Table 1 Correlations between EGFR mutations and clinicopathological features

Characteristics	Total	No. of patients					
		EGFR status		p[a]	KRAS status		p[a]
		Mutation	Wild-type		Mutation	Wild-type	
	(n = 388)	(n = 185, 47.7%)	(n = 203, 52.3%)		(n = 33, 8.5%)	(n = 355, 91.5%)	
Mean age, yr ± SD[b]	66.6 ± 10.0	65.1 ± 10.3	67.9 ± 9.57	0.462	68.6 ± 9.11	66.4 ± 10.1	0.553
Gender				<0.001			0.552
Male	228	75	153		21	207	
Female	160	110	50		12	148	
Histological type				<0.001			0.059
Adenocarcinoma	302	183	119		30	272	
Others	86	2	84		3	83	
Vascular invasion							
Ly -	314	155	159	0.172	25	289	0.429
Ly +	74	30	44		8	66	
V-	261	151	110	<0.001	23	238	0.756
V+	127	34	93		10	117	
p-stage				<0.001			
I	293	155	138		22	271	0.217
II / III	95	30	65		11	84	
T-factor				<0.001			
T1	197	114	83		14	183	0.316
T2 / 3	191	71	120		19	191	
Tumor diameter (cm)	3.03 ± 1.43	2.68 ± 0.92	3.35 ± 1.71	<0.001	3.46 ± 1.99	2.99 ± 1.36	0.001
N-factor				0.348			
N0	322	157	165		29	293	0.435
N1 / 2	66	28	38		4	62	
Smoking status				<0.001			0.107
Non-smoker	157	106	51		9	148	
Smoker	231	79	152		24	207	
Pre-existing cardiopulmonary comorbidity	203	86	117	0.028	20	183	0.319

[a]$p < 0.05$ statistically significant.
[b]SD, standard deviation.
EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; ND, lymph node dissection.

younger than 80 years (younger group) and compared EGFR status and clinicopathological features between these age groups (Table 2). The younger group comprised 359 patients (92.5%), and the older group comprised 29 (7.5%). The proportion of patients with EGFR mutations was significantly higher in the younger group (178/359, 49.6%) than in the older group (7/29, 24.1%; P = 0.008). In contrast, KRAS mutations were more common in the older group (6/29, 20.7%) than in the younger group (27/359, 7.5%; P = 0.014). The proportion of smokers was significantly lower in the younger group (208/359, 57.9%) than in the older group (23/29, 79.3%; P = 0.024). Elderly patients had more pre-existing cardiopulmonary comorbidities than younger patients (P = 0.024). Gender, histopathological type, vascular invasion, pathological stage, and tumor diameter did not differ significantly between the groups. We omitted lymph-node resection in the older group (P < 0.001). Table 3 shows the region of EGFR mutation according to age group. Although the study group was small, there were no exon 20 mutations in the older group.

Relations between EGFR status and outcomes

Kaplan-Meier curve analysis showed that EGFR mutation status was significantly associated with survival (Figure 1). The 5-year overall survival rate was significantly higher in patients with EGFR mutations (90.2%) than in those with wild-type EGFR (75.2%) in the younger group (P < 0.001; Figure 1A). The 5-year overall survival rate was slightly, but not significantly higher

Table 2 Correlations between age group and clinicopathological features, including EGFR status

Characteristics	No. of patients			
	Total (n = 388)	≥80 years (n = 29, 7.5%)	<80 years (n = 359, 92.5%)	p[a]
Mean age, yr ± SD[b]	66.6 ± 10.0	82.6 ± 2.41	65.3 ± 9.29	<0.001
Gender				0.246
Male	228	20	208	
Female	160	9	151	
Histology				0.034
Adenocarcinoma	302	18	284	
others	86	11	75	
Biomarker				
EGFR wild type	203	22	181	0.008
EGFR mutation	185	7	178	
KRAS wild type	355	23	332	0.014
KRAS mutation	33	6	27	
Vascular invasion				
Ly -	314	26	288	0.214
Ly +	74	3	71	
V-	261	18	243	0.535
V+	127	11	116	
p-stage				0.080
I	293	18	275	
II / III	95	11	84	
T-factor				0.506
T1	197	13	184	
T2/3	191	16	175	
Tumor diameter (cm)	3.03 ± 1.43	3.00 ± 1.44	3.40 ± 1.24	0.629
N-factor				0.584
N0	322	23	299	
N1/2	66	6	60	
Operation				0.155
Limited resection (wedge/segmentectomy)	80	3	77	
Standard surgery (lobectomy, pneumonectomy)	308	26	282	
Lymph node resection				<0.001
ND0/1/sampling	109	25	156	
ND2	278	4	203	
Smoking				0.024
Non-smoker	157	6	151	
Smoker	231	23	208	
Pre-existing cardiopulmonary comorbidity	203	21	182	0.024

[a]$p < 0.05$ statistically significant.
[b]SD, standard deviation.
EGFR, epidermal growth factor receptor; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; ND, lymph node dissection.

in patients with EGFR mutations (100%) than in those with wild-type EGFR (66.2%) in the older group ($P = 0.226$; Figure 1B).

Discussion

In the present study, we first evaluated EGFR mutations in resected NSCLC tissue by LH-MSA. LH-MSA is a

Table 3 Region of EGFR mutation according to age group

	No. of patients		
EGFR mutations	Total	≥80 years	<80 years
Exon 19	73	3	70
Exon 20	13	0	13
Exon 21	97	4	93
Combined	2	0	2

highly sensitive polymerase chain reaction-based method. Sakuma et al. previously evaluated EGFR mutations by LH-MSA in our hospital. EGFR mutations were detected in 53.2% of NSCLCs and were significantly associated with adenocarcinoma, female sex, and no smoking history [14]. In the present study, we detected EGFR mutations in 47.7% of NSCLCs (Table 1). The presence of an EGFR mutation is closely linked to several clinicopathological factors, such as gender, smoking history, and pathological findings. Our results are consistent with those of recent studies reporting that the rate of EGFR mutations is higher among Asians (including Japanese), females, nonsmokers, and adenocarcinomas [14,15]. Although LH-MSA yet has not been generally performed, it is known to be a sensitive and low cost method in scanning the known gene mutation. Furthermore, we can treat many samples in a short time by LH-MSA. Nakajima et al. analyzed EGFR mutations using LH-MSA, and confirmed the results by direct sequencing. They concluded that LH-MSA has a high detection capability compared with direct sequencing [16]. Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology indicate that LH-MSA compares favourably with the other method [17].

We then studied the relations between EGFR status and clinicopathological factors according to age group (Table 2). Past report suggested the impact of age on EGFR mutation, and concluded that age was associated with EGFR mutation in lung cancer [18]. In this study, if we analyze the EGFR status using the median age of 66 years old as a cutoff, there is no difference between younger and elderly group. Next, we divided the cohort in every ten years old, and we found that the rate of EGFR mutation suddenly decreased in a group 80 years or older. Because aging of the population is a global problem, the average life-span older than 80 years old in Japan was worthy of mention to the world. Due to the above reasons, we thought that the age of 80 years old is turning point in consideration of gene profile change, and divided the patients into two groups at 80 years of age. The older group (≥80 years) of patients with NSCLC included significantly higher rates of non-adenocarcinoma, wild-type EGFR, KRAS mutations, and smokers. There was no difference between the older group and younger group in tumor size, T-factor, or pathological stage. Moreover, in Japan, females outlive males (males 79 years, females 86 years). Of the 29 elderly patients, 9 are females include 7 adenocarcinomas and 4 smokers. EGFR mutations were detected in 3 females. The 5-year overall survival rate was 100% regardless of EGFR mutation or wild type. When we examined the region of EGFR mutation according to age group (Table 3), no exon 20 mutations were found in the older group. Although our study group was small, our results suggest that EGFR mutation status might differ

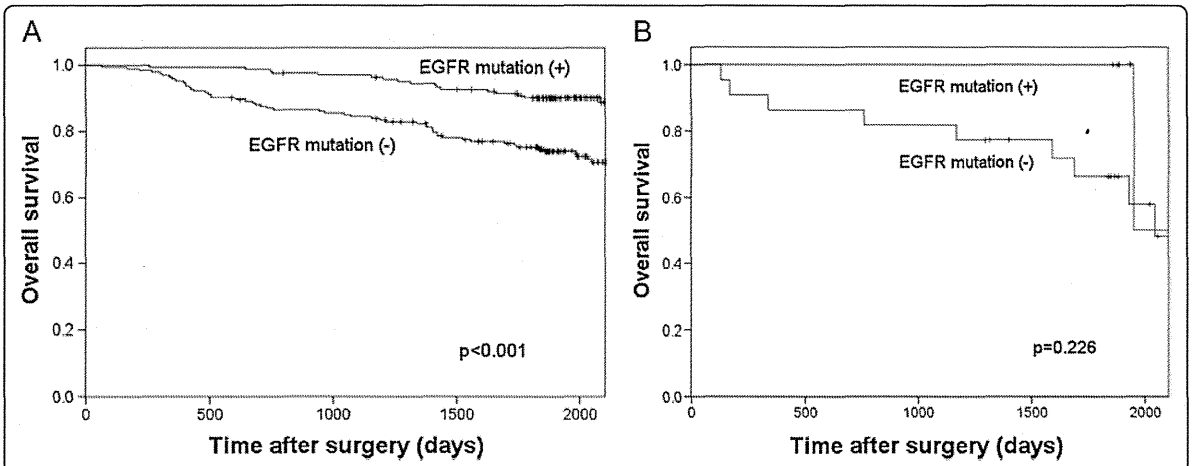

Figure 1 Relations between EGFR mutations and outcomes. Kaplan-Meier curve analysis showed that EGFR mutation status was significantly associated with survival. (A) The 5-year overall survival rate was higher in patients with EGFR mutations (90.2%) than in those with wild-type EGFR (75.2%) in the younger group ($P < 0.001$). (B) In the older group, the 5-year disease free survival rate was 100% among patients with EGFR mutations; however, the difference between the two groups was not significant.

between elderly and younger patients with NSCLCs. Given that smoking is one of the causes of the low rate of EGFR mutations in the older group, the rate of EGFR mutations may increase in the future owing to enlightenment movements such as the WHO Framework Convention on Tobacco Control [19]. Recently, smoking prevalence in Japan is decreasing generally. In particular, the drop of the smoking prevalence in young generation is remarkable. On the other hands, lung cancer mortality in Japan rises, probably it depends on the increase of the lung cancer in an elderly person who had been a smoker [20]. If the low rate of EGFR mutations is unrelated to smoking, it is very interesting that EGFR status might be affected by aging. Furthermore, it is reported that the response rate of gefitinib in elderly (aged 70 years or older) patients with advanced EGFR mutated NSCLC was 45.5%. EGFR-TKI is more effective than conventional chemotherapy in elderly patients, if we could pay attention to drug discontinuation and dose reduction due to age-related organ dysfunction [21]. On the other hand, NSCLC with exon 20 mutation is resistant for EGFR-TKI. Although our result has no statistical significance due to a small population of elderly patients, the lack of exon 20 mutations might be a characteristic of elderly patients. Large clinical trials are needed to investigate the relation between age group and the response to EGFR-TKI.

Finally, we assessed the relations between the EGFR status and outcomes. EGFR mutations were associated with significantly better survival than wild-type EGFR in the younger group (Figure 1). In the older group, however, the 5-year overall survival rate did not differ significantly according to EGFR mutations, and wild-type EGFR status and was 100% in patients with EGFR mutations. EGFR-TKIs are obviously beneficial in patients with advanced or recurrent NSCLC, but several studies have suggested that EGFR mutations might be an independent positive prognostic factor [22]. Our results suggest that elderly patients with NSCLC who have EGFR mutations are especially likely to have good outcomes after complete lung resection.

Conclusion

Our results suggest that the EGFR status of patients with NSCLC differs according to age group (>80 years vs. ≤80 years). EGFR mutation status might be a prognostic marker in elderly patients with completely resected NSCLC.

Additional file

Additional file 1: Table S1. PCR Primers and LH-G Probes Used for Detection of Mutations in EGFR

Competing interests
The authors declare that they have no competing interest.

Authors' contributions
Study design: TN, TY, YM and YD; sample collection: TN, HI, TI, KI, Shuji M, TK, HS, FO, KY, MT, and HN; experiments: TN, TY, YM, YD, and Shoichi M; data analysis: TN and TY; preparation of the manuscript: TN, TY, HN, and MM. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Mosapride Citrate Increases Postprandial Glucagon-Like Peptide-1, Insulin, and Gene Expression of Sweet Taste Receptors

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Abstract
Background and Aim Mosapride citrate—a prokinetic agent—improves hemoglobin A1c levels in diabetic patients; however, the underlying mechanism is unclear. We aimed to clarify this mechanism.
Methods Preprandial and postprandial (90 min after a meal) blood was obtained from 12 healthy men, and serum insulin and plasma active glucagon-like peptide-1 concentrations were measured. Measurements were also taken after the administration of 5 mg of mosapride citrate three times per day after every meal for 14 days. In addition, C57BL/6 mice were permitted free access to water containing 0.04 % domperidone (D group) or 0.02 % mosapride citrate (M group) for 2 weeks (four mice per group). T1r2 (taste receptor, type 1, member 2), T1r3, and Gnat3 (guanine nucleotide-binding protein, alpha transducing 3) mRNA expression levels of the stomach, duodenum, and proximal and mid-jejunum were evaluated.
Results In human subjects, postprandial plasma active glucagon-like peptide-1 and serum insulin concentrations after administration of mosapride citrate were significantly higher than those pre-administration (4.8 ± 2.2 pmol/L, 45.6 ± 41.6 l IU/mL, and 3.7 ± 1.2 pmol/L, 34.1 ± 28.4 l IU/mL, respectively). The mouse expression levels of T1r2 and Gnat3 in the proximal jejunum and mid-jejunum in the M group (4.1 ± 1.8-fold, 3.1 ± 1.6-fold, and 4.6 ± 0.8-fold, 3.1 ± 0.9-fold increases, respectively), were significantly higher than those of the control group.
Conclusions The administration of mosapride citrate for 2 weeks enhanced postprandial plasma active glucagon-

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like peptide-1 and serum insulin concentration and increased the expression of sweet taste receptors in the upper intestine.

Keywords Mosapride · Glucagon-like peptide-1 · Incretins · L cells · Taste receptors, type 1

Abbreviations

BMI	Body mass index
ER	Endoplasmic reticulum
GLP-1	Glucagon-like peptide-1
GNAT3	Guanine nucleotide-binding protein, alpha transducing 3
$t_{1/2}$	Half-life
HbA1c	Hemoglobin A1c
IP3	Inositol 1,4,5-triphosphate
IP3R3	Inositol 1,4,5-triphosphate receptor type 3
PLCb2	Phospholipase Cb2
RT-PCR	Reverse transcription PCR
T1R2	Taste receptor, type 1, member 2
T1R3	Taste receptor, type 1, member 3
TRPM5	Transient receptor potential channel 5
VGCC	Voltage-gated calcium channel

Introduction

Mosapride citrate is a 5-HT4 receptor agonist that acts as a prokinetic agent [1]. The mechanisms of mosapride citrate efficacy were suggested to involve an agonistic action on 5-HT4 receptors and enhancement of cholinergic excitatory neurotransmission [2]. This drug has been widely used in Japan, and its significance was again highlighted when it was found to be effective against functional gastrointestinal disorders in a large, randomized clinical trial [3]. The drug has pharmacological properties of reducing hemoglobin A1c (HbA1c) levels in patients with type 2 diabetes [4, 5]. Ueno et al. [4] demonstrated that mosapride citrate intake increases sugar utilization through enhanced insulin sensitivity in muscle, and Nam et al. [6] showed that the drug increased the mobilization of glucose transporters from intracellular pools in muscle; however, the causative mechanisms are unclear.

The major property of GLP-1 is insulin secretion from pancreatic beta cells. It is also well recognized that GLP-1 increases glucose uptake in muscles by enhancing insulin sensitivity [7]. GLP-1 is secreted from L cells on the mucosa of gastrointestinal tract [8, 9], and the secretion of GLP-1 from L cells has also been described to occur via the sweet receptor system [10–12]. Heterodimers of taste receptor type 1, member 2 and taste receptor type 1, member 3 (T1R2/T1R3)—a G-protein-coupled receptor located in taste buds that acts as a receptor for sweet tastants—exist in L cells of the intestinal tract mucosa [13, 14]. We hypothesized that the effect of mosapride citrate on glucose metabolism was GLP-1 mediated and caused by the upregulation of sweet taste receptor system components in L cells. In this study, we evaluated the effect of mosapride citrate on glucose metabolism—particularly the level of GLP-1—in healthy human volunteers, as well as the expression levels of taste receptors in the mouse gastrointestinal tract before and after mosapride citrate treatment.

Methods

Plasma Active GLP-1 and Serum Insulin Before and After 2-Week Mosapride Citrate Administration

Twelve healthy male volunteers without gastrointestinal symptoms or a history of abdominal surgery were enrolled in the study. This study was approved by the medical ethics board of the Graduate School of Medicine, Chiba University and performed at Chiba University Hospital in accordance with the Declaration of Helsinki. All of the subjects received clear explanations of the study and were provided written informed consent. The study protocol was registered in the UMIN Clinical Trial Registry (UMIN 000009704). Their mean age was 31.9 ± 7.8 years, mean height was 1.75 ± 0.06 m, mean weight was 70.3 ± 7.0 kg, and mean body mass index (BMI) was 23.2 ± 3.3 kg/m^2. The levels of serum insulin, plasma glucose, glucagon, active GLP-1 concentrations, HbA1c [National Glycohaemoglobin Standardisation Program (NGSP)], and blood chemistry in the preprandial and postprandial (90 min after the test meal) state were measured. All volunteers consumed the test meal, JANEF E460F18® that included creamed chicken, crackers, and pudding [460 kcal (1,927 kJ); protein, 18.0 g; fat, 18.0 g; carbohydrate 56.5 g; Kewpie Corp., Tokyo, Japan]. Blood was drawn into collection tubes that contain DPP-4 inhibitors (P700; Becton, Dickinson and Company, New Jersey, USA), mixed, and centrifuged at 1,200g for 15 min at 4 °C. Plasma samples were stored temporarily at - 20 °C prior to GLP-1 measurement. The plasma active GLP-1 concentration was measured using an ELISA kit (Millipore Corp., MA, USA) at SRL, Inc. (Tokyo, Japan) [15]; the other components were also analyzed by SRL, Inc. After administration of 5 mg of mosapride citrate orally three times per day for 14 days, blood samples were again drawn for analysis before and after subjects consumed the test meal. The last dose of mosapride citrate was also 5 mg and was administered in the evening of the day before the blood sampling.

Expression Levels of Sweet Taste Receptors in the Mouse Gastrointestinal Tract

Male C57BL/6 mice aged 9 weeks (CLEA Japan Inc., Tokyo, Japan) had free access to water and a standard rodent diet. This study was performed in accordance with the guidelines of the Animal Ethics Committee of the Graduate School of Medicine at Chiba University. The freely accessible water contained 0.04 % domperidone (D group) or 0.02 % mosapride citrate (M group) for 2 weeks (four mice per group). We determined the administered domperidone dosage to the mice as fairly low dosage that could be administered without any adverse effects (e.g., liver and renal function disorders) [16]. The administered mosapride citrate dosage was half the quantity of the domperidone dosage because the regular mosapride citrate dosage in humans is half the quantity of the domperidone dosage. Mice were fasted overnight and killed. Administration of water containing mosapride citrate or domperidone was discontinued, and plain water was administered instead 12 h before sampling tissues. The stomach, duodenum, proximal jejunum (4 cm from the ligament of Treitz), and mid-jejunum (8 cm from the end of the proximal jejunum) were collected (Table 1).

The gastrointestinal samples were disrupted in a glass mortar and pestle and homogenized in TRIzol® reagent (Life Technologies Corp., CA, USA). Total cellular RNA was isolated using the TRIzol® Plus RNA Purification Kit (Life Technologies Corp., CA, USA), and mouse RNA was reverse transcribed into cDNA by reverse transcription PCR (RT-PCR) using the SuperScript™ III First Strand Synthesis SuperMix (Life Technologies Corp., CA, USA) according to the manufacturer's instructions. A DNase step, using the DNase I (Life Technologies Corp., CA, USA), was incorporated into the RNA isolation protocol to prevent any trace genomic DNA contamination. The cDNA was then purified using a QIAquick PCR Purification Kit (Qiagen Inc., CA, USA). The cDNA (equivalent to 50 ng of RNA) was amplified by real-time RT-PCR using the StepOne™ sequence detection system and StepOne™ Software version 2.0.2 (Life Technologies, CA, USA). Mouse primers were purchased as TaqMan® gene expression assays [Mm00499716_m1 for T1r2, Mm00473459_g1 for T1r3, Mm01165313_m1 for Gnat3, Mm00498453_m1 for Trpm5, Mm00801714_m1 for Gcg, and Mm99999915_g1 for glyceraldehyde phosphate dehydrogenize (Gapdh); Life Technologies, CA, USA]. The cycling conditions were one cycle at 95 °C for 20 s, followed by 50 cycles of 95 °C for 1 s, and 60 °C for 20 s (using fast ramp speed and fast reagents). The Gapdh primers were used as a positive control to normalize the results obtained with the taste gene-specific primers. Each sample was examined in triplicate. Relative

Table 1 Ct values for each gene according to organs and drugs

Ct value	Stomach			Duodenum			Proximal jejunum			Mid-jejunum		
	C	M	D	C	M	D	C	M	D	C	M	D
Gapdh	25.9 ± 0.2	24.7 ± 0.3	25.0 ± 0.4	24.7 ± 0.6	24.6 ± 0.5	23.9 ± 0.1	24.8 ± 0.7	24.8 ± 0.9	23.9 ± 0.6	24.0 ± 0.5	23.5 ± 0.5	23.6 ± 0.2
T1r2	*	*	*	40.4 ± 0.7	37.8 ± 0.7	39.6 ± 0.4	39.0 ± 0.4	36.8 ± 0.8	38.9 ± 0.6	39.1 ± 0.3	37.2 ± 0.9	39.3 ± 1.2
T1r3	36.1 ± 0.5	34.9 ± 1.1	37.2 ± 0.5	33.9 ± 0.6	34.2 ± 0.9	36.4 ± 0.4	34.3 ± 0.8	33.6 ± 0.7	35.8 ± 0.4	33.5 ± 0.4	32.5 ± 0.5	36.0 ± 0.4
Gnat3	33.5 ± 0.4	30.5 ± 0.5	35.1 ± 0.3	37.3 ± 1.1	34.9 ± 1.2	39.3 ± 0.2	37.1 ± 0.6	34.4 ± 0.9	39.1 ± 0.5	35.2 ± 0.7	33.2 ± 0.4	37.7 ± 0.2
Trpm5	35.5 ± 0.2	34.8 ± 0.5	35.9 ± 0.6	33.9 ± 0.7	34.2 ± 0.8	34.2 ± 0.2	33.7 ± 0.6	33.6 ± 0.9	33.7 ± 0.5	32.7 ± 0.6	32.1 ± 0.3	33.2 ± 0.4
Gcg	33.8 ± 0.6	34.3 ± 0.4	33.1 ± 0.5	31.6 ± 0.4	32.8 ± 0.5	31.7 ± 0.4	30.5 ± 0.4	31.7 ± 0.8	30.2 ± 0.6	29.8 ± 0.3	30.0 ± 0.8	29.4 ± 0.2

Data are presented as mean ± SEM

C control, M mosapride citrate, D domperidone, and GLP-1 glucagon-like peptide-1

* The expression of T1r2 was absent in the stomach

gene expression levels across conditions were computed using the 2^{-DDC_T} method described previously [17]. The expression level of each gene in the D group and M group was expressed as a ratio with respect to the control group (C group; four mice per group).

Statistical Analysis

All results are presented as mean ± SD. The paired t test and the Mann–Whitney U test were used to analyze the data. A p value of < 0.05 was considered statistically significant. All statistical analyzes were performed using SPSS statistics, version 20, software package (SPSS Inc., IBM Japan, Tokyo, Japan).

Results

Change in Plasma Active GLP-1 and Serum Insulin Before and After Mosapride Citrate Administration

None of the subjects developed any side effects or a change in body weight (data not shown). There was no significant change in HbA1c before and after administration of mosapride citrate [5.13 ± 0.18 % (32.6 ± 1.9 mmol/mol) vs. 5.10 ± 0.17 % (32.2 ± 1.9 mmol/mol); p = 0.22, paired t test]. Values of < 2.0 pmol/L were treated as 1.9 pmol/L (the lower limit). In the preprandial state, the levels of plasma active GLP-1 before and after mosapride citrate administration were 2.1 ± 0.4 and 1.9 ± 0.0 pmol/L, respectively, which showed no significant difference (p = 0.07, paired t test; Fig. 1a). In the postprandial state, the levels of plasma active GLP-1 before and after mosapride citrate administration were 3.7 ± 1.2 and 4.8 ± 2.2 pmol/L, respectively, which were significantly different (p = 0.026, paired t test; Fig. 1b).

Additionally, the postprandial serum insulin concentration after mosapride citrate administration was significantly higher than that before administration (45.6 ± 41.6 and 34.1 ± 28.4 l IU/mL, respectively; p = 0.030, paired t test), whereas there was no significant change in preprandial serum insulin concentration before (6.2 ± 3.5 pmol/L) and after mosapride citrate administration (5.4 ± 2.6 pmol/L; p = 0.29, paired t test; Fig. 2). In contrast, the preprandial plasma glucagon concentration was significantly lower before mosapride citrate administration (68.3 ± 18.4 pg/mL) compared with that after administration (77.6 ± 13.7 pg/mL; p = 0.013, paired t test), and the postprandial plasma glucagon concentration after administration (74.4 ± 18.5 pmol/L) appeared lower than that before administration (81.6 ± 17.5 pmol/L; p = 0.20, paired t test), although the difference was not statistically significant. There was no difference in preprandial and postprandial plasma glucose concentration before and after administration (data not shown). Two patients had BMI values [25 kg/m^2; however, their preprandial and postprandial plasma glucose and serum insulin levels were within the normal ranges.

Expression Levels of Sweet Taste Receptors in the Mouse Gastrointestinal Tract After 2-Week Mosapride Citrate Administration

There was no significant change in body weight and no adverse reaction induced by mosapride citrate in any of the mice. The expression levels of T1r2, T1r3, Gnat3, Trpm5, and Gcg in mice were analyzed, and the expression ratios are presented in comparison with those of mice who did not undergo drug administration. The expression levels of T1r2 are shown in Fig. 3a. The expression of T1r2 was absent in the stomach, which is consistent with a previous report [18]. The expression of T1r2 did not change significantly in the D group. However, the expression of T1r2 in the proximal jejunum and mid-jejunum was significantly higher in the M group (4.1 ± 1.8- and 3.1 ± 1.6-fold increases, respectively; p = 0.021 and 0.021, respectively, Mann–Whitney U test). The expression levels of T1r3 are presented in Fig. 3b. Whereas the expression of T1r3 in any digestive tract region in the D group was significantly lower than that in the control group (0.1-fold–0.2-fold increase; all p = 0.021, Mann–Whitney U test), the expression of T1r3 in the proximal jejunum and mid-jejunum tended to be higher in the M group than in the control group. The expression levels of Gnat3 are shown in Fig. 3c. The expression of Gnat3 in the stomach, proximal jejunum, and mid-jejunum in the M group was also significantly higher than in the control group (3.2 ± 0.7-, 4.6 ± 0.8-, and 3.1 ± 0.9-fold increases, respectively; p = 0.021, 0.034, and 0.034, respectively, Mann–Whitney U test), whereas the expression in the stomach, duodenum, and proximal jejunum was significantly lower in the D group (0.1-fold–0.2-fold increase; all p = 0.021, Mann–Whitney U test). There was no trend of increased expression of Trpm5 or Gcg in the M group (Fig. 3d, e).

Discussion

The administration of a conventional dose of mosapride citrate increased the postprandial concentration of plasma active GLP-1 and serum insulin in healthy volunteers in the current study. Gastric dysfunction and constipation are associated with diabetes at a high frequency, and mosapride citrate improves these clinical conditions [19–21].

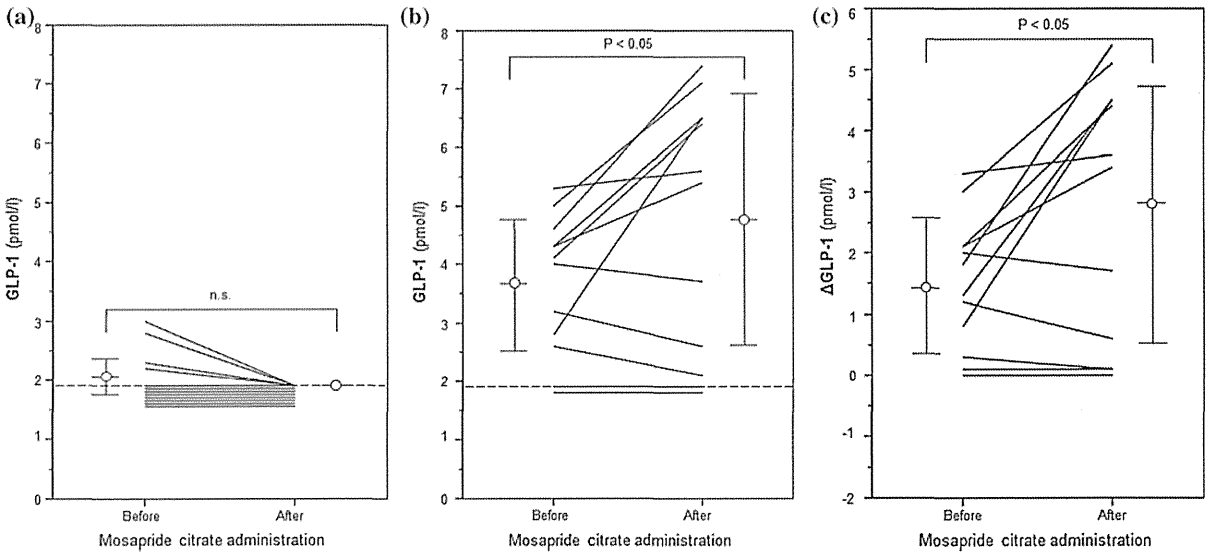

Fig. 1 Comparison between concentrations of plasma active GLP-1 before and after administration of 5 mg of mosapride citrate orally three times per day for 2 weeks in male human subjects (n = 12). *P\ 0.05 determined by paired t test. Data are presented as mean ± SD. Values of \ 2.0 pmol/L were treated as 1.9 pmol/L. a Comparison between concentrations of preprandial plasma active GLP-1 at pre-administration (2.1 ± 0.4 pmol/L) and post-administration (1.9 ± 0.0 pmol/L; p = 0.07, paired t test). b Comparison between concentrations of postprandial plasma active GLP-1 at pre-administration (3.7 ± 1.2 pmol/L) and post-administration (4.8 ± 2.2 pmol/L; p = 0.07, paired t test). c Comparison between DGLP-1 (the difference between preprandial and postprandial plasma active GLP-1) at pre-administration (1.5 ± 1.1 pmol/L) and post-administration (2.8 ± 2.1 pmol/L; p = 0.03, paired t test)

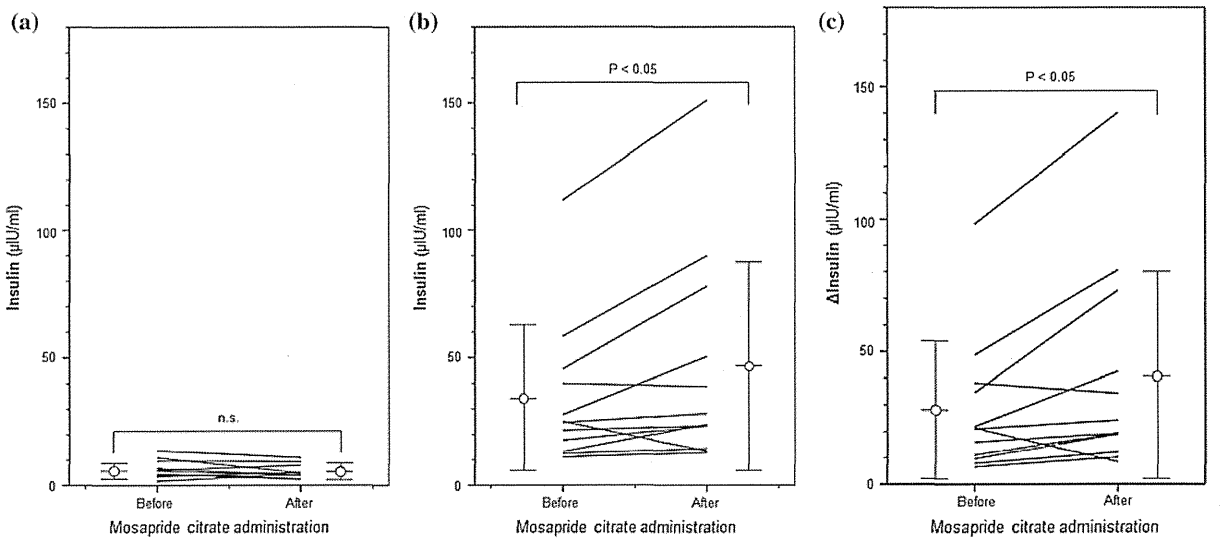

Fig. 2 Comparison between concentrations of serum insulin before and after administration of 5-mg mosapride citrate orally three times per day for 2 weeks in male human subjects (n = 12). P\ 0.05, as determined by paired t test. Data are presented as mean ± SD. a Comparison between concentrations of preprandial serum insulin at pre-administration (6.2 ± 3.5 l IU/mL) and post-administration (5.4 ± 2.6 l IU/mL; p = 0.29, paired t test). b Comparison between concentrations of postprandial serum insulin at pre-administration (34.1 ± 28.4 l IU/mL) and post-administration (45.6 ± 41.6 l IU/mL; p\ 0.05, paired t test). c Comparison between Dinsulin (the difference between preprandial and postprandial serum insulin) at pre-administration (27.9 ± 25.7 l IU/mL) and post-administration (40.3 ± 39.3 l IU/mL; p = 0.02, paired t test)

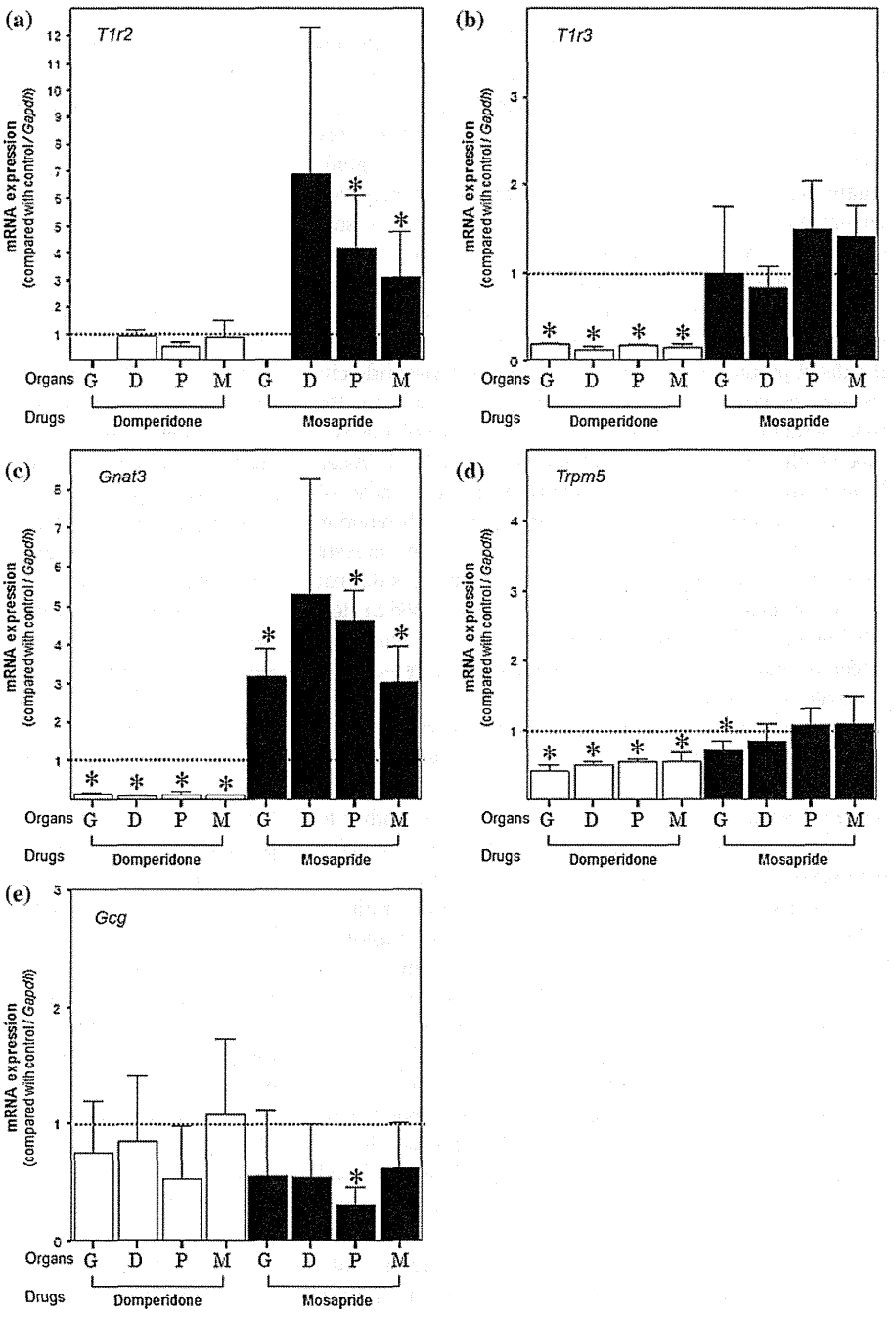

Fig. 3 Gene expression of taste molecules in the fasting male mice digestive tract which was permitted free access to water containing 0.04 % domperidone or 0.02 % mosapride citrate for 2 weeks compared with an unmedicated control group. The gene expression of T1r2 (a), T1r3 (b), Gnat3 (c), Trpm5 (d), and Gcg (e) is visualized in the stomach (G), duodenum (D), proximal jejunum (P), and mid-jejunum (M) of mice (n = 4, in each group). *P\ 0.05, as determined by Wilcoxon signed-rank test. Data are presented as mean ± SD

The administration of mosapride citrate can improve not only gastrointestinal symptoms in diabetic patients, but also glucose metabolism, if the effects on plasma active GLP-1 and serum insulin—as noted in the current study—are observed in patients with diabetes. Monosodium glutamate [22], a-glucosidase inhibitors [23, 24], and IL-6 [25] were reported to increase postprandial plasma GLP-1 concentrations. Metformin was observed to cause higher meal-related incremental changes in GLP-1, and in cases where metformin was combined with a DPP-4 inhibitor, the increase in concentrations of active GLP-1 was more than that observed with DPP-4 inhibitor alone [26]. However, the mechanisms of GLP-1 upregulation by the use of these drugs remain unclear.

Aoki et al. [27] reported that a single administration of 20 mg of mosapride citrate 2 h before breakfast significantly reduced postprandial blood glucose and increased postprandial plasma active GLP-1. Based on the findings that the GLP-1 concentration after a meal test is reportedly increased after a sleeve gastrectomy [28] and is significantly higher in patients after gastrectomy compared with patients without gastrectomy [29]; Aoki et al. [27] suggested that mosapride citrate—a gastrokinetic agent—facilitates the entry of a large amount of food into the small intestine and thus increases the plasma active GLP-1 concentration. Because the predominant location of L cells is the distal portion of the gut [30], 20 mg of mosapride citrate—a comparatively high dosage—may lead to the transportation of nutrients to the distal portion of gut at a much earlier phase and increase secretion of GLP-1. When healthy male subjects are administered a single dose of 5 mg of mosapride citrate, the apparent plasma elimination half-life ($t_{1/2}$) is 2.0 ± 0.2 h [31]. Thus, in the current study of both humans and mice, the study subjects did not receive mosapride citrate on the day they consumed the test meal and had blood samples drawn. As a result, the direct effect of mosapride citrate—a prokinetic agent—should dissipate by the time of the test. However, the gene expression of sweet taste receptors in the gastrointestinal tract was increased in our mouse study, suggesting that the changes in plasma active GLP-1 may not be related to the temporal enhancement of gastric emptying, but rather to the increased gene expression of sweet taste receptors expressed on L cells in the gastrointestinal tract.

In the taste cell, the a-subunit of the G-protein gustducin mediates the downstream signaling of sweet taste through T1R2/T1R3. This downstream signaling is triggered by a-gustducin (coded by Gnat3) [32] and transmitted through transient receptor potential channel 5 (TRPM5) [33], and the voltage-gated calcium channel (VGCC). This sweet taste receptor signaling system is suggested to function in the gastrointestinal tract according to a large number of previous studies [13, 18, 32, 34–37]. GLP-1 which is liberated from its precursor proglucagon (coded by GCG) and is secreted from L cell is via this sweet taste receptor system [10–12].

The administration of mosapride citrate significantly increased T1r2 and Gnat3 mRNA expression and potentially increased T1r3 mRNA expression, which may result in increased activity of sweet taste receptors and downstream signaling after sweet tastants bind to the sweet taste receptors. Thus, we speculated that the improvement of glucose metabolism by mosapride citrate may be mediated through the sweet taste receptor and its downstream signaling in the postprandial phase. On the other hand, the expression of T1r3 appeared to increase, but the result was not significant, and the expression of Trpm5 and Gcg mRNA did not increase. We thought this result of T1r3 may occur because T1R3 is not only a component of the receptor for sweet tastants but also for umami tastants (heterodimer of taste receptor, type 1, member 1 and taste receptor, type 1, member 3) [38]. However, the reason underlying the unchanged expression of Trpm5 and Gcg mRNA was unclear, but we speculate that this signaling system may not require increased expression of these components to induce greater GLP-1 secretion from L cells.

The release of GLP-1 secretion primarily from L cells is believed to occur immediately upon exposure to ingested nutrients (i.e., L cells in the upper intestine) [39–41]. In the current study, the expression of sweet taste receptors in the duodenum, proximal jejunum, and mid-jejunum—very upper intestine—was increased by the administration of mosapride citrate but not by domperidone, which is also a prokinetic agent. This result corresponds to the observation that only mosapride citrate has the property of enhancing insulin sensitivity and glucose utilization in muscle, unlike domperidone and metoclopramide [4, 42], whereas domperidone is effective for diabetic gastropathy as is mosapride citrate [5, 43, 44]. This enhancement of insulin sensitivity in muscle may be caused by GLP-1 which was increased by the administration of mosapride citrate. However, the mechanism by which the administration of mosapride citrate increases the expression of sweet taste receptors is unclear. The differences in gene expression between groups treated with mosapride citrate and domperidone might be due to the specific mechanism of action of each drug. That is, mosapride citrate is a 5-HT4 receptor agonist, and domperidone is a dopamine receptor antagonist. To our knowledge, there are no studies reporting that domperidone exacerbates diabetes. Nakagawa et al. [45] reported that the sweet taste receptor T1R2/T1R3 is expressed in mouse pancreatic islet beta cells, and they mentioned the possibility that sweet tastants stimulate glucose-induced insulin secretion. In the current study, there is also the possibility of an increase in the expression of sweet taste receptors in pancreatic beta cells that may directly cause increased secretion of insulin in addition to that stimulated by increased GLP-1 secretion from the intestine.

In the present study, the administration of mosapride citrate for 2 weeks enhanced the postprandial plasma active GLP-1 and serum insulin concentrations and increased the expression of sweet taste receptors in the upper intestine. In addition, mosapride citrate may increase the expression of sweet taste receptors in the gastrointestinal tract which may lead to increased sweet tastants binding to the receptors, and a resulting high abundance of postprandial plasma active GLP-1. In the current study, we analyzed human blood samples and mucosa samples from the gastrointestinal tract of mice. We would have preferred

to perform both studies in human subjects. However, since peroral double-balloon enteroscopy is an invasive procedure, this issue remains to be addressed. Additionally, what is important is that the activation of the sweet taste receptor is necessary but not sufficient to secrete GLP-1 from L cell. First, compared with the control group, oral ingestion of artificial sweetener augmented GLP-1 release by more than one-third after a load of oral glucose in healthy humans [46]. This suggests a potential synergy between artificial sweeteners and glucose in stimulating GLP-1 secretion. On the other hand, oral ingestion of sugar by rats increased the secretion of GLP-1 in the digestive tract, but the ingestion of artificial sweetener alone did not increase the secretion of GLP-1 [47]. Similarly, in healthy humans, the level of GLP-1 did not increase after the intake of artificial sweetener alone [46, 48]. Furthermore, the sweet taste receptor antagonist, lactisole, or siRNA against a-gustducin attenuates glucose-stimulated GLP-1 secretion, which suggests that activation of the sweet taste receptor is required for the stimulation of secretion of GLP-1 from L cells [49]. To summarize, activation of the sweet taste receptor is a necessary, but not a sufficient condition for the secretion of GLP-1 from L cells. A causal relationship between the increased expression of sweet taste receptors and the increased levels of postprandial plasma active GLP-1 in the current study is not completely certain from past scientific knowledge by reference to previous studies. Furthermore, we think that it is important to determine the postprandial plasma active GLP-1 and serum insulin levels of patients with diabetes after mosapride citrate administration. We believe that further study is needed to evaluate the benefit of mosapride citrate for patients with diabetes.

Conflict of interest None.

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Surgical outcomes of non-small-cell lung carcinoma in patients previously treated for gastric cancer

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Abstract

OBJECTIVES: Although the incidence of non-small-cell lung cancer (NSCLC) as a second malignancy is increasing, the prognosis remains controversial. Therefore, the present study aimed to determine the prognosis of patients with NSCLC who had previously been treated for gastric cancer (PGC).

METHODS: The clinicopathological records of patients who underwent complete surgical resection for NSCLC in three institutions from 2000 to 2013 were retrospectively investigated.

RESULTS: A total of 4651 patients were eligible for this study: 100 (2.1%) were patients with PGC and 4551 (97.9%) were patients with NSCLC who had not previously been treated for gastric cancer (NGC). The populations of older patients ($P < 0.001$), males ($P < 0.001$), limited resection for NSCLC ($P = 0.015$) and non-adenocarcinoma ($P = 0.024$) were significantly higher in the PGC, than in the NGC group. Overall survival did not significantly differ between the PGC and NGC groups (76.4 vs 74.5% $P = 0.82$). Multivariate analysis revealed that more advanced age, male sex, higher serum carcinoembryonic antigen levels, more advanced clinical stage of lung cancer and nonadenocarcinoma were independent factors for a poor prognosis, whereas a history of gastric cancer was not. None of the factors associated with gastric cancer affected the survival of patients with PGC.

CONCLUSIONS: After surgical treatment for lung cancer, a history of gastric cancer treatment had low impact on survival and no factors related to gastric cancer influence the outcomes. Curative surgery for NSCLC should be recommended when previously treated gastric cancer is well controlled.

Keywords: Gastric cancer • Non-small-cell lung cancer • Prognosis • Second primary tumours

INTRODUCTION

The incidence of non-small-cell lung cancer (NSCLC) as a second malignancy is increasing [1–3] because the survival of patients with many other types of malignancy has improved. Treatment for NSCLC as a second malignancy was determined considering the general status and the type or prognosis of previous cancer. However, whether surgical resection was the appropriate treatment for these patients is unclear because previous cancer might have affected the surgical outcomes of lung cancer. Although the prognosis of NSCLC as a second primary malignancy has been studied in detail [1–8], it remains controversial. One possible reason for this is population heterogeneity in the previous studies due to a variety of previous malignancies. Little survival data are available about patients with NSCLC and prior specific malignancies have been reported. We highlighted gastric cancer because it is a common malignancy [9] and it could affect the survival rates of lung cancer because it is associated with higher fatality rates [10]. The incidence rate of a second primary tumour developing after gastric cancer is 4.2% and such second primary tumours comprise lung cancer in 28.4% of these patients [11]. A few studies [1, 11] have addressed the clinical behaviour and survival of small cohorts of patients with NSCLC who had previously been treated for gastric cancer (PGC). Thus, the clinicopathological characteristics and the prognosis of patients with PGC remain unknown.

Here, we evaluated the clinicopathological features of PGC, assessed the prognosis of PGC and determined whether a history of gastric cancer influences the prognosis of NSCLC.

MATERIALS AND METHODS

Patient population

This study included 4782 patients who underwent complete surgical NSCLC resection at Hiroshima University Hospital (Hiroshima, Japan), the National Cancer Center Hospital East (Chiba, Japan) and Juntendo University Hospital (Tokyo, Japan) between January 2000 and March 2013. We reviewed medical records and obtained clinicopathological information about gastric cancer and lung cancer for each patient. The surgical indications for primary lung cancer were discussed by each institutional cancer board. Sublobar resection was performed in cases of complete resection of the disease with appropriate surgical margins for small peripheral tumours. If lymph node metastasis was confirmed on an intraoperative frozen section of any lymph node, the procedure was converted to standard lobectomy. All stages were reclassified according the TNM classification of Malignant Tumors, 7th Edition [12]. The Institutional Review Boards at the participating hospitals approved the study and waived the requirement for the provision of written informed consent by individual patients.

Patients with NSCLC were assigned to groups according to whether they had been previously treated for gastric cancer (PGC) or not (NGC). Patients were excluded if they had incompletely resected NSCLC, missing information about treatment dates or stage of gastric cancer, stage IV gastric cancer and/or gastric cancer that was detected after surgery for lung cancer (Fig. 1). Data from the remaining 4651 patients were retrospectively analysed.

Pathological studies

Sections were fixed with 10% formalin and embedded in paraffin. Consecutive 4-μm sections were pathologically assessed using microscopy. Histological type was determined by staining with haematoxylin-eosin (H-E) and if the findings were inconclusive, the sections were immunohistochemically stained. Whether tumours were histologically second primary tumours or metastases was determined by pathologists from each institution based on immunohistochemical staining for TTF-1, CK7, CK20 SPA or Napsin A.

Statistical analysis

Data were statistically analysed using EZR (Saitama Medical Centre, Jichi Medical University; Kanda, 2012), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). Summarized data are presented as numbers or as means ± standard deviation unless otherwise stated. Categorical and continuous variables were compared using the χ^2 test and an unpaired *t*-test, respectively. Overall survival (OS) was defined as the interval between the date of lung surgery and that of death or the last follow-up visit. OS curves were calculated using the Kaplan-Meier method and survival differences among patients were assessed using the log-rank test. OS was assessed by univariate and multivariate analyses using the Cox proportional hazards model. A *P*-value of <0.05 was considered statistically significant.

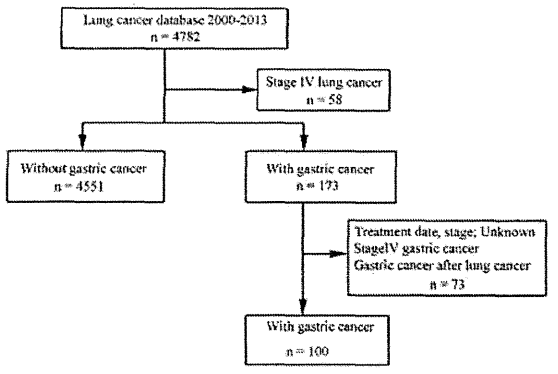

Figure 1: Flow of study cohort.

RESULTS

Clinical outcomes of patients with non-small-cell lung cancer who had previously been treated for gastric cancer

We assigned 100 (2.1%) and 4551 (97.9%) patients into PGC and NGC groups, respectively. The median follow-up duration was 62.3 months. A total of 952 patients died (PGC, $n = 16$; NGC, $n = 936$). The proportions of patients who were older (70.9 ± 7.6 vs 66.2 ± 9.7%, $P < 0.001$) and male (85 vs 61%, $P < 0.001$), and had limited resection of NSCLC (24 vs 15%, $P = 0.015$) and nonadenocarcinoma were significantly higher (39 vs 28%, $P = 0.024$) in the PGC, than in the NGC group. Serum CEA levels, clinical and pathological stages of lung cancer and adjuvant therapy for lung cancer did not significantly differ between the groups (Table 1).

Most patients in the PGC group had early-stage gastric cancer (78% had Stage I), and had been surgically treated for gastric cancer (73%). Only 3 patients had recurrent gastric cancer. Sixteen (16%) patients died (lung cancer, $n = 1$; gastric cancer, $n = 9$; other diseases, $n = 6$; Table 2). The median interval between gastric cancer and lung cancer was 3.2 (range 0–21.1) years. Thirty-two (41%) patients had undergone surgical lung cancer resection within 2 years of treatment for Stage I gastric cancer, and 31 (40%) had undergone such surgery over 5 years after treatment for gastric cancer (Fig. 2A). On the other hand, 7 (32%) and 8 (36%) patients underwent surgical resection for lung cancer within 2 and over 5 years after treatment for Stage II + III gastric cancer (Fig. 2B).

Prognosis of patients with non-small-cell lung cancer according to a history of treatment for gastric cancer

The 5-year OS rates did not significantly differ between the PGC and NGC groups (76.4 vs 74.5%, $P = 0.82$; Fig. 3), or between those in the two groups with Stages I and II + III gastric cancer (74.4 vs 74.5%, $P = 0.83$ and 83.0 vs 74.5%, $P = 0.93$). Univariate analysis of predictive factors for OS did not uncover a significant association with a history of gastric cancer (Table 3). Multivariate Cox analysis identified more advanced age, male sex, higher serum CEA levels and a higher clinical stage (II + III) of NSCLC as independent prognostic factors for poor OS, but not a history of gastric cancer [hazard ratio (HR) 1.17; 95% confidence interval (CI) 0.71–1.92; $P = 0.528$] (Table 3). We also examined predictive factors for OS in patients with PGC. Multivariate analysis did not associate OS with any of the factors related to gastric cancer, namely, pathological stage, method of treatment, adjuvant therapy and interval between gastric cancer and lung cancer (Table 4). Furthermore,

Table 1: Patients' characteristics

	All, $n = 4651$	PGC, $n = 100$ (%)	NGC, $n = 4551$ (%)	P-value
Age (years), means ± SD	66.3 ± 9.7	70.9 ± 7.6	66.2 ± 9.7	<0.001
Sex				
Male	2880	85 (85)	2795 (61)	<0.001
Female	1771	15 (15)	1756 (39)	
CEA (ng/ml), means ± SD	8.9 ± 65.1	9.6 ± 28.6	8.9 ± 65.7	0.912
cStage of lung cancer				
I	3678	80 (80)	3598 (79)	0.294
II	614	16 (16)	598 (13)	
III	359	4 (4)	355 (8)	
Surgical procedure				
Limited	694	24 (24)	670 (15)	0.015
Standard	3957	76 (76)	3881 (85)	
Histology				
Adenocarcinoma	3325	61 (61)	3264 (72)	0.024
Nonadenocarcinoma	1326	39 (39)	1287 (28)	
pStage of lung cancer				
I	3195	70 (70)	3125 (69)	0.725
II	711	17 (17)	694 (15)	
III	745	13 (13)	732 (16)	
Adjuvant therapy for lung cancer				
Yes	902	12 (12)	661 (15)	0.566
No	749	88 (88)	3890 (85)	

CEA: carcinoembryonic antigen; cStage: clinical stage; NGC: NSCLC who had not previously been treated for gastric cancer; PGC: NSCLC who had previously been treated for gastric cancer; pStage: pathological stage; SD: standard deviation.

Table 2: Clinicopathological characteristics of patients with previous gastric cancer

Factors	$n = 100$
pStage of gastric cancer	
I	78
II	13
III	9
Treatment methods for gastric cancer	
Endoscopic	27
Surgery	73
Interval between gastric cancer and lung cancer (years)	
≤5	61
>5	39
Adjuvant therapy for gastric cancer	
Yes	15
No	81
Recurrent gastric cancer	
Yes	3
No	97
Cause of death	
Lung cancer	9
Gastric cancer	1
Other	6

pStage: pathological stage.

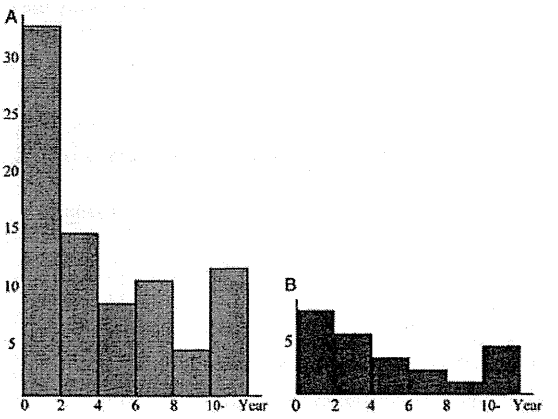

Figure 2: Number of patients based on interval from gastric cancer to lung cancer in the PGC group. Stage I (A) and Stage II/III (B) gastric cancer ($n = 78$ and $n = 22$, respectively). PGC: NSCLC who had previously been treated for gastric cancer.

OS did not significantly differ among intervals between gastric cancer and lung cancer (Table 5).

DISCUSSION

The present study found a higher ratio of older and male patients in the PGC, than in the NGC group and similar prognoses between the two groups after complete surgical resection of lung cancer (5-year OS: 76.4 vs 74.5%; $P = 0.82$). Furthermore, multivariate analysis showed that a history of gastric cancer had low impact on the prognosis of patients after complete NSCLC resection.

Very few reports have described the survival of patients with PGC [1, 11]. Ikeda *et al.* [11] have shown that the prognosis of patients with gastric cancer and a second primary cancer is more negatively influenced by the second primary tumour than by the primary gastric cancer. Our findings were consistent with these results. Although lung cancer accounted for a high ratio of second primary cancers among patients with gastric cancer, the study by Ikeda *et al.* included many types of malignancies such as

colorectal, liver and oesophageal cancers, as well as other malignancies including lung cancers. On the other hand, Pages *et al.* [1] described particularly low OS rates among patients with NSCLC and a history of upper gastrointestinal malignancies. However, these studies included small sample cohorts and the prognosis of NSCLC with previous gastric cancer has remained controversial.

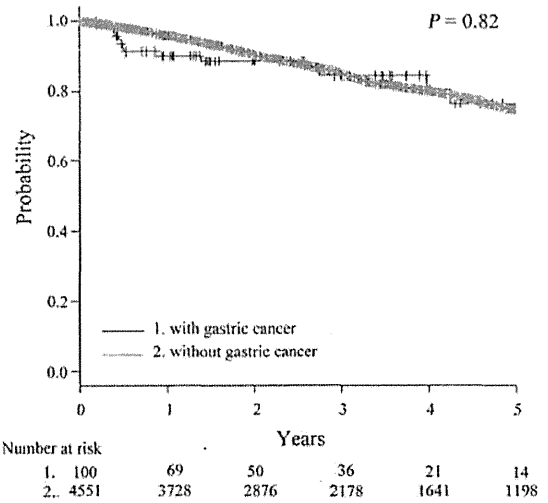

Figure 3: Kaplan–Meier overall survival curves according to history of gastric cancer. Five-year OS do not significantly differ between PGC and NGC (76.4 vs 74.0%, $P = 0.82$). OS: overall survival; PGC: NSCLC who had previously been treated for gastric cancer; NGC: NSCLC who had not previously been treated for gastric cancer.

The present multivariate analysis found that having had gastric cancer had low impact on the survival of patients with NSCLC when gastric cancer was curatively treated. Our multivariate analysis showed that patients without previous gastric cancer had slightly poorer outcomes than patients with previous gastric cancer (HR 1.17; without versus with previous gastric cancer), for unknown reasons. Variables that were not included in the analysis might have been involved. In addition, multivariate analysis did not uncover any factors related to gastric cancer that were associated with OS in the PGC group and the findings of multivariate analysis were similar in both the PGC and the NGC groups (data not shown). Thus, if gastric cancer was considered controlled, then it appeared not to influence prognosis after surgical resection of lung cancer. Although the criteria for controllable gastric cancer were not defined herein, Stage I gastric cancer was considered controllable. The reason is that OS did not significantly differ between patients in the PGC group with Stage I gastric cancer and the NGC group. The results were similar for Stage II + III gastric cancer, but these findings should be carefully considered in light of the following. Firstly, only 22 patients had Stage II + III gastric cancer. Since survival rates were worse for patients with advanced than early-stage gastric cancer, a second cancer might occur less frequently in those with Stage II + III than Stage I gastric cancer because the follow-up term is short. Secondly, about 40% of surgical resections to treat lung cancer in the PGC group with Stage I gastric cancer were performed within 2 years after gastric cancer treatment. On the other hand, surgical resection to treat lung cancer in the PGC group with Stage II + III gastric cancer might be avoided even if

Table 3: Univariate and multivariate Cox regression analysis of OS of all patients

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (≥75 vs <75 years)	1.79	1.55–2.07	<0.001	1.79	1.54–2.07	<0.001
Sex (male versus female)	2.17	1.86–2.52	<0.001	1.56	1.33–1.84	<0.001
CEA (>5.0 vs ≤5.0 ng/ml)	2.10	1.85–2.39	<0.001	1.71	1.50–1.95	<0.001
cStage of lung cancer (II + III versus I)	3.90	3.42–4.44	<0.001	2.17	1.88–2.50	<0.001
History of gastric cancer (without versus with)	0.94	0.57–1.54	0.819	1.17	0.71–1.92	0.528
Lung cancer (non-ad versus ad)	2.04	1.79–2.31	<0.001	1.29	1.10–1.46	0.001
Adjuvant therapy for lung cancer (yes versus no)	1.16	0.95–1.42	0.134	1.13	0.92–1.39	0.233

Ad: adenocarcinoma; CI: confidence interval; cStage: clinical stage; HR: hazard ratio; OS: overall survival.

Table 4: Univariate and multivariate Cox regression analysis of OS of patients with previous gastric cancer

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
pStage of gastric cancer (II + III versus I)	0.92	0.26–3.27	0.901	0.75	0.17–3.32	0.708
Treatment for gastric cancer (endoscopic versus surgery)	0.97	0.33–2.82	0.961	1.16	0.35–3.86	0.802
Adjuvant therapy for gastric cancer (yes versus no)	2.90	0.74–11.36	0.124	3.47	0.70–17.15	0.125
Interval between gastric and lung cancer (≥5 vs <5 years)	0.93	0.36–2.54	0.900	0.93	0.31–2.77	0.904

CI: confidence interval; HR: hazard ratio; OS: overall survival; pStage: pathological stage.

Table 5: Five-year overall survival rates of patients according to interval between gastric cancer and lung cancer

Interval (years)	N	5-year OS (%)	P-value[a]
≤1 vs >1	31/69	68.3 vs 79.8	0.897
≤2 vs >2	39/61	77.2 vs 75.9	0.965
≤3 vs >3	49/51	78.7 vs 74.7	0.519
≤5 vs >5	62/38	75.9 vs 77.2	0.9

OS: overall survival.
[a]Log-rank test.

lung cancer is diagnosed at the early stage after gastric cancer treatment.

Several reports [3, 13, 14] have suggested that the incidence of early-stage lung cancer is higher in patients with previous malignancy because of follow-up assessments after treatment for malignant tumours. Quadrelli *et al.* [3] described that patients with a previous malignancy were more frequently diagnosed at Stage I than those without a previous malignancy. One study found a 72% likelihood of developing Stage I + II lung cancer as a second primary tumour [13]. However, the present study found no significant differences in the clinical stages of lung cancer between the PGC and NGC groups. This is because about 40% patients with a history of gastric cancer had been surgically treated for lung cancer over 5 years after undergoing treatment for gastric cancer. These findings indicate that the frequency of routine follow-up was insufficient for many of these patients.

We found that the interval between gastric cancer and lung cancer did not influence the prognosis. A previous multivariate analysis [13] also found that the disease-free interval between the appearance of the first and the primary tumours does not significantly impact the survival of patients with lung cancer as a second primary malignancy. Thus, a previous controllable malignancy seems not to substantially influence the prognosis of lung cancer.

We also found a higher ratio of older and male patients in the PGC, than in the NGC group, which was consistent with published findings [1, 2, 11, 15]. Since cancer incidence increases with age, patients with a second primary malignancy are often older [1, 2, 11]. The incidence of secondary primary cancers is high in patients with lung, colorectal, hepatic and gastric cancers that are common among Japanese males [16]. Information about smoking was not available, but we are aware that smoking is an important factor in the development of lung and gastric cancers [17], and it is a potential risk factor for the development of a second tumour [18, 19].

The present study has some limitations. The retrospective design is one and another is the selection bias imposed by not including patients whose surgeons found no indication for surgically treating lung cancer due to the patient having had previous gastric cancer. Cox regression analysis did not reveal previous gastric cancer as a significant factor. Regardless, our findings suggested that if surgeons considered that a previous gastric cancer had been controllable, then it minimally influenced the prognosis of patients with lung cancer. However, the upper limit of the 95% CI was broad, and we should interpret this conclusion to allow the exclusion of only the effect of a history of gastric cancer history beyond a HR of 1.92.

In summary, we showed that a history of treatment for gastric cancer had low impact on postoperative survival after complete surgical resection of a second lung cancer and that no factor associated with gastric cancer influenced the surgical outcomes of lung cancer. Curative surgery for lung cancer should be recommended when gastric cancer has been curatively treated regardless of the interval between the onset of both types of cancer. Furthermore, patients with Stage I gastric cancer in particular might be considered suitable for inclusion in clinical trials. Patients with previous gastric cancer and other types of cancer should be prospectively investigated in the future.

Conflict of interest: none declared.

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特　集

肺癌治療の最近の動向

3. 術後補助化学療法

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キーワード　非小細胞肺癌，術後補助化学療法，シスプラチン併用療法，5-FU系抗癌剤，ERCC1

I. 内容要旨

2003年のIALT，JLCRGの発表以降，II期，III期非小細胞肺癌完全切除例に対するシスプラチンと第三世代以降の抗癌剤を併用する術後補助化学療法とは世界的に標準的治療として認知され，本邦でも同様に標準的治療として位置付けられている．また，IB期あるいはIA期の一部については，本邦のみのエビデンスに基づいてテガフール・ウラシル配合剤（UFT）による術後補助療法が標準的治療の枠組みに入っている．進行がんの治療実績からある種の抗がん剤が組織型によって異なることが示されたことを受けて，最近では病期に加えて組織型を加味した対象に臨床試験が行われている．肺癌に対し標準的に使用される殺細胞性抗がん剤に関して，効果予測あるいは無効予測可能なバイオマーカーは同定されていない．こういったバイオマーカーが確立すれば，症例個々の術後補助化学療法においてより安全でより高い再発防止効果を期待できるであろう．

II. はじめに

本稿では，非小細胞肺癌（NSCLC）完全切除例に対する術後補助化学療法のエビデンスと現状について述べる．

III. エビデンスと推奨グレード

1．II-III期に対するシスプラチン（CDDP）併用療法

1995年にNon-small Cell Lung Cancer Collaborative Groupより手術単独群と術後補助化学療法群のランダム化比較試験のメタアナリシスが報告され，CDDP併用療法の術後補助化学療法で相対死亡危険率を13%減少し，有意差は認めないが5年生存率を5%改善するとの結果であった．このmeta-analysisの結果を基にInternational Adjuvant Lung Cancer Trial Collaborative Group（IALT），JBR.10及びAdjuvant Navelbine International Trial association（ANITA）trialなどの比較試験が行われ，いずれもCDDP併用療法を術後補助化学療法として行うことで無病生存率及び5年生存率の向上が得られた．これらの比較試験に，Adjuvant Lung Cancer Project Italy（ALPI），Big Lung Trial（BLT）を加えた，5つの比較試験，4,584症例の個々のデータに基づくメタアナリシスが行われた（Lung Adjuvant Cisplatin Evaluation（LACE）[1]．その結果，術後生存に対するハザード比（HR）0.89（95%信頼区間（95% CI）：0.82-0.96）と，術後補助療法による有意な延命効果が示された．病期別のハザード比では，IA期でHR：1.40（95%CI：0.95-2.06），IB期でHR：0.93（95%CI：0.78-1.10），II期でHR：0.83（95%CI：0.73-0.95），III期でHR：0.83（95%CI：0.72-0.94）という結果であった．また，このLACEのCDDP+ビノレルビン（VNR）に限った

CURRENT STATUS OF POSTOPERATIVE ADJUVANT CHEMOTHERAPY FOR COMPLETELY RESECTED NON-SMALL LUNG CANCER

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