± 2.7 pmol/L, P = 0.0039 and 5.3 ± 0.9 to 6.7 ± 2.7 pmol/L, P = 0.0332, and 648.9 ± 91.6 to 843.3 ± 336.9 pmol h/L, P = 0.0137, respectively). Active GLP-1 levels peaked 30 min after a meal at week 0 and 60 min after a meal at week 12. In group B, there was no significant difference in

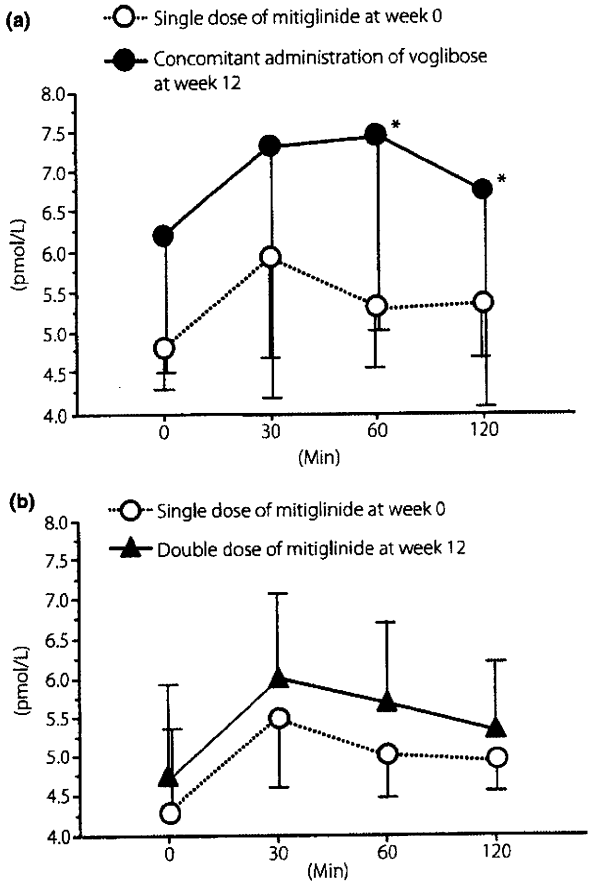

Figure 4 | Change in active glucagon-like peptide-1 level after meal tolerance tests from week 0 to 12 in (a) group A and (b) group B. A significant increase at 60 and 120 min after a meal was observed at week 12 in group A. *$P < 0.05$ *vs* before addition of voglibose (Wilcoxon signed-rank test).

active GLP levels between week 0 and 12. Active GLP-1 levels peaked 30 min after a meal at weeks 0 and 12.

There was no significant change in total GIP levels between week 0 and 12 in either group (Table 2, Figure 4).

DISCUSSION

In the present study, when mitiglinide was given concomitantly with voglibose for 12 weeks, the peak plasma glucose level after a meal decreased significantly and the time required for plasma glucose level to reach the peak value was prolonged (Figure 2a). Although there was no significant difference in AUC_{0-120} of plasma insulin levels, a significant decrease was observed in AUC_{0-30} ($P = 0.0273$), namely during the early phase of insulin secretion (Figure 3). It has been shown that voglibose inhibits the postprandial increase in plasma glucose level and thereby decreases plasma insulin levels.

It was recently reported that the acute postprandial increase in plasma glucose level (postprandial glucose spike) promotes arteriosclerosis and increases the risk of cardiovascular disease, including myocardial infarction[3]. It has also been reported that hyperinsulinemia impairs vascular endothelial function and increases the risk of ischemic heart disease[4]. In the present study, concomitant administration of mitiglinide and voglibose (group A) more markedly inhibited the postprandial glucose spike and decreased insulin secretion than did a single dose of mitiglinide. This suggests that concomitant use of mitiglinide with voglibose might have a beneficial effect in preventing arteriosclerosis.

In group A, the active GLP-1 levels at 60 and 120 min after a meal were significantly increased at week 12 (Figure 4a). This finding is of particular interest, because there has been no previous report of voglibose significantly increasing the active GLP-1 level in patients with type 2 diabetes mellitus.

Active GLP-1 level was reported to increase when voglibose was given to *ob/ob* mice for 3–4 weeks[5]. It appears that continuous administration of voglibose evoked chronic glucose absorption from the small intestine and increased the amount of undigested carbohydrates, which results in constant stimulation of the lower small intestine and the large intestine, thus promoting differentiation and proliferation of GLP-secreting cells (L-cells)[6]. This mechanism of action appears to explain why the GLP-1 levels at 60 and 120 min after a meal were significantly increased at week 12 in group A. These findings suggest that concomitant use of mitiglinide and voglibose could spare excessive insulin secretion, and that the increase in GLP-1 level might protect the function of pancreatic β-cells and regulate postprandial plasma glucose levels.

It has been reported that GLP-1 improved abnormal glucagon secretion, particularly the paradoxical rise in glucagon secretion[7]. However, in the present study, no relationship between GLP-1 secretion and pancreatic glucagon secretion was observed in either group (Table 2). Further investigation is necessary to elucidate whether the beneficial effects of the concomitant use of α-GI and mitiglinide treatment, on better long-term glucose control, would depend on the suppression of glucagon secretion.

In contrast, in group B, HbA_{1c}, GA and 1,5-AG levels significantly improved at week 12 (Table 2). In a double-blind comparative phase III clinical study of mitiglinide in China[8], HbA_{1c} levels improved when the mitiglinide dose was increased from 10 to 20 mg, which is similar to the results of the present study. However, meal tolerance tests at week 12 showed no significant change in plasma glucose level in group B (Figure 2). It is quite difficult to explain the discrepancy; the plasma glucose level 120 min after a meal in group B showed no significant decrease at week 12, but did tend to decrease compared with that of week 0. In the present study, we investigated the plasma glucose levels only until 120 min after a meal. However, there was a great difference in plasma glucose levels at 120 min or later (Figure 2). Therefore, the HbA_{1c} level might have been significantly improved at 120 min or later after a meal in group B.

In the present study, we randomly allocated the subjects to two groups; incidentally, the background characteristics were significantly different between the groups (Table 1). The duration of diabetes was shorter and the blood glucose control was worse in group A participants on entry to the study.

Mean BMI was 26.0 in participants of group A, which shows that they were slightly more obese than the Japanese patients with type 2 diabetes. Because impairment of early insulin secretion is closely related to the pathogenesis of type 2 diabetes in Japanese patients and the secretory capacity of pancreatic β-cells is weaker in Japanese patients than those in the USA and Europe[9–11], concomitant use of mitiglinide with voglibose could be more useful even in fairly well-controlled obese Japanese patients with type 2 diabetes mellitus as long as they were switched to concomitant treatment at an early stage.

In contrast, in group B in the present study, mean BMI was 23.2; that is, they were non-obese, plus their mean duration of diabetes was much longer (12.4 years).

In non-obese Japanese type 2 patients whose blood glucose levels are fairly well controlled with mitiglinide (30 mg/day) alone, a double dose of mitiglinide treatment could be expected to improve the plasma glucose level without causing bodyweight gain and/or hypoglycemia. This might support the potential for a double dose of mitiglinide to still be effective in non-obese, long-standing type 2 diabetes patients with low insulin secretory capacity.

Because the sample size of the present study was limited, a large-scale study should be carried out to confirm the patient benefits by treatment regimen.

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ORIGINAL RESEARCH

Incretin Responses to Oral Glucose Load in Japanese Non-Obese Healthy Subjects

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ABSTRACT

Introduction: Recently, incretin-related therapy has been developed for the new treatment of diabetes mellitus; however, incretin response to glucose ingestion in normal glucose tolerant (NGT) subjects has not been clarified in detail with special reference to the role of incretin hormones, glucagon, and a family history of diabetes. ***Methods:*** We conducted a 75 g oral glucose tolerance test in 30 NGT subjects. ***Results:*** The total glucose-dependent insulinotropic peptide (GIP)-$AUC_{0\text{-}120}$ (area under the curve over a period of 0-120 minutes) was correlated with immunoreactive insulin (IRI)-$AUC_{0\text{-}120}$ ($P<0.05$), insulinogenic index (II; $P<0.05$), ΔIRI between 0 and 120 minutes ($P<0.05$). Active glucagon-like peptide-1 (GLP-1) $AUC_{0\text{-}120}$ was correlated inversely both with Δ glucose between 0 and 30 minutes ($P<0.01$) and with Δ immunoreactive glucagon between 0 and 30 minutes ($P<0.05$). Δ Total GIP between 0 and 15 minutes ($P<0.01$), Δ total GIP between 0 and 30 minutes ($P<0.05$), and the total GIP-$AUC_{0\text{-}120}$ ($P<0.05$) in the subjects with a family history of type 2 diabetes were significantly higher than those in the subjects without a family history. ***Conclusion:*** These results suggest that GIP possibly facilitates insulin secretion in response to oral glucose load directly and active GLP-1 may exert the glucoregulatory action via the suppression of glucagon secretion in NGT subjects. Notably, the subjects with a family history of diabetes exert significantly higher GIP response in the early phase of glucose load compared with those without a family history.

Keywords: diabetes mellitus; glucagon; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide; incretin; insulin

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INTRODUCTION

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones that are released from enteroendocrine cells within minutes after food intake. GIP is secreted from enteroendocrine cells (K cells) primarily in the proximal small intestine (duodenum and jejunum), while GLP-1 is secreted from enteroendocrine cells (L cells) that are scattered throughout the small bowel (primarily in the distal portion) and colon.[1] Both GIP and GLP-1 have been shown to stimulate insulin secretion in response to glucose and nutrient ingestion.[2] The primary role of GIP is supposed to stimulate the secretion of insulin from pancreatic β cells, especially in the postprandial state. In addition to its insulinotropic effect, GLP-1 has a potent glucagonostatic action, inhibits gastric emptying, decreases food intake, and slows the rate of endogenous glucose production, all of which would ameliorate the blood glucose levels in type 2 diabetes mellitus (T2DM). GLP-1 shows direct effects through GLP-1 receptors in the hypothalamus, thereby regulating the appetite, food intake, and body weight in the "gut-brain axis" (the reciprocal system between the gastrointestinal tract and central nervous system to regulate short- and long-term energy homeostasis).[1]

The earlier studies on the response of both incretin hormones to an oral glucose tolerance test (OGTT) and test meal revealed that the incretin effect of Caucasian patients with T2DM is significantly lower than healthy subjects.[3,4] Conversely, in the three studies demonstrating incretin responses after ingestion of glucose and/or nutrients in Japanese subjects,[5-7] any difference between T2DM patients with or without obesity and nondiabetic control subjects could not be found out.

Whether the abnormality of the incretin response to the nutrient might be involved in the pathophysiological mechanisms of the development of T2DM or not awaits further investigation.

At the first step of the analysis of the mechanism, it is necessary to reveal the response of incretin to glucose or food intake in normal glucose-tolerant (NGT) subjects in detail with special reference to the relationship between incretin hormone responses and insulin, glucagon secretion, gender, metabolic parameters inclusive of body mass index (BMI), waist circumference, and a family history of diabetes in particular.

In this study, the various parameters were investigated after an intake of 75 g glucose in order to clarify the relationship between the responses of incretin hormones and glucose, insulin, or glucagon in 38 healthy subjects who are currently NGT.

MATERIALS AND METHODS

Materials

Thirty healthy subjects, who were a group of medical students within a pregraduate course, participated in this study. All of them had NGT. The exclusion criteria were subjects who had any major illness or premenopausal women who were pregnant or nursing. This study has been approved by the Ethics Committee of Hyogo College of Medicine. All participants provided written informed consent before the start of the study.

Methods

To identify the baseline characteristics of incretin secretion in Japanese subjects, the influence of body weight, BMI, height, gender, and a family

history of diabetes on the hormonal response to oral glucose ingestion were also examined.

The OGTT consisted of ingesting 75 g glucose (Toleran G®, Ajinomoto Pharma, Tokyo, Japan) in the overnight fasting state within 5 minutes. For active GLP-1 and total GIP analysis, catheters were placed in cubital veins and blood samples were withdrawn directly into the blood collection ethylenediaminetetraacetic acid-disodium salt coated tubes (1.25 mg/mL blood) containing aprotinin and an inhibitor of dipeptidyl peptidase-4 (10 μL/mL blood; Linco Research Inc., MO, USA) before the start of the OGTT and 15, 30, 60, and 120 minutes after ingestion, respectively. The levels of plasma glucose, immunoreactive insulin (IRI), immunoreactive glucagon (IRG), total GIP, and active GLP-1 were measured at each time interval. Plasma glucose concentrations were measured immediately by the glucose oxidase method. IRI was measured using chemiluminescent enzyme immunoassay (Fujirebio Inc., Tokyo, Japan). Plasma levels of glucagon were measured using a radioimmunoassay via the glucagon kit daiichi-II (TFB Corp., CA, USA). Plasma levels of active GLP-1 were measured using an enzyme-linked immunosorbent assay (ELISA), using the GLP-1 active ELISA kit (Millipore Corp., MA, USA) according to the manufacturer's instructions. Plasma levels of the total GIP were also measured using an ELISA, using the human GIP (total) ELISA kit (Millipore Corp., MA, USA).

Statistical Analysis

The results of statistical analysis were shown as the mean ± standard deviation (SD) unless otherwise specified. The trapezoidal method was used to calculate all areas under the curves (AUCs). The homeostasis model assessment was used both for assessing insulin resistance (HOMA-R) and insulin secretion (HOMA-β).[8] HOMA-R was calculated as fasting plasma glucose (mg/dL) × fasting serum insulin (μIU/mL)/405. HOMA-β was calculated as (360× fasting serum insulin [μIU/mL]) / (fasting plasma glucose [mg/dL] –63). The insulinogenic index (II) was calculated as the ratio of the increment of serum insulin to plasma glucose concentration 30 minutes after an oral glucose load (IRI $\Delta_{0\text{-}30\ \text{minutes}}$/ glucose $\Delta_{0\text{-}30\ \text{minutes}}$).[9] Single Pearson correlation analysis was used to examine bivariate relationships. The unpaired *t*-test was used when two separate sets of samples (family history [+]/[–]) were obtained. A *P* value of less than 0.05 was taken to indicate statistically significant difference.

RESULTS

As shown in Table 1, the age of subjects was 24.7±2.3 years and BMI was 21.8±3.5 kg/m^2. The average BMI of women (10 subjects) was 19.3 kg/m^2 and men (20 subjects) was 23.1 kg/m^2. The mean plasma glucose, IRI, total GIP, active GLP-1, and IRG levels in the subjects are summarized in Figure 1. The mean plasma glucose and IRI peaked at 30 minutes (Figure 1A).

The mean active GLP-1 peaked at 15 minutes and the peak level of active GLP-1 was 10.6 pmol/L. The level of total GIP increased steeply in 15 minutes, and thereafter increased gradually with time, whereas the IRG gradually decreased during the observation period. The peak level of total GIP was 265.5 pg/mL (Figure 1B).

The total GIP-$AUC_{0\text{-}120}$ was related to the IRI-$AUC_{0\text{-}120}$ (R=0.39, P<0.05), II (R=0.40, P<0.05), and Δ IRI between 0 and 120 minutes (R=0.40, P<0.05), as shown in Figure 2A, B, and C, respectively. Conversely, the active GLP-1-$AUC_{0\text{-}120}$ was inversely correlated with Δ glucose between 0 and 30 minutes (R=–0.47,