population [15]. Such thromboembolic event was rare at 2 % in the current Japanese population. Other severe adverse events associated with bevacizumab were grade 2 bleeding event (2 %) and grade 3 GI perforation (2 %), all of which occurred during the first-line chemotherapy. Thus, no critical events associated with bevacizumab were observed during the second-line therapy. It is of note that a higher incidence of new or worsening hypertension was observed during the second-line therapy compared with the first-line therapy. The higher cumulative incidence of hypertension in the BBP group was not unexpected, given that the risk of developing bevacizumab-associated hypertension appears to accumulate over time and that the BBP results in substantially longer bevacizumab exposure. The type and frequency of other grade 3/4 events (including neutropenia, diarrhea, vomiting, and asthenia) were consistent with the known safety profile of the chemotherapy regimens.

Our study is merely hypothesis-generating regarding the efficacy of BBP because of the one-arm design and relatively small sample size. However, it does imply that the BBP strategy is beneficial to the Japanese population with the 2nd PFS nearly 10 months longer than that observed in the Tournigand study and SBP and OS that is similar to the survival data observed in the BRiTE study and the ARIES study. Data regarding safety of the BBP strategy was more robust, in which only hypertension was to be carefully taken care of. From these encouraging data, it can now be recommended that a randomized study involving a larger numbers of patients be performed in Japan to obtain hard evidence regarding the efficacy of BBP.

In summary, the planned continuation of bevacizumab during the second-line treatment is feasible for the Japanese mCRC patients. A prospective randomized control study to confirm the efficacy is warranted.

Conflict of interest The authors have declared no conflicts of interest.

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Phosphorylation of 4E-BP1 predicts sensitivity to everolimus in gastric cancer cells

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ABSTRACT

We studied the effect of everolimus, an inhibitor of the mammalian target of rapamycin (mTOR) on human gastric cancer cell lines. Cell proliferation in 3 of 8 cell lines was effectively inhibited by everolimus. Basal phosphorylation level of 4E-BP1 (T37/46, T70) was significantly higher in everolimus-sensitive cells than in everolimus-resistant cells. In subcutaneous xenograft model, immunohistochemistry analysis revealed that everolimus-sensitive cells expressed high levels of phospho-4E-BP1 (T37/46). In conclusion, phosphorylation of 4E-BP1 may be a predictive biomarker of everolimus sensitivity in gastric cancer.

1. Introduction

Gastric cancer is the fourth most common cancer worldwide and it is the second most common cause of cancer deaths [1]. Most patients with gastric cancer commonly present with advanced unresectable disease and over 60% of them eventually experience relapse even after curative surgical resection [2]. Systemic chemotherapy has been attempted in patients with unresectable and recurrent gastric cancer [2,3]. At present, although fluoropyrimidine-based therapy is used worldwide, no standard chemotherapeutic regimen has been accepted globally for advanced gastric cancer. Combination regimens including 5-fluorouracil, taxanes, irinotecan, and platinum derivatives achieved median overall survival of only up to 12 months in phase III trials [4–9]. Further efforts to explore new and more effective drugs or treatment regimens, or biomarkers to find a cohort that would benefit from specific drugs are warranted, as exemplarily shown by the recent application of trastuzumab to the HER2-positive gastric cancer.

The mammalian target of rapamycin (mTOR) is a key downstream protein kinase of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, which has been recognized to play a central role in regulating cell growth, cell cycle progression, cell proliferation, cell metabolism and angiogenesis [10–12]. mTOR forms with the adaptor protein raptor named mTOR complex 1 (mTORC1), and a rictor named mTOR complex 2 (mTORC2). mTORC1 regulates mRNA translation by activating ribosomal protein S6 kinase 1 (S6K1) and inhibiting a translational repressor, 4E-binding protein 1 (4E-BP1) [13]. In particular, mutations, deletion or ectopic expression of mTOR signal-related genes affects protein synthesis by eIF4E/4E-BP1 and S6K1, a phenomenon commonly observed in several types of cancer [14]. The translational repressor 4E-BP1 binds tightly to eIF4E. When 4E-BP1 is phosphorylated at multiple sites, it prevents the formation of eIF4E translation initiation complex at the 5′ end of cap-bearing mRNA [15]. eIF4E overexpression in mammalian cell culture is enough to increase cell size, and this is counteracted by co-overexpression of 4E-BP1 [16]. These observations are consistent with the reports that eIF4E expression is high in several types of cancer [14]. Subsequent activation of eIF4E results in translation of multiple

Abbreviations: 4E-BP1, 4E-binding protein 1; DCR, disease control rate; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PFS, progression-free survival; PI3K, phosphoinositide 3-kinase; S6K1, S6 kinase 1.

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malignancy-associated proteins such as c-myc [17]. Abnormal expression of growth receptors frequently activates PI3K/AKT/mTOR pathway in human gastric cancer [18–22]. The mTOR pathway is, therefore, a promising new therapeutic target in the treatment of gastric cancer.

Rapamycin and its derivatives including everolimus inhibit mTOR, thereby preventing phosphorylation of its downstream molecules including S6K and 4E-BP1. mTOR inhibitors have been shown to exhibit potent preclinical activities against a wide variety of cancers, including neuroblastoma, glioblastoma, small cell lung cancer, renal cell carcinoma, pancreatic cancer and leukemias [17]. Everolimus has recently been approved for treatment of renal cell carcinoma and neuroendocrine tumor of pancreas in Japan. Everolimus has shown to be active also against gastric cancer in the preclinical studies [23–25], and has been investigated in a phase I/II clinical trial for patients with advanced gastric cancer [26,27]. In this phase II trial, although the disease control rate was 56% and a decrease in tumor size from baseline was obtained in 45% of patients, in the phase III trial, the overall survival rate was not superior to that of gastric cancer patients who were treated only with best supportive care [28]. It is imperative that biomarkers for everolimus be found to optimize patients who should be treated with this drug. But little is known about the details of the signaling pathways that predict the effect of everolimus for gastric cancer. The present study was performed to assess the antiproliferative effect of everolimus on gastric cancer cell lines and to determine the predictable molecule which is correlated with sensitivity to everolimus *in vitro* and *in vivo*.

2. Materials and methods

2.1. Reagents

Everolimus, purchased from Sigma–Aldrich (St. Louis, MO), was initially dissolved in ethanol at a concentration of 0.05 mg/ml and stored at 4 °C.

2.2. Cell lines

Eight cell lines derived from human gastric cancer were examined. NUGC-2, NUGC-4 and SC-2-NU were established and maintained at the Department of Surgery II, Nagoya University Graduate School of Medicine. AZ521, MKN28 and MKN1 were provided by the Japanese Cancer Research Resource Bank (Tokyo, Japan). H111 and SC-6-JCK were established and kindly donated by the Department of Surgery, Research Institute for Microbial Disease, Osaka University and Central Institute for Experimental Animals, respectively. All cells were maintained at 37 °C in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and with 1% penicillin–streptomycin (GIBCO, Paisley, UK) in a humidified atmosphere of 5% CO_2 in air.

2.3. MTT assay

Cells (5×10^3 in 200 μl/well) were seeded on 96-well plates and incubated with everolimus at concentrations as indicated. After treatment, medium was replaced by 50 μl medium containing 0.05% 3-[4.5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] (Sigma–Aldrich) and incubated for 1 h at 37 °C. The 570 nm absorbance was recorded by a kinetic microplate reader Vient XS (DS Pharma Biomedical, Osaka, Japan). All experiments were performed in triplicate.

2.4. Flow cytometric analysis for cell apoptosis

2×10^5 cells were treated with 10 and 100 ng/ml everolimus for 48 h on 6-well plates. Cells were collected and stained with FITC labeled Annexin-V and PI (BD, Franklin Lakes, NJ). Apoptotic cell death was measured by counting the number of cells that stained positive for Annexin V by flow cytometry. All experiments were performed in triplicate.

2.5. Western blot analysis

2×10^5 cells were treated with various amounts of everolimus and lysed in 150 μl lysis buffer (Cell Signaling Technology, Danvers, MA). In xenograft tumor tissue study, frozen tumor tissue was sonicated on ice in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.1% SDS) with protease and phosphatase inhibitor (Sigma–Aldrich). Samples separated on a 10–12.5% SDS–PAGE were transferred to nitrocellulose membranes (Millipore, Billerica, MA). Membranes were incubated with antibody against AKT, phospho-AKT (Ser473), mTOR, phospho-mTOR (Ser2448), S6 K, phospho-S6 K (Thr389), phospho-S6 (Ser235/236), 4E-BP1, phospho-4E-BP1 (Thr37/46), phospho-4E-BP1 (Ser65), phospho-4E-BP1 (Thr70), 4E-BP1, ERK, phospho-ERK (Thr202/Tyr204), GAPDH (all are from Cell Signaling Technology) and S6 (Santa Cruz Biotechnology, Santa Cruz, CA) with a working dilution in Can Get Signals Solution 1 (TOYOBO, Tokyo, Japan) at 4 °C overnight. Primary antibodies were detected by HRP-anti-rabbit IgG (Cell Signaling Technology). Signals were observed via ECL® Western Blotting Detection Reagents (GE Healthcare, Little Chalfont, UK). Antibody concentrations were 1:2000 for the anti-Akt antibody and 1:1000 for the others.

2.6. Tumor xenograft studies

1×10^7 cells (SC-2-NU and NUGC-4) in 0.2 ml PBS were injected subcutaneously into the left shoulder of 6–7 week-old male nude mice of KSN/Slc strain (Chubu Kagaku Shizai, Nagoya, Japan) maintained under specific-pathogen-free conditions. Mice ($n = 8$) were orally administered everolimus via a gastric tube at a dose of 0 or 5 mg per kg per day from day 10 after inoculation, five times per week for 4 weeks. Tumor maximum diameter (L) and the right angle diameter to the axis (W) were measured 3 times a week. Tumor volume was estimated by the following formula, $L \times W \times W \times 1/2$. Animal welfare was strictly monitored by the Committee for Ethics of Animal Experimentation, and the experiments were carried out in accordance with the Guidelines for Animal Experiments at Nagoya University.

2.7. Immunohistochemistry

Paraffin-embedded tissue sections were heated in a microwave at 500 W for 10 min in citric acid buffer, pH 6.0, for antigen retrieval and then incubated in 0.3% H_2O_2 in methanol for 15 min at room temperature to suppress the endogenous peroxidase activity. After blocking nonspecific reactions, these slides were further incubated with primary antibodies overnight in a moist chamber at 4 °C. Anti-4E-BP1 and anti-p-4E-BP1 (T37/46) antibodies (Cell Signaling Technology; 1:200 dilution) were used for primary antibodies. The sections were incubated for 30 min at room temperature with the secondary antibodies (biotinylated goat anti-rabbit IgG; Dako, Denmark) followed by incubation with streptavidin-peroxidase complex (HISTOFINE SAB-PO Kit, Nichirei Biosciences, Tokyo, Japan). The chromogen was developed with 0.03 mol/L diaminobenzidine, and the sections were counterstained with hematoxylin.

2.8. Statistical analysis

For comparison between groups, data were analyzed by t test. Differences in western blot analysis between the groups were analyzed with Mann–Whitney-U test. Differences between groups were considered statistically significant at $p < 0.05$ except for the tumor xenograft studies, in which differences between groups were considered statistically significant at $p < 0.01$.

3. Results

3.1. Antiproliferative and apoptotic effects of everolimus in gastric cancer cells

Eight gastric cancer cell lines were incubated with serial concentrations of everolimus (0.1–100 ng/ml) for up to 4 days. Everolimus inhibited proliferation of these cells in a concentration dependent manner, but with varying effectiveness (Fig. 1A). For almost all cell lines, the growth inhibitory effect diminished at 4 days after initiation of the treatment. The 50% growth inhibition of each cell line by everolimus was therefore determined after 3 days of exposure. The concentration of everolimus required to inhibit growth of AZ521, H111 and SC-2-NU cells was less than 100 ng/ml (10 ng/ml, 25 ng/ml, 100 ng/ml, respectively) whereas other cells (NUGC-2, SC-6-JCK, NUGC-4, MKN1, MKN28) did not reach the point of 50% inhibition even with 100 ng/ml (Fig. 1B). We therefore classified the cell lines AZ521, H111 and SC-2-NU as sensitive to everolimus and the other 5 cell lines as resistant. Apoptosis analysis by flow cytometry was performed in both the everolimus-sensitive cells (AZ521, SC-2-NU) and the everolimus-resistant cells (NUGC-4, MKN28) (Fig. 1C and D). Regardless of the sensitivity to everolimus, no increase in apoptotic cells was observed. Taken together, we confirmed that everolimus exhibits

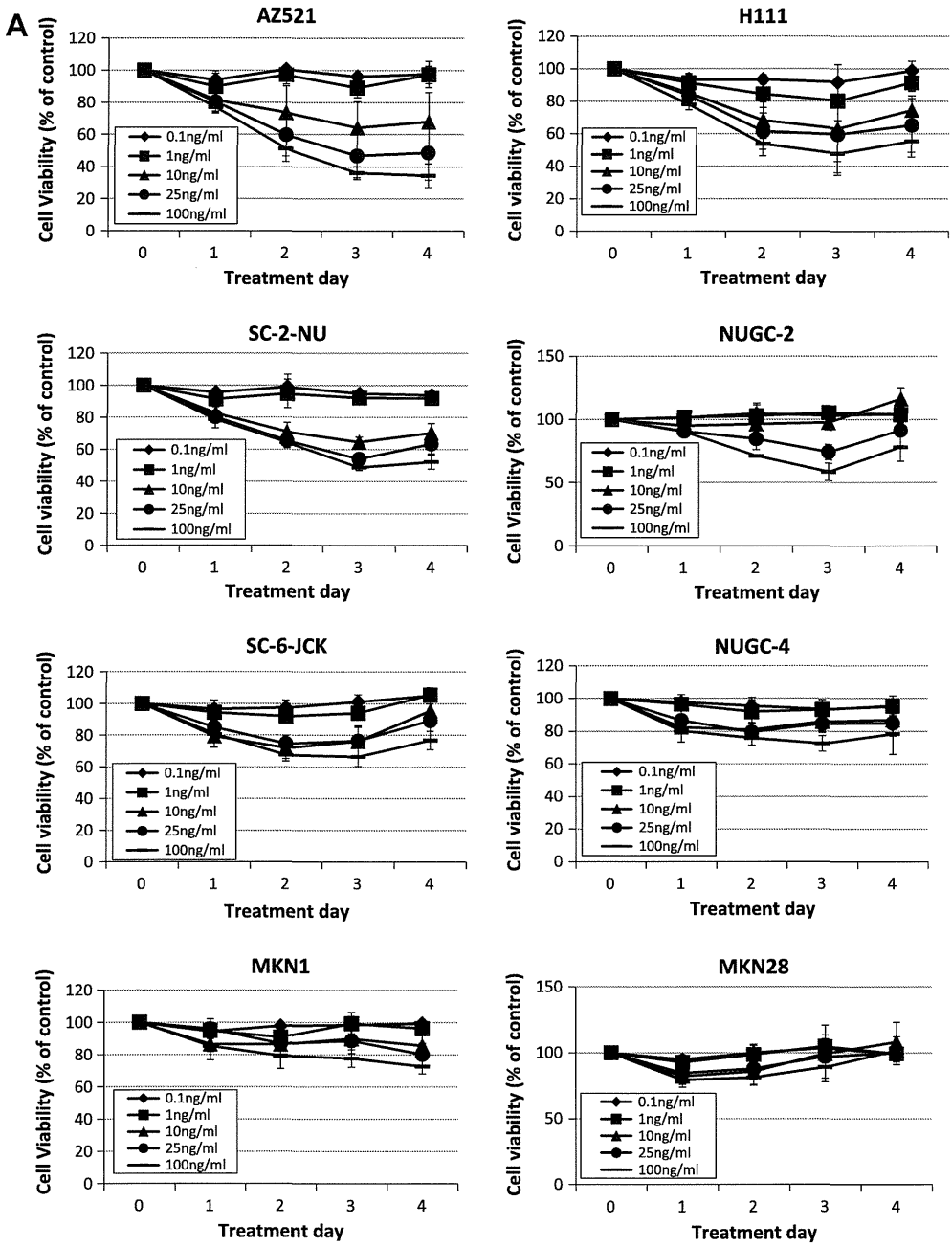


Fig. 1. Inhibitory effects of everolimus on gastric cancer cell growth and proliferation. (A) Eight different gastric cancer cells were seeded into a 96-well plate. Cells were treated with different amounts of everolimus from 0.1 to 100 ng/ml for 4 days. The data represents mean ± SEM ($n = 3$). (B) Cell viability was determined daily, and compared at 3 days by MTT assay. The data represents mean ± SEM ($n = 3$). (C) Gastric cancer cells were treated with 10 and 100 ng/ml everolimus for 48 h. Apoptotic cells were analyzed by Annexin-V/PI staining assay. Representative flow cytometry results using AZ521, H111, SC-2-NU, NUGC-4 were shown. Each population was separated by Quadrant, and each percentage was calculated. (D) Bars compare differences in the percentage of apoptosis cells between controls and everolimus-treated cells. Values are expressed as mean ± SD ($n = 3$). Everolimus did not induce apoptosis nor showed protective effect, although the differences in apoptosis levels were observed among cancer cells.

various levels of growth inhibitory effect on gastric cancer cell lines, and this is not through induction of apoptosis.

3.2. Effect of everolimus on mTOR-related genes and ERK in gastric cancer cell lines

To investigate the effect of everolimus on mTOR pathway, genes related to the pathway were analyzed. AZ521, SC-2-NU, NUGC-4 and MKN28 cells were treated for 24 h by various concentrations of everolimus. Cells were then harvested and subjected to western blotting to estimate the respective levels of phosphorylated and non-phosphorylated forms of S6K, S6 and 4E-BP1. Fig. 2 shows that the treatment with everolimus reduces phosphorylation levels of S6 and 4E-BP1 (T70) in a dose-dependent manner in all cell lines. But there were no significant effects of everolimus on the phosphorylated S6K and 4E-BP1 (T37/46). In addition, everolimus at 100 ng/ml concentration resulted in a decrease of the phosphorylated 4E-BP1 (S65) only for MKN28. We then examined the levels of phosphorylated and non-phosphorylated ERK, the downstream component of the Ras/MEK signaling which has been considered to be one of major pathways involved in cell proliferation and tumorigenesis. In AZ521, the phosphorylation of ERK was increased in a dose-dependent manner after the treatment. But in other cell lines, the phosphorylation of ERK was not affected by

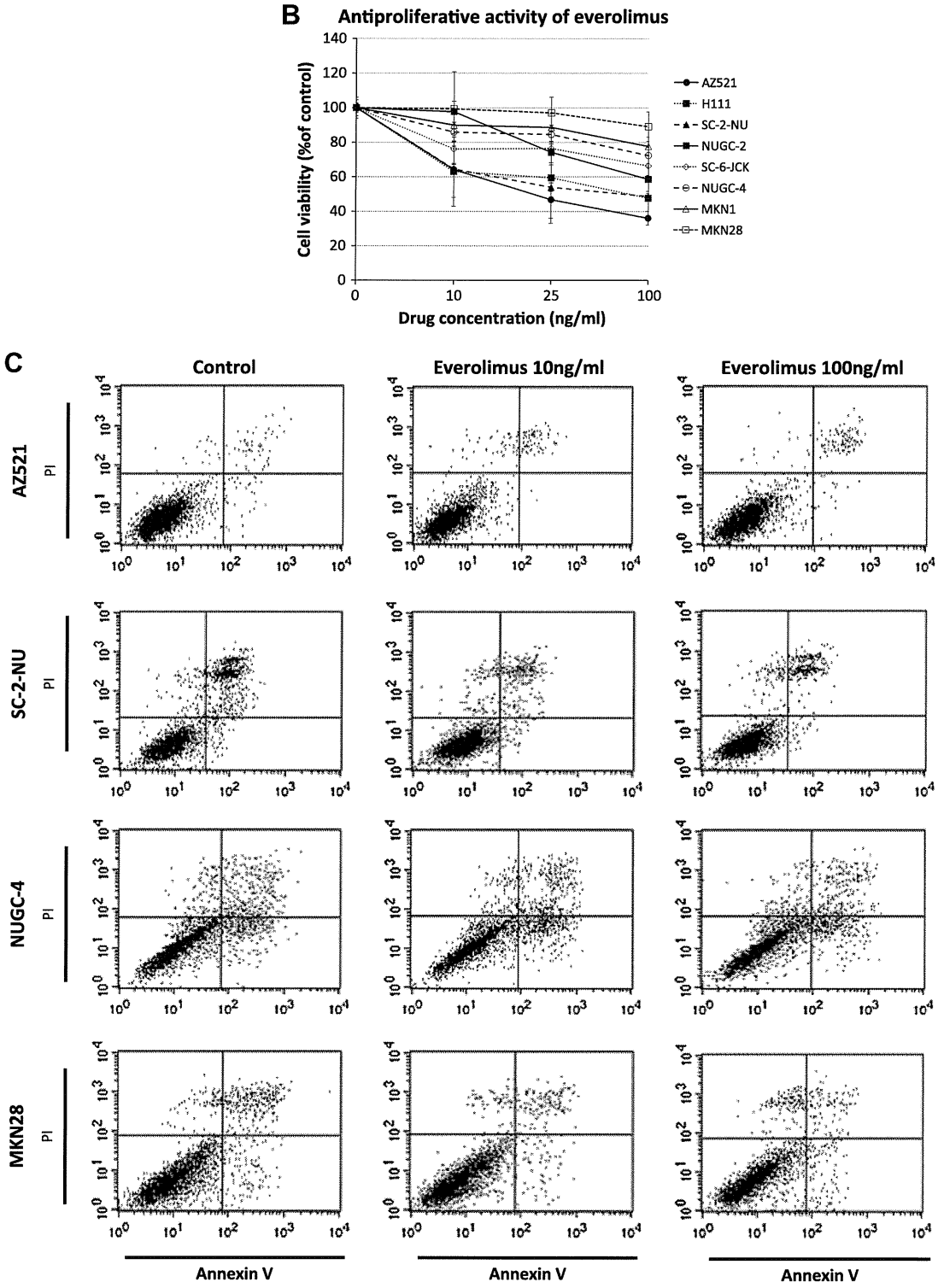
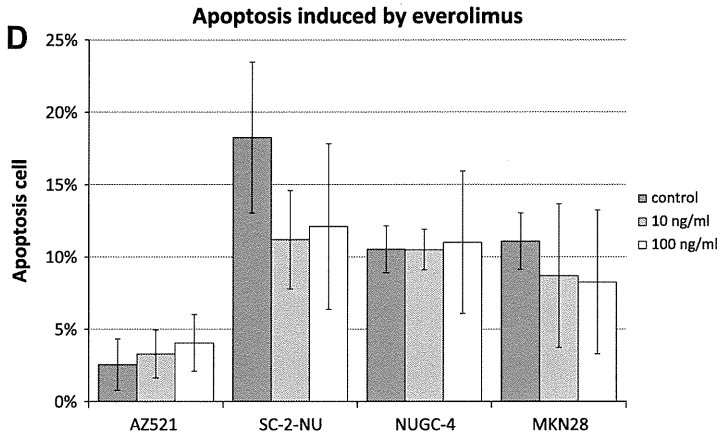


Fig. 1. (*continued*)

everolimus. Taken together, the effects of everolimus on the phosphorylation levels of proteins related to mTOR signaling pathway did not correlate with the sensitivity of tested cell lines to the growth inhibitory effect of everolimus.

3.3. Different expression of 4E-BP1 and ERK phosphorylation between everolimus-sensitive and everolimus-resistant gastric cancer cells

To further characterize sensitivity to mTOR inhibition, we conducted a conventional quantitation-approach for component proteins of mTOR signaling pathway. All 8 cell lines were lysed and subjected to western blotting to estimate basal phosphorylation levels of AKT, mTOR, S6K, S6, 4E-BP1 and ERK (Fig. 3A). The levels of AKT, mTOR, S6K and S6 did not exhibit any relation with everolimus sensitivity. Statistical analysis showed significant positive correlations between everolimus sensitivity and phosphorylation levels of 4E-BP1 (T37/46) and 4E-BP1 (T70) (Fig. 3B). In contrast, the level of phosphorylation of ERK tended to be lower in everolimus-sensitive cell lines than in resistant ones, although not significantly so.



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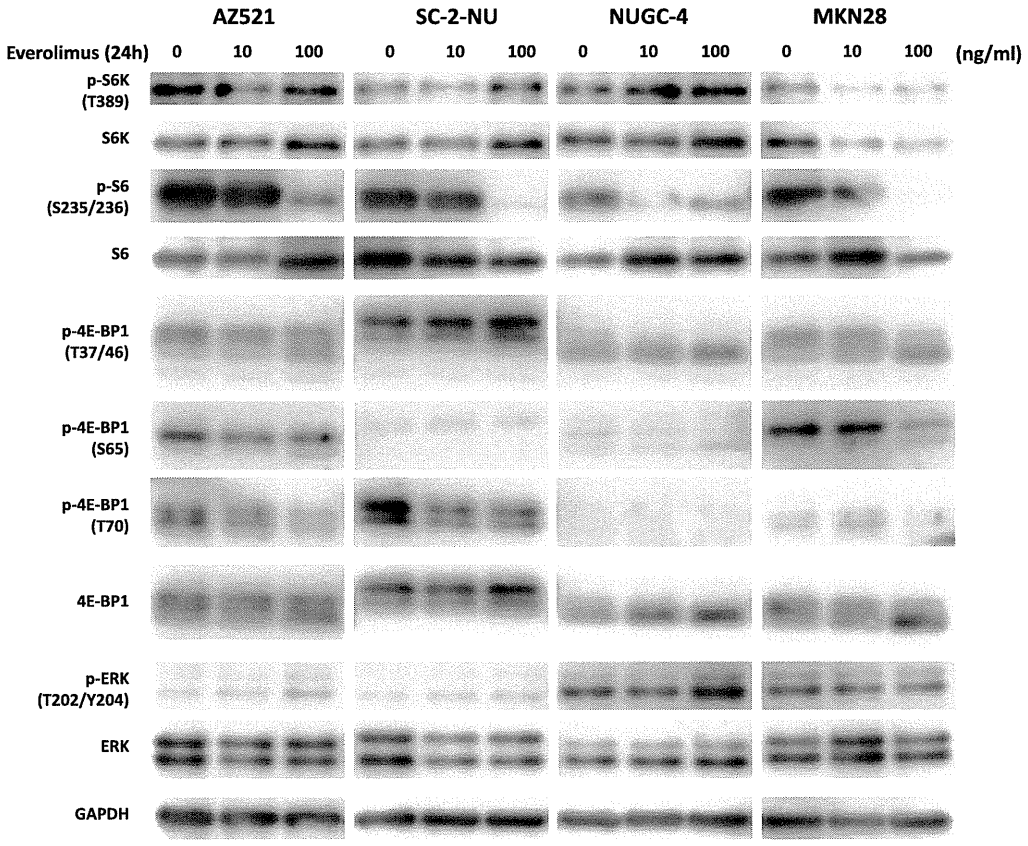


Fig. 2. Activity of ERK and downstream molecule of mTOR pathway. AZ521, SC-2-NU, NUGC-4 and MKN28 cells were treated with 10 and 100 ng/ml everolimus for 24 h. Cells were harvested and subjected to western blotting with antibody against indicated proteins.

3.4. Effects of everolimus on the growth of phospho-4E-BP1-overexpressing cell and phospho-ERK overexpressing cell in vivo

To verify the *in vitro* results under *in vivo* conditions, SC-2-NU (higher 4E-BP1 phosphorylation and lower ERK phosphorylation) and NUGC-4 (lower 4E-BP1 phosphorylation and higher ERK phosphorylation) cells were implanted subcutaneously into nude mice. The tumor-bearing mice were treated with everolimus 5 days a week for 4 weeks, and the tumor size was measured 3 times a week. After 2 weeks of treatment, everolimus significantly inhibited the growth of tumors derived from both cell lines (Fig. 4). Everolimus more powerfully inhibited tumor growth in SC-2-NU cells (79% decrease) than in NUGC-4 cells (26% decrease). The results showed that everolimus indeed was more effective against the everolimus-sensitive cells in the xenograft model.

Xenograft tumor tissue samples were lysed and subjected to western blotting to analyze the phosphorylation levels of 4E-BP1 (T37/46, T70). As observed by the immunohistochemical staining, the phosphorylation of 4E-BP1 (T37/46, T70) was higher in SC-2-NU than in NUGC-4 in western blotting analysis (Fig. 5A). Immunohistochemical analysis of tumor tissue samples showed higher expression of p-4E-BP1 (T37/46) in SC-2-NU than in NUGC-4 (Fig. 5B), though the levels of p-4E-BP1 (T37/46) were not decreased by everolimus treatment. We could not obtain a reliable

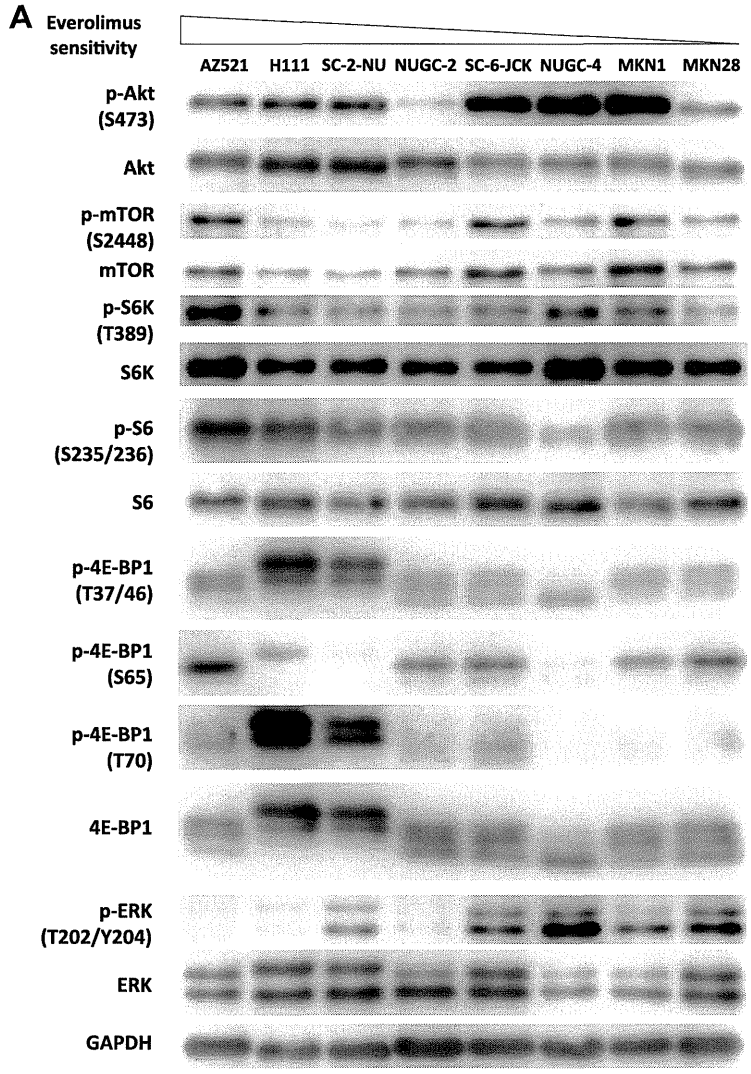


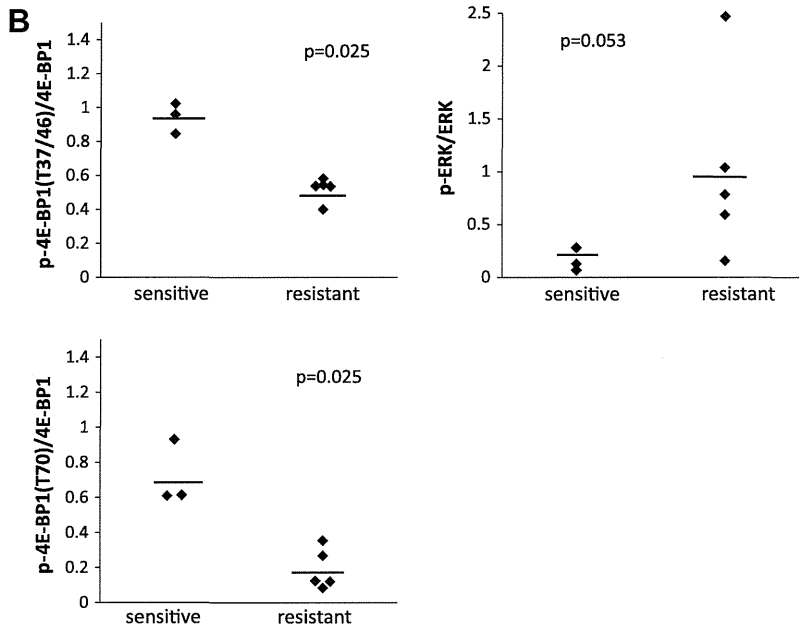
Fig. 3. Comparison of basal activity of ERK and mTOR signal-related molecules among eight different cells. (A) Eight non-treated human gastric cancer cells were seeded and incubated overnight. Cells were harvested and subjected to western blotting with antibody against indicated antibodies. (B) The ratio of the strength of phospho-protein to total protein was quantified and shown. Each bar represents the mean ratio. Sensitive: 3 everolimus-sensitive cell lines (AZ521, H111 and SC-2-NU), Resistant: 5 everolimus-resistant cell lines (NUGC-2, SC-6-JCK, NUGC-4, MKN1 and MKN28).

result regarding the p-4E-BP1 (T70) expression level, because a high-quality antibody for immunohistochemical analysis was not available.

4. Discussion

As in a previous study [29], the results of the present investigation suggested that the anti-proliferative efficacy of everolimus depends on the type of cell line. It is considered that inhibiting mTOR pathway results in G1 phase arrest and inhibition of S phase initiation [23], and that the anti-proliferative effect of everolimus is induced not by cell apoptosis or cell death, but by cell-cycle inhibition [25,29–31]. In our study, everolimus treatment did not lead to induction of cell apoptosis and cell death, even in cell lines that are sensitive to everolimus. Everolimus reduced the phosphorylation of S6 and 4E-BP1 (T70), but did not inhibit the phosphorylation of S6K. These effects on the mTOR signaling pathway were not associated with the sensitivity to everolimus. The Ras/MEK pathway, which is acknowledged to contribute to growth and proliferation of cancer, is a competitive pathway known to be activated by inhibiting the mTOR pathway [32]. Since the interaction between these two pathways might be why an inhibitory effect of everolimus on phosphorylation of S6 and 4E-BP1 did not lead to suppression of cell proliferation, we investigated the effect of everolimus on ERK, the target protein of the Ras/MEK pathway. However, everolimus did not activate Ras/MEK pathway in most cell lines.

To examine in further detail the relation between the basal activity of signaling pathways related to cell proliferation and the sensitivity to everolimus, we investigated phosphorylation levels of the mTOR and Ras/MEK pathway components. The results showed that the phosphorylation of 4E-BP1 (T37/46) and 4E-BP1 (T70) were significantly enhanced in everolimus-sensitive cell lines. 4E-BP1 is a key protein of mTOR pathway but its function remains unclear. Dowling et al. [33] reported that 4E-BP1 mediated cell proliferation and S6K, another key protein of mTOR pathway, regulated cell size, and when 4E-BP1 was knocked down, the anti-proliferative effect of everolimus was decreased. In line with this report, our results suggested that downregulation of 4E-BP1 reduced the impact of mTOR on cell proliferation, resulting in the attenuated effect of everolimus. On the other hand, since



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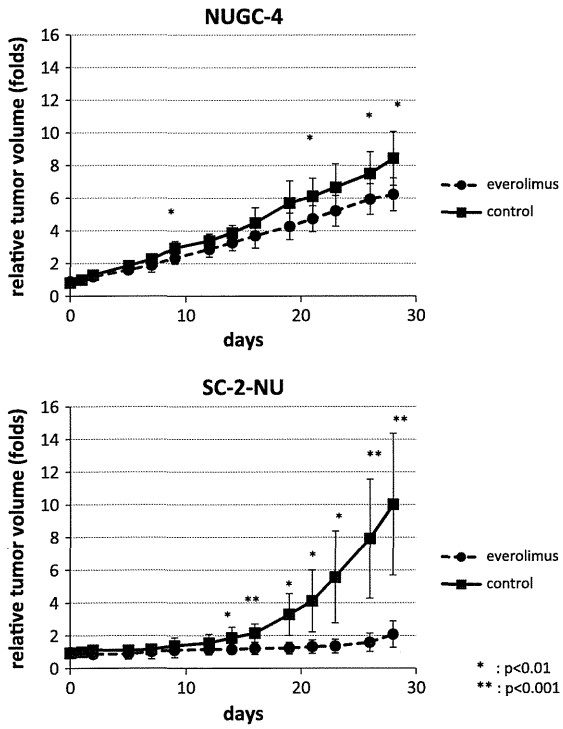

Fig. 4. Everolimus inhibitory effects on gastric cancer cells *in vivo*. Tumor growth was assessed in control and everolimus-treated nude mice bearing s.c. NUGC-4 and s.c. SC-2-NU xenograft for 28 days, and the tumor volume measured. Tumor volume is expressed as the mean fold increase over time. Data represents mean ± SEM ($n = 8$). $^{*}p < 0.01$, $^{**}p < 0.001$.

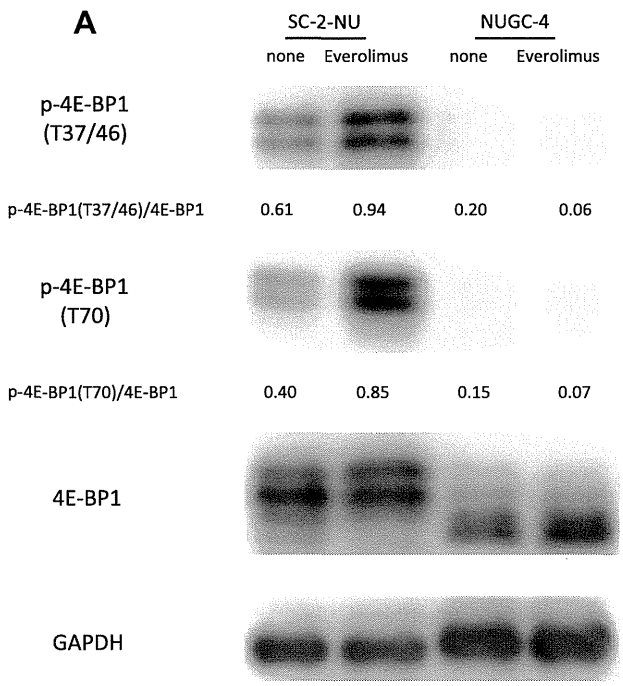

Fig. 5. Phospho-4E-BP1 expression in subcutaneous xenograft tumor. SC-2-NU (everolimus-sensitive cells) or NUGC-4 (everolimus-resistant cells) were subcutaneously injected into nude mice. Tumor tissue sections in xenograft were harvested after 28 days treatment with either a placebo or everolimus. (A) Tumor cells in xenograft were subjected to western blotting with anti-phospho-4E-BP1 (T37/46, T70) antibody. The ratio of phospho-4E-BP1 to total 4E-BP1 was quantified and shown just below p-4E-BP1 panels. (B) Immunohistochemical staining of phospho-4E-BP1 (T37/46) was assessed. Left and right panels show unstained and stained tissue sections, respectively.

phosphorylation of ERK tended to be stronger in everolimus-resistant cells, the basal level of p-ERK could be used as an additional marker predicting everolimus sensitivity.

One of our hypotheses is that the dominant action of Ras/MEK pathway including that of ERK over mTOR pathway in cell proliferation, results in the insufficient anti-tumor effect of everolimus and the resistant properties of these cells. Although more research is needed to clarify the reason for the difference between everolimus-sensitive cells and everolimus-resistant cells, it is suggested that combination therapy with everolimus and other drugs like Ras/MEK inhibitor could improve the anti-tumor effect.

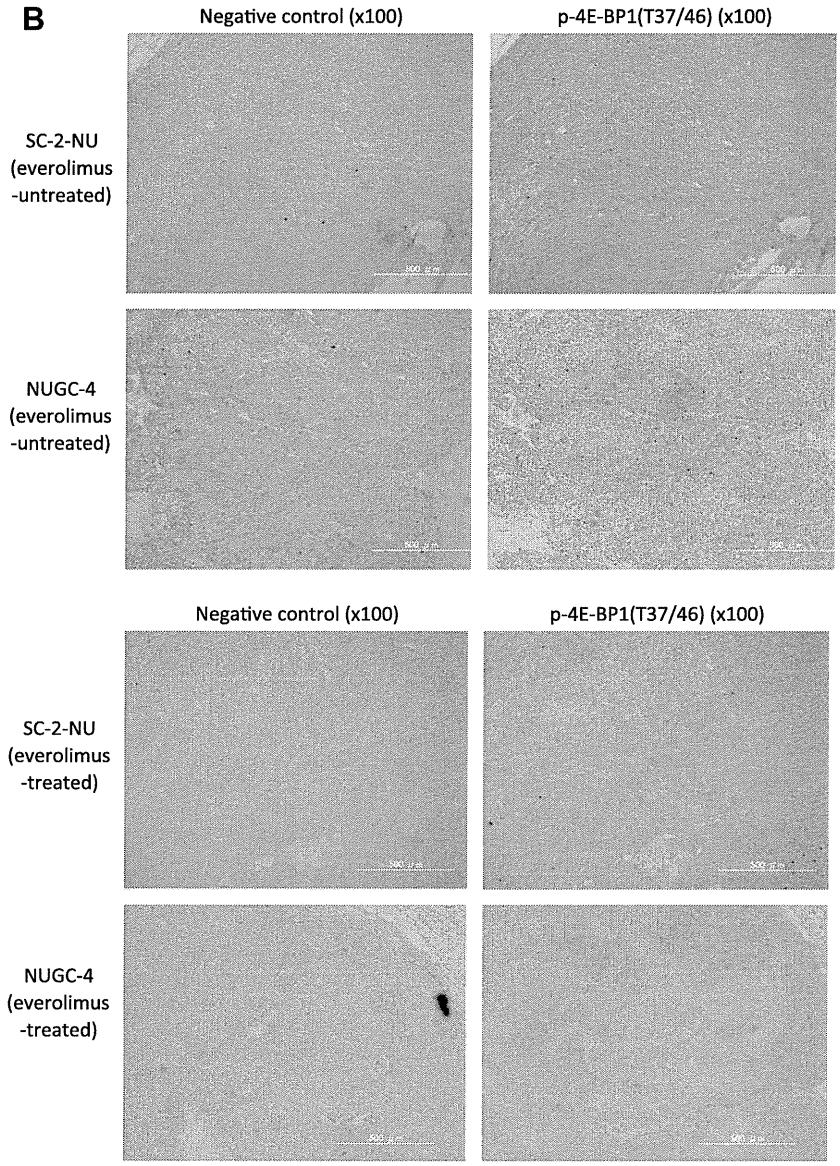

Fig. 5. (*continued*)

Similar to the *in vitro* examination, everolimus had a more powerful anti-tumor effect in SC-2-NU, an everolimus-sensitive cell line, than in NUGC-4, an everolimus-resistant cell line, in a subcutaneous xenograft model. Immunohistochemical staining showed that the phosphorylation of 4E-BP1 (T37/46) was stronger in SC-2-NU than in NUGC-4. From these results, the sensitivity to everolimus could be predicted by examining the phosphorylation of 4E-BP1 (T37/46). However, we obtained the intriguing finding that everolimus treatment did not actually decrease the phosphorylation of 4E-BP1 (T37/46), which corresponds with a previous study on rapamycin [34]. 4E-BP1 phosphorylation events at T37/46 reportedly exist without mTORC1 or mTORC2 expression, which indicates the existence of a potential third mTOR complex [35,36]. As the full phosphorylation of four sites (T37, T46, S65 and T70) in 4E-BP1 is required for the release of eIF4E and promotion of cell cycle [34], the dephosphorylation of 4E-BP1 at T70 alone by everolimus would be enough to prevent cell proliferation even when other 4E-BP1 sites (including T37/46) are still phosphorylated. Therefore, strong expression of phosphorylation at T37/46 of 4E-BP1 might mean that cell proliferation properties are highly dependent on mTOR signaling pathway via mTORC1, C2 and others, which could create a high susceptibility to everolimus. *In vitro* experiment demonstrated that everolimus reduced the phosphorylation of 4E-BP1 (T70). In contrast, p-4E-BP1 (T70) was enhanced in SC2-NU by everolimus treatment in the xenograft model. Initial inhibition of 4E-BP1 phosphorylation by mTOR inhibitor has reportedly been recovered within 6 h [37], although time course of effect would be dependent on cell type and condition. It is also speculated that the properties of cancer cells might be changed by everolimus treatment for 28 days, because long-term suppression of signaling pathway could incidentally cause the activation of another pathway such as Akt [38], which may be responsible for acquired resistance to such a pathway targeted drug. Taken together, the sensitivity to everolimus (i.e., inhibitory effect on cell proliferation) may be ascribed not to the capability to inhibit mTOR pathway, but to be the proliferation properties of cells; that is, the dominant cell growth pathway would be mTOR or others such as Ras/MEK.

The phase II trial of the everolimus effect on gastric cancer showed an overall disease control rate (DCR) of 56%, but an objective response rate of 0%. This suggests that the major benefit of everolimus in clinical use is disease stabilization, not tumor

shrinkage. Looking back on past clinical trials of molecular-targeted agents, effects on gastric cancer, the ToGA study with biomarker-based patient selection showed prolonged overall survival [39], whereas the AVAGAST study, in which all patients were registered without established biomarkers, failed to show significant increases in progression-free survival (PFS) and overall response rate [40]. It seems likely, therefore, that a biomarker to predict an anti-tumor effect of everolimus is clinically important. A previous study [41] reported that the high expression of phosphorylated S6 (S240/244) was associated with higher DCR and PFS, but further clinical trials and analyses were deemed necessary. The proposal of a novel candidate of the biomarker predicting effects of everolimus is timely. In conclusion, the phosphorylation status of 4E-BP1 might predict sensitivity to everolimus in gastric cancer. Thus, close evaluation of this biomarker in the tumor tissues and selecting patients accordingly may indeed help improve the efficacy of everolimus treatment for gastric cancer. Further studies using clinical samples are warranted.

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Clinical Trial Note

Effect of Daikenchuto (TJ-100) on Postoperative Bowel Motility and on Prevention of Paralytic Ileus after Pancreaticoduodenectomy: A Multicenter, Randomized, Placebo-controlled Phase II Trial (The JAPAN-PD Study)

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We conducted a multicenter, randomized, controlled trial in patients with pancreaticoduodenectomy to investigate the efficacy of Daikenchuto (TJ-100), which is a Kampo medicine (traditional Japanese herbal medicine), for its effect on postoperative bowel motility and for prevention of postoperative paralytic ileus. This clinical trial primarily evaluates the co-primary endpoints: (i) the incidence rate of postoperative paralytic ileus lasting over 72 h after surgery and (ii) time to having the first postoperative passage of flatus. The secondary endpoints are the incidence of postoperative paralytic ileus in cases that combined with/without enteral alimentation, QOL assessment by the Gastrointestinal Symptom Rating Scale (GSRS) Score (Japanese Version) and visual analogue scale, the change ratio of abdominal circumference, the incidence of postoperative complication, the number of postoperative hospital days, the incidence of surgical site infection and the incidence of postoperative small bowel obstruction within 2 years after surgery. Two hundred and twenty patients are required in the study (110 patients per group).

Key words: pancreaticoduodenectomy – Japanese herbal medicine (TJ-100) – postoperative paralytic ileus – surgical site infection

INTRODUCTION

Pancreaticoduodenectomy (PD) is one of the most extensive surgical procedures with high incidence of morbidity for patients with periampullary disease. Improved surgical skills and modern perioperative care reduced the mortality rate, but there is still a high morbidity rate, which remains about 40–50% (1, 2). In these days, several investigators have reported that a fast-track program reduced the incidence of morbidity and the postoperative hospital days in PD (3, 4). To keep normal state of the digestive function is an essential factor affecting the recovery of postoperative paralytic ileus in the fast-track program. Daikenchuto (TJ-100), which is a traditional Japanese herbal medicine, has been used for the prevention and treatment of postoperative ileus in Japan (5, 6). TJ-100 extract powder (Tsumura & Co., Tokyo, Japan) is manufactured as an aqueous extract containing

2.2% Japanese pepper, 5.6% processed ginger, 3.3% ginseng and 88.9% maltose syrup powder. To date, there has been no prospective study investigating the effect on the normalization of bowel peristalsis after PD. Therefore, we have started a multicenter, randomized, placebo-controlled phase II trial of TJ-100 to evaluate its efficacy for supporting postoperative bowl motility and preventing postoperative paralytic ileus after PD.

PROTOCOL DIGEST OF THE STUDY

Objective

Postoperative paralytic ileus after surgery for intraperitoneal organ is one of the common complications (>90% in many series) and recognized as an inevitable response to intraperitoneal surgery (7–9). The JAPAN-PD study is a multicenter, randomized, double-blinded, placebo-controlled, phase II trial, and planned to implement for patients with periampullary tumors (extrahepatic bile duct tumor, tumors of ampulla of Vater and duodenal tumor) and pancreatic tumors (pancreatic cancer, intraductal papillary mucinous neoplasm of the pancreas, pancreatic endocrine tumor and pancreatic neuroendocrine tumor) of the head of pancreas who are expected to undergo PD to investigate an enhancement effect of the bowel motility and the prevention effect of TJ-100 for postoperative paralytic ileus after PD.

Resources

A research grant from a non-profit organization: epidemiological and clinical Research Information Network (ECRIN).

Endpoints

This clinical trial primarily evaluates the co-primary endpoints: (i) the incidence rate of postoperative paralytic ileus lasting over 72 h after surgery and (ii) the time to having the first postoperative passage of flatus. In this study, the postoperative paralytic ileus is defined as the delay of the first postoperative flatus for over 72 h (3.0 days) after surgery (7–9), or the status requires some intervention of treatment for ileus. Every 12 h are counted as 0.5 postoperative day and 24 h as 1.0 postoperative day. The secondary endpoints are the incidence of postoperative paralytic ileus in cases that combined with/without enteral alimentation, QOL assessment by the GSRS Score (Japanese Version) and visual analogue scale about abdominal pain and abdominal distention, the change ratio of abdominal circumference on postoperative day 3 and operative day just after surgery, the incidence of postoperative complication, the number of postoperative hospital days, the incidence of surgical site infection and the incidence of postoperative small bowel obstruction within 2 years after surgery.

Eligibility Criteria

Inclusion Criteria

(i) Patients with periampullary tumors (extrahepatic bile duct tumor, tumors of ampulla of Vater and duodenal tumor) and pancreatic tumors (pancreatic cancer, intraductal papillary mucinous neoplasm of the pancreas, pancreatic endocrine tumor and pancreatic neuroendocrine tumor) of the head of the pancreas who are scheduled to undergo PD.
(ii) Age of at least 20 years and older at the time of registration.
(iii) All patients provided written informed consent before initiation of study-related procedures.

Exclusion Criteria

(i) Clinically problematic cardiac disease.
(ii) Liver cirrhosis or active hepatitis.
(iii) Severe pulmonary disease (interstitial pneumonia, pulmonary fibrosis, pulmonary emphysema etc.).
(iv) Chronic renal failure requiring hemodialysis.
(v) Other malignant disease that can influence the adverse effect.
(vi) Patients with tumors requiring resection of colon.
(vii) Patients who are expected to have severe intra-abdominal adhesion due to past surgical history or past peritonitis history.
(viii) Patients who had used gastrointestinal prokinetic medication, antipsychotic medication or antidepressants.
(ix) Patients who had used Japanese herbal (Kampo) medicines within 4 weeks before registration.
(x) Pregnant or lactating women.
(xi) Any other medical condition that makes the patient unsuitable for including into the study according to the opinion of the investigator.

Registration

An eligibility report form is sent to the registration center at ECRIN. Eligible patients are centrally randomized to either Arm A (TJ-100) or Arm B (placebo) using primary disease, the presence of preoperative therapy, the presence of pylorus ring in the remnant stomach and the institution as balancing variables. Information regarding the necessary follow-up tests is then sent from the registration center at ECRIN.

Treatment Methods

ARM A

In the TJ-100 group, TJ-100 at a dose of 5 g was administered orally as a solution three times daily immediately before meals or every 8 h for 17 consecutive days (15 g/day from preoperative day 3 to postoperative day 14). On the operative day (only once immediately after operation) and

postoperative day 1, TJ-100 were administered as a diluent via enteral feeding tube (10 Fr), which terminates in jejunum to prevent aspiration pneumonia.

ARM B

In the placebo group, placebo at a dose of 5 g was administered orally as a solution three times daily immediately before meals or every 8 h for 17 consecutive days (15 g/day from preoperative day 3 to postoperative day 14). On the operative day (only once immediately after operation) and postoperative day 1, placebo were administered as a diluent via enteral feeding tube (10 Fr), which terminates in jejunum to prevent aspiration pneumonia.

Criteria of Dose Reduction and Discontinuation of the Protocol Treatment

In cases where Grade 2 postoperative diarrhea or other clinical adverse effects (CTCAE version 4.0 criteria) are observed, the patient will be administered reduced dose of the test drug to a dose of 2.5 g, and in case where Grade 3 postoperative diarrhea or other clinical adverse effects (CTCAE version 4.0 criteria) are observed, the protocol treatment will be immediately discontinued.

Data Collection

Data will be collected prospectively for all patients including history, physical examination, laboratory data, pathologic examination, perioperative clinical information and complications.

Study Design and Statistical Analysis

This clinical trial primarily evaluates the co-primary endpoints: (i) the incidence rate of postoperative paralytic ileus for over 72 h after surgery and (ii) the time to have the first postoperative passage of flatus. The multiplicity issue (inflation of the type I error) due to analyzing two endpoints is dealt with using the Bonferroni method. That is, the significance levels for both tests are set at 2.5% to control the overall type I error rate. The sample size was calculated on the basis that the incidence rate of postoperative paralytic ileus for 72 h after surgery was expected to be 90% for the placebo group. In case the effect of reducing the incidence of postoperative paralytic ileus is assumed to be 20% for the TJ-100 group (that is, incidence rate = 70%), the least number of patients to provide the 85% power necessary to confirm the superiority of a group was calculated to be 94 per group for a two-sided 2.5% significance level test. Furthermore, given the number of patients, 84% statistical power is retained to prove the superiority in terms of time to occurrence of postoperative paralytic ileus for the hazard ratio of 0.62. The significance level for this inference is also set at 2.5%. Taking exclusion from analysis of about 15% into account, the number of patients to be accrued was set at 110 per treatment arm (220 in total). The first primary endpoint, incidence rate of postoperative paralytic ileus for 72 h after surgery, will be compared between the two treatment groups using the χ^2 test. The second primary endpoint, time to having the first postoperative passage of flatus, will be analyzed by constructing Kaplan–Meier curves as time-to-event plots. Differences between the curves are tested for significance using log-rank statistics.

PARTICIPATING INSTITUTIONS

Eleven leading Japanese institutions and hospitals (all of them are high volume center in pancreatic surgery) for PD are participating in this trial.

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Conflict of interest statement

None declared.

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CASE REPORT

Fatal hypermagnesemia induced by preoperative colon preparation in an elderly woman: report of a case

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Abstract An 85-year-old woman with rectal carcinoma was referred to our hospital for surgical treatment. She had a history of constipation treated with oral magnesium oxide. She received 34 g of magnesium citrate (Magcolol P®) orally for 2 days as a mechanical bowel preparation prior to the operation. Just before the operation, she suddenly developed nausea, vomiting, and cyanosis and went into cardiac arrest. Despite support by mechanical ventilation, dopamine, dobutamine, and norepinephrine, she exhibited repeated bradycardia that was nearly fatal and required temporary pacing. The following day, her laboratory tests revealed marked hypermagnesemia (14.3 mg/dL). After a hemodialysis session, she recovered dramatically and all vasopressors were withdrawn. We conclude that preoperative mechanical bowel preparation with magnesium-containing cathartics can cause fatal hypermagnesemia in elderly patients even if their renal function is normal.

Keywords Hypermagnesemia · Dialysis · Preoperative preparation · Rectum carcinoma · Magnesium citrate

Introduction

Hypermagnesemia is a rare complication caused by intravenous or oral administration of magnesium (Mg) as an antacid, cathartic, or antiarrhythmic; however, when the serum Mg concentration exceeds 9 mg/dL, potentially fatal symptoms can occur such as severe bradycardia, cardiac arrest, paralysis, and respiratory failure [1]. Although symptomatic hypermagnesemia usually occurs in patients with renal dysfunction, people with normal renal function can develop Mg toxicity after therapeutic doses of Mg-containing medications [1–4]. Here, we report the case of a patient who developed fatal hypermagnesemia after mechanical bowel preparation for surgery.

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Case report

An 85-year-old woman suffering from constipation underwent a colonoscopy. After several examinations, she was diagnosed with non-obstructive rectal carcinoma and referred to our hospital for surgical treatment (Fig. 1). She had a history of long-term constipation but had achieved a regular defecation habit via treatment with a daily 1 g oral dose of magnesium oxide and 24 mg of sennoside. She also had a history of abdominal artery aneurysm, cerebral infarction, and femoral fracture. Her renal function was normal (Cr, 0.46 mg/dL; eGFR, 94 mL/min 1.73 m^2). For preoperative mechanical bowel preparation, she received a daily oral dose of 34 g magnesium citrate (Magcolol P®; Horii Pharmaceutical Ind. Ltd, Osaka, Japan) in 500 mL of water containing 2,710 mg of Mg. Although she had a loose stool passage after the first dose, the accumulation of remnant feces was suspicious. She received a total of 68 g of magnesium citrate slowly over 2 days and produced soft bowel movements. On the morning of the operation day (day 1), she vomited and went into shock and was transferred to the intensive care unit. She did not have a heart murmur, but lung crackles were audible from her right chest. Her abdomen was hard and distended, and bowel sounds were diminished. Her deep tendon reflexes were

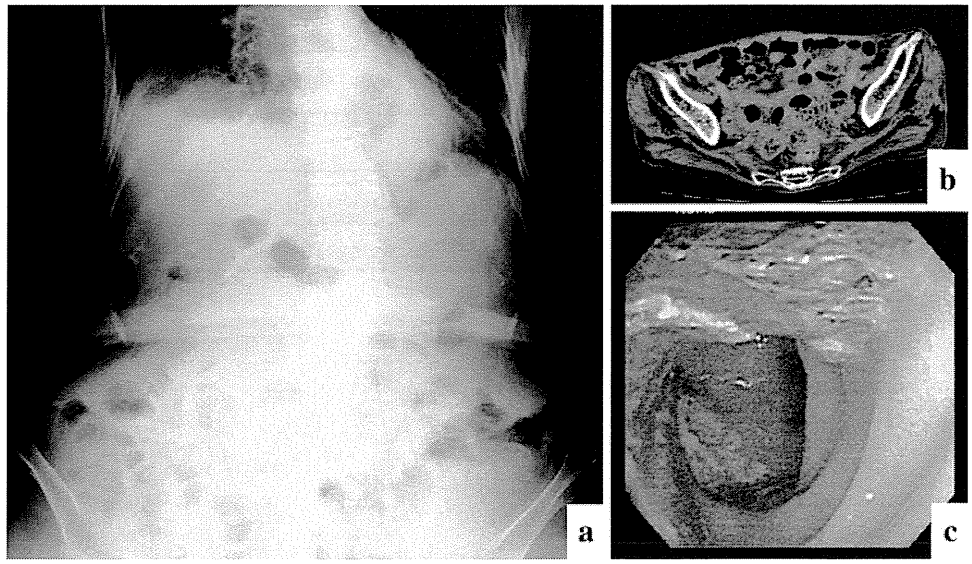

Fig. 1 **a** Preoperative radiograph showing no signs of bowel obstruction. **b** Bowel distension was not detected on abdominal computed tomography. **c** Colonoscopy revealed carcinoma located in the rectosigmoid portion

absent. Radiography showed consolidation in the right lung, indicating aspiration pneumonia, and massive air in the small bowel, suggesting paralytic ileus. Considering ileus and aspiration pneumonia, she was then intubated and supported by mechanical ventilation. To maintain stable hemodynamics, a 10 μg/kg min dopamine drip infusion was started. However, bradycardia occurred repeatedly, and the electrocardiogram showed sinus arrest with junctional escape rhythm. After recovery from cardiac arrest by chest compression, additional administration of dobutamine and norepinephrine was necessary to improve her hemodynamics. Protracted bradycardia required temporary cardiac pacing, and laboratory data showed elevated inflammatory reactions and a decreased anion gap (Table 1). On day 2, the patient showed marked hypermagnesemia (14.3 mg/dL), which strongly suggested that hypermagnesemia was the cause of her refractory bradycardia and cardiac arrest. Although urinary Mg level (232.1 mg/dL) and the Mg excretion fraction (52.4 %) indicated adequate clearance ability, we decided to perform hemodialysis aiming for rapid recovery from severe symptoms. During the hemodialysis session, her hemodynamics dramatically stabilized, and dopamine, dobutamine, and norepinephrine were rapidly withdrawn (Fig. 2). After a single 4-h session of hemodialysis, her serum Mg level decreased to 5.3 mg/dL, and she was returned to the surgery ward on day 9. On day 45, she underwent a laparoscopic low anterior resection with preparation by sodium picosulfate. She had a good postoperative course and was discharged on day 63 without any major medical problems. Interestingly, her serum calcium level was not decreased despite an abnormally high Mg level, and her intact parathyroid hormone (PTH) level remained high even after normalization of Mg and calcium (Table 1). Further examination by ultrasonography and methoxyisobutylisonitrile scintigraphy revealed an adenoma of the parathyroid gland and hyperparathyroidism. However, we did not perform any invasive surgical procedures for her parathyroid adenoma because of her normal calcium level and her age.

Table 1 Laboratory tests

	Day 1	Day 2	Day 3	Day 9	3 months later
WBC (/μL)	21,600	7,600	4,900	5,300	3,900
Neutrophils (%)	65.9	76.9	77.5	62.0	75.2
Na (mEq/L)	138	142	142	143	142
K (mEq/L)	2.9	4.3	3.8	3.9	3.6
Cl (mEq/L)	100	103	108	109	110
Ca (mg/dL)	–	10.2	9.6	10.4	9.5
Alb (g/dL)	3.2	3.5	2.9	2.6	2.6
Mg (mg/dL)	–	14.3	5.3	1.7	1.9
FEMg (%)	–	52.4	–	–	3.9
BUN (mg/dL)	22.0	34.8	18.6	17.0	13.4
Cr (mg/dL)	0.46	0.84	0.71	0.42	0.4
CRP (mg/dL)	0.8	10.4	17.8	1.6	2.9
iPTH (pg/mL)	–	87	–	–	77
pH	7.417	7.608	7.466	–	–
PaO_2 (mmHg)	32.0	110	144	–	–
$PaCO_2$ (mmHg)	57.9	24.7	37.4	–	–
HCO_3 (mEq/L)	36.6	28.1	26.6	–	–
AG (mEq/L)	1.4	10.9	7.4	–	–
Urinary Mg (mg/dL)	–	232.1	–	–	14.4

Discussion

We examined a case of severe hypermagnesemia despite normal renal function. The patient showed severe bradycardia refractory to medical therapy, which thus required

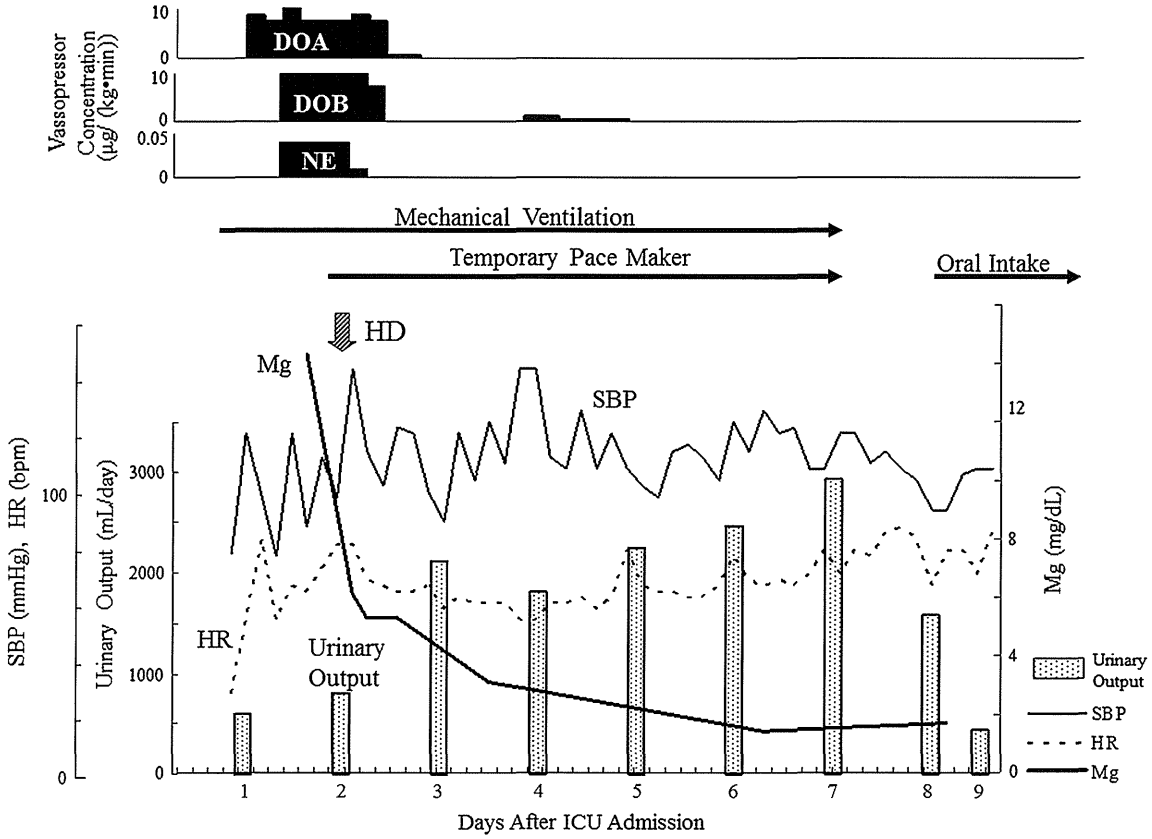

Fig. 2 Clinical course of the patient. *SBP* systolic blood pressure, *HR* heart rate, *Mg* magnesium, *HD* hemodialysis, *DOA* dopamine, *DOB* dobutamine, *NA* norepinephrine, *ICU* intensive care unit

temporary pacing; however, she dramatically recovered by hemodialysis.

Mg is the second most abundant intracellular cation with 67 % of the total body stores found in bone, 31 % found in intracellular spaces, and only 2 % found in extracellular regions. Disorders resulting in Mg elevation include acute renal failure, chronic renal failure, Addison disease, hyperparathyroidism, and familial hypocalciuric hypercalsemia. Lithium administration also causes an increase in Mg levels.

Mg is absorbed through the small intestine by passive paracellular transport through Claudin-16/19 and by active transcellular transport through TRPM6 [5]; the former is thought to be dominant. In a patient with constipation, increasing Mg absorption may cause hypermagnesemia. High levels of extracellular Mg inhibit acetylcholine release from the neuromuscular end-plate and thereby cause paralysis of smooth muscles, resulting in intestinal ileus. Furthermore, elevated intrabowel pressure can promote Mg absorption, creating a vicious cycle. The necessity of preoperative mechanical bowel preparation is still controversial, although it is commonly used in Japan to prevent postoperative infection or anastomotic leak. The cathartics we used contained 5,420 mg of Mg, and although this was an appropriate dose increase recommended by the pharmaceutical manufacturer, it could cause life-threatening hypermagnesemia for patients at high risk for increased absorption or for decreased excretion of Mg. A periodic examination of serum Mg level is necessary when Mg-containing agents are administered for a long time even if these agents are administered at a routine dosage level.

The kidneys are primarily responsible for the excretion of Mg. The mechanism of renal handling of filtered Mg is similar to that of the intestine and involves passive paracellular transport in the proximal tubule (approximately 20 %) and in the thick ascending limb of the loop of Henle (approximately 70 %), as well as active transcellular transport in the distal convoluted tubule (approximately 10 %) [4]. The rate of decline in serum Mg concentration has been reported to follow a logarithmic decay pattern if renal function is normal [5]. The elderly are at a risk for Mg toxicity as kidney function declines with age [11]. Older persons with hypermagnesemia are likely to develop reduced blood flow leading to a prerenal type of renal dysfunction, which creates a vicious cycle. In our case, the patient's renal Mg excretion was remarkably increased with a Mg excretion fraction of 52.4 % (normal value

Table 2 Literature review

References	Age/sex	Clinical examination			Baseline disease	Mg load		Symptom				
		Serum Mg (mg/dL)	Serum Cr (mg/dL)	eGFR (mL/min 1.73 m^2)		Mg quantity	Cause	Vomiting	Abdominal pain	Bradycardia	Hypotension	Somnolence
Schelling [12]	81/F	21.2	1.8	20	AAA COPD	1 g	Operation	+	+	+	+	
Onishi [13]	89/M	12.6	1.1	47	Unilateral kidney	2.7 g	Colonoscopy	+	+		+	+
Weber [14]	77/F	10.3	1.5	25	COPD (theophylline intake)	1.3 g	Treatment for theophylline toxicity					+
McLaughlin [15]	42/F	9.1	0.9	51	Schizophrenia ileus	4.6 g	Constipation	+	+		+	
Kontani [4]	76/F	16.6	1.4	21	Constipation	2.7 g	Constipation		+		+	+
Fung [3]	69/F	16.2	1.7	22	Constipation	24 g	Constipation					
Kikuchi [16]	79/F	11.4	1.3	29	Constipation	0.5 g/day + 2.7 g	Colonoscopy		+	+	+	+
Golzarian [17]	67/F	8.1	2.6	13	Constipation	3 g	Constipation	+				
Weber [14]	61/F	6.9	0.6	60	COPD (theophylline intake)	55 g	Treatment for theophylline toxicity				+	+
Golzarian [17]	65/F	5.1	1	41	Constipation	5 g	Constipation	+	+			
Our case	85/F	14.3	0.5	89	Constipation	0.5 g/day + 5.4 g	Regular intake + operation	+		+	+	+

References	Age/sex	Symptom					Therapy						Outcome
		Absent deep tendon reflexes	Respiratory depression	Paralysis	Complete heart block	Cardiac arrest	Calcium administration	High-volume infusion	Hemodialysis	Mechanical ventilation	Vasopressor	Pacing	
Schelling [12]	81/F						+	+	+				Dead
Onishi [13]	89/M		+			+	+	+			+	+	Dead
Weber [14]	77/F						+	+		+	+		Dead
McLaughlin [15]	42/F					+	+	+		+	+		Dead
Kontani [4]	76/F		+			+		+		+	+	+	Alive
Fung [3]	69/F			+	+			+		+			Alive
Kikuchi [16]	79/F						+	+	+	+	+		Alive
Golzarian [17]	67/F							+					Alive
Weber [14]	61/F						+	+		+	+		Alive
Golzarian [17]	65/F		+					+					Alive
Our case	85/F	+	+		+	+		+	+	+	+	+	Alive

AAA abdominal aortic aneurysm, *COPD* chronic obstructive pulmonary disease

3–5 %), indicating that her renal function was normal and her kidney was unlikely to be the cause of the hypermagnesemia. However, PTH, which affects renal Mg reabsorption and is discussed later in this paper, may have contributed to her onset of hypermagnesemia.

To date, there is no hormone known to primarily regulate serum Mg concentration. However, Mg homeostasis is influenced by many factors including PTH, vitamin D, antidiuretic hormone, 17β-estradiol, glucagon, acid–base status, FK506, cyclosporine, diuretics, epithelial growth factor, the anticancer agent cetuximab, and receptors for activated C-kinase 1 [6]. PTH is thought to activate TRPM6 and thereby increase renal Mg reabsorption from the distal convoluted tubule [6]. In this case, autonomously secreted PTH from a benign adenoma increased both calcium and Mg absorption in the thick ascending limb [7]. Furthermore, metabolic alkalosis caused by the absorption of citric acid contained in magnesium citrate may have also decreased renal Mg excretion in the distal tubule. Administered citric acid is metabolized to bicarbonate in the liver [8]. Acute and chronic metabolic alkalosis may have consistently led to a decrease in urinary Mg excretion [8]. Hyperparathyroidism and metabolic alkalosis, which were observed in this case, might have caused the patient's pathogenesis despite her normal renal function. Our case is unique because although there have been several reports of fatal hypermagnesemia, here we have demonstrated a possible association between hyperparathyroidism and the development of fatal hypermagnesemia. Clinical manifestations of hypermagnesemia vary according to the serum Mg concentration. Hypotension, nausea, vomiting, facial flushing, urinary retention, and ileus occur at levels ranging from 5–8 mg/dL, while the absence of deep tendon reflexes and somnolence occur at 9–12 mg/dL. Respiratory depression, paralysis, and complete heart block occur at levels >15 mg/dL, and cardiac arrest occurs in asystole at levels >20 mg/dL [1, 4]. Although details of the inhibitory effect of Mg on cardiac function have not been fully elucidated, several mechanisms have been suggested, including inhibition of norepinephrine release by sympathetic postganglionic nerves [1] and blocking of calcium and potassium channels [9].

This case exhibited typical manifestations of severe hypermagnesemia; however, we did not recognize these manifestations until day 2. Indeed, the incidence of hypermagnesemia as identified based on physician-initiated requests is reported to be approximately 13 % because most symptoms of hypermagnesemia are nonspecific, and serum Mg is not routinely measured [10]. In our patient, considering her elevated renal Mg excretion, hypermagnesemia would have recovered spontaneously within several days without hemodialysis. In cases such as this, hypermagnesemia would never be diagnosed if not suspected. In our case, severe bradycardia was a prominent feature of hypermagnesemia. Additionally, a decreased serum anion gap indicated an increase in unmeasured cations such as calcium and Mg.

Treatments for hypermagnesemia include high-dose calcium administration, Mg washout via high-volume normal saline infusion, and administration of a loop diuretic and hemodialysis. Loop diuresis inhibits tubular reabsorption of Mg in the thick ascending limb of Henle's loop. Hemodialysis is extremely effective in cases with severe symptoms or renal failure. The expected change in serum Mg level after a 3- to 4-h dialysis session is approximately one-third to one-half between the dialysate Mg concentration and the predialysis ultrafilterable serum Mg [11]. In our patient, serum Mg decreased from 14.2 to 5.3 mg/dL; in fact, her hemodynamics improved remarkably during (rather than after) the hemodialysis session.

We searched the term 'hypermagnesemia' in English literature published between 1989 and 2011 by using the National Library of Medicine's PubMed database (http://www.pubmed.gov) [3, 4, 12–17] and found 10 cases in addition to our case which are summarized in Table 2. Almost all the patients were elderly and had insufficient renal function. Some cases showed extremely high serum Mg levels, and 4 cases succumbed to death. Kontani et al. [4] suggested that severe hypermagnesemia could occur even in the absence of pre-existing renal dysfunction, particularly if the patient is elderly.

In conclusion, Mg-containing preoperative preparations may lead to the development of hypermagnesemia even in patients with normal renal function. Although hypermagnesemia requires early diagnosis and a rapid response, it is often diagnosed only when the condition is severe. The monitoring of serum Mg concentration and/or the serum anion gap before and after the administration of Mg-containing cathartics would be useful for avoiding life-threatening hypermagnesemia. Mg-free cathartics are the ideal choice of mechanical bowel preparations for patients with renal dysfunction, severe constipation, or a long-term history of Mg-containing treatment.

Conflict of interest The authors declare that they have no conflict of interest.

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