Surgery Today (2019) 49:785–794
https://doi.org/10.1007/s00595-019-01792-y

ORIGINAL ARTICLE

Intraperitoneal chemotherapy for peritoneal metastases using sustained release formula of cisplatin-incorporated gelatin hydrogel granules

Kota Yamashita[1,2] · Shigeru Tsunoda[1] · Shutaro Gunji[1] · Takahide Murakami[1,2] · Takahisa Suzuki[1,2] · Yasuhiko Tabata[2] · Yoshiharu Sakai[1]

Received: 15 October 2018 / Accepted: 13 February 2019 / Published online: 7 March 2019

Abstract

Purpose We previously reported the effectiveness of gelatin microspheres incorporating cisplatin in a mouse model of peritoneal metastases. In this study, we report our new complete sustained-release formula of gelatin hydrogel granules incorporating cisplatin (GHG–CDDP), which exerted a good anti-tumor effect with less toxicity.

Methods GHG–CDDP was prepared without organic solvents to enable its future clinical use. The pharmaceutical characterization of GHG–CDDP was performed, and its in vivo degradability was evaluated. The anti-tumor effect was evaluated using a murine peritoneal metastasis model of the human gastric cancer MKN45-Luc cell line.

Results Our new manufacturing process dramatically reduced the initial burst of CDDP release to approximately 2% (wt), while the previous product had a 25–30% initial burst. In intraperitoneal degradation tests, approximately 30% of GHG–CDDP remained in the murine abdominal cavity 7 days after intraperitoneal injection and disappeared within 3 weeks. GHG–CDDP significantly suppressed the in vivo tumor growth ($p = 0.02$) and prolonged the survival time ($p = 0.0012$) compared with the control. In contrast, free CDDP did not show a significant therapeutic effect at any dose. Weight loss and hematological toxicity were also significantly ameliorated.

Conclusions GHG–CDDP is a promising treatment option for peritoneal metastases through the complete sustained-release of CDDP with less systemic toxicity.

Keywords Gelatin hydrogel · Peritoneal metastases · Intraperitoneal chemotherapy · Cisplatin · Gastric cancer

Introduction

Peritoneal metastases are one of the most frequent and life-threatening forms of metastases and recurrence in patients with gastric, pancreatic, colorectal, appendiceal, and ovarian tumors, and malignant peritoneal mesothelioma [1]. Several types of therapy for peritoneal metastases have been investigated, including systemic and hyperthermic intraperitoneal chemotherapy (HIPEC), and cytoreductive surgery [2–4]. Although systemic chemotherapy has been used as a standard treatment, inevitable adverse systemic effects have been reported. Accordingly, local treatment is expected to reduce systemic toxicity. Intraperitoneal chemotherapy is one choice for the treatment of ovarian cancer, but it is not the standard of care. Furthermore, it is expected that local treatment will be effective for mesothelioma, low-grade appendiceal cancer, and colon cancer with peritoneal metastases [4–9]. In addition, recent studies have shown that cytoreductive surgery to remove macroscopic lesions, in combination with HIPEC, is effective for the treatment of peritoneal metastases in gastric and colorectal tumors [10, 11].

Cisplatin (cis-dichlorodiammineplatinum (II), CDDP) is one of the strongest cytotoxic agents and has conventionally been used in the treatment of cancer [12, 13]. CDDP continues to play an important role as the first-line treatment for some malignancies. However, its clinical application is often restricted because it causes severe systemic adverse effects,

✉ Shigeru Tsunoda
tsunoda@kuhp.kyoto-u.ac.jp

[1] Department of Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan

[2] Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

such as nephrotoxicity, myelosuppression, and gastrointestinal disturbances. In terms of intraperitoneal injection, CDDP has been routinely used in conjunction with cytoreductive surgery for peritoneal mesothelioma. However, the effectiveness of intraperitoneal CDDP injection for gastric cancer remains unclear [14, 15].

Drug delivery systems have been intensively studied with the aim of achieving higher local concentrations and prolonged retention of CDDP at the tumor site. Polymeric micelles, microspheres, and liposomes have primarily been used as the drug delivery vehicles for CDDP [16–19]. We have already demonstrated the therapeutic efficacy of gelatin hydrogel as a carrier for the sustained release of CDDP via intraperitoneal injection [20]. However, we observed an initial burst of CDDP, which is a phenomenon caused by the early drug release of non- or weakly bound drugs to macromolecule materials. This result is often observed in sustained release drug formulations and is closely related to adverse effects. Therefore, it is important to reduce the initial burst.

We herein report a new preparation of gelatin hydrogel granules (GHGs) incorporating CDDP (GHG–CDDP) for the sustained release of CDDP with a minimal initial burst along with our pharmacological assessment.

Materials and methods

Materials

A gelatin sample with a low level of endotoxin (beMatrix® LS-H) and an isoelectric point of 5.0, prepared by an alkaline process using porcine skin, and collagenase L were kindly supplied by Nitta Gelatin, Inc. (Osaka, Japan). A cell line of poorly differentiated human gastric adenocarcinoma cells expressing luciferase (MKN45-luc) was purchased from the JCRB Cell Bank, National Institutes of Biomedical Innovation, Health and Nutrition (Osaka, Japan). The cells were maintained in GIBCO® RPMI1640 (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 10% (vol) fetal bovine serum (Thermo Fisher Scientific Inc.) and 50 U/mL of penicillin and streptomycin (Nacalai Tesque, Inc., Kyoto, Japan) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Balb/c nude mice (female; 16–20 g body weight; 6 weeks old) and ddY (female; 16–20 g body weight; 4 weeks old) mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan).

All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institute of Health. The study protocol received approval from the Institutional Animal Experiment Committee (No. F-15-196).

Preparation of GHGs

GHGs were prepared by glutaraldehyde (GA; Wako Pure Chemical Industries, Osaka, Japan) cross-linking of an aqueous gelatin solution, as described previously [21]. In brief, after mixing various amounts (20, 40, 80, 160, and 320 μL) of aqueous GA solution [25% (wt)] with an aqueous gelatin solution [5% (wt)] preheated at 40 °C, the mixed aqueous solution was cast into a polystyrene dish, which was then left for 12 h at 4 °C for gelatin cross-linking. The resulting hydrogel sheets were then placed in a 100 mM aqueous solution of glycine (Nacalai Tesque), followed by agitation at room temperature for 1 h to block the residual aldehyde groups of unreacted GA. The cross-linked hydrogel sheets were washed twice with double-distilled water (DDW), homogenized with a Polytron PT-13000DM system (Central Scientific Commerce Inc., Tokyo, Japan), and separated via sieves to obtain GHGs with diameters ranging from 52 to 75 μm in the DDW-swollen state.

Preparation of GHG–CDDP

To prepare GHG–CDDP, cross-linked GHGs (2 mg) were immersed in 100 μL of CDDP solution (2 mg/mL) at 37 °C, followed by overnight incubation at 37 °C to allow the formation of CDDP–gelatin conjugates, which were then freeze-dried for 48 h. The samples were swollen and then repeatedly washed in DDW and centrifuged (20 °C, 5 min, 5000 rpm) to remove uncombined cisplatin from the gelatin hydrogels incorporating CDDP before freeze-drying. This rinsing process was repeated twice. Thereafter, GHG–CDDP was freeze-dried for 48 h and sterilized with ethylene oxide gas. The prepared 2.5 mg of GHG–CDDP contained 100 μg CDDP.

Characterization of GHG–CDDP

GHG–CDDP dispersed in water was observed by light microscopy (CKX41; Olympus, Tokyo, Japan), and dried GHG–CDDP was imaged by scanning electron microscopy (Model SU8000; Hitachi, Ltd., Tokyo, Japan) after platinum coating.

To evaluate the in vitro GHG degradability, GHGs (5 mg) were fully swollen in 500 μL of DDW for 12 h at 25 °C, suspended in 500 μL of 2 N HCl aqueous solution, and incubated at 25 °C. At different time intervals, 200 μL of supernatant was collected after centrifugation. Fresh 1N HCl aqueous solution (200 μL) was added, and the preparation was incubated at 25 °C. The absorbance

of each supernatant at 260 nm was measured on an ultraviolet–visible spectroscopy (DU 800 Spectrophotometer; Beckman Coulter, Inc, Fullerton, CA, USA) to obtain a time profile for GHG degradation.

To evaluate the CDDP release, GHG–CDDP was suspended in 10 mL of DDW containing 0.01% (wt) Tween-80 (Wako Pure Chemical Industries), followed by reciprocal shaking at 60 strokes/min at 37 °C. The supernatant was pipetted 1 and 24 h later, and the same volume of DDW was immediately added. After 24 h, the same volume of phosphate-buffered saline (PBS) containing 500 μL of 50 mg/mL collagenase L solution was added to each sample. A similar collection process was performed 1 h later. The concentration of CDDP released in the supernatant was measured by measuring platinum (Pt) detected on a polarized Zeeman Z-8000 atomic absorption spectrophotometer (Hitachi, Ltd.). Five different replicates were performed for each experiment.

The in vitro cytotoxicity assay

The cytotoxic effects of GHG–CDDP were assessed at different CDDP-equivalent concentrations up to 0.1 mg/mL (CDDP equivalents). GHG–CDDP was digested in PBS with 0.8 mL of 100 μg/mL collagenase L solution for 24 and 72 h to prepare GHG–CDDP samples at different stages of degradation. MKN45 Luc (5000 cells/well) was treated with either GHG–CDDP, digested GHG–CDDPs, or free CDDP solution for 48 h, and the numbers of living cells were counted. Cell viability was evaluated according to the conventional mitochondrial assay using a tetrazolium compound (2-(2-methoxy-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) and 1-methoxy phenazine methosulfate. Formazan absorbance was measured at 450 nm on a microtiter plate reader (Spectra Max i3x; Molecular Devices LLC, San Jose, CA, USA). Cell viability was evaluated as the percentage of live cells relative to that of PBS-treated control cells. Each experiment was performed in five replicate wells.

The evaluation of the in vivo GHG–CDDP degradability

The in vivo degradability of GHG–CDDP after intraperitoneal injection was evaluated using ^{125}I-labeled GHG–CDDP. PBS was used to swell GHG–CDDP, and Bolton–Hunter reagent (PerkinElmer Inc., Waltham, MA, USA) was added for radioiodination, followed by several washes with PBS for 5 days to remove the unbound Bolton–Hunter reagent. Female ddY mice (5 weeks old) were anesthetized, and ^{125}I-labeled GHG–CDDP (2.5 mg) was injected intraperitoneally. Mice were killed at each time point, and 2 mL of PBS was injected intraperitoneally. Ascitic fluid was collected to check the radioactivity. The retention of radiolabeled GHG–CDDP was evaluated as the percentage of radioactivity compared with that of samples collected immediately after injection. Each experiment was independently performed in triplicate.

The in vivo retention of CDDP after GHG–CDDP injection

Degraded GHG–CDDP was prepared in the same manner as that in the in vitro cytotoxicity assay. GHG–CDDP, degraded GHG-CDDP, and free CDDP were injected intraperitoneally at a dose of 5 mg/kg of CDDP equivalent. Blood was taken directly from the heart at each time point. The CDDP concentrations in plasma were measured with an atomic absorption spectrophotometer (Hitachi, Ltd.).

In vivo toxicity study

To observe weight changes, mice were injected intraperitoneally with GHG–CDDP at doses of 5 and 10 mg CDDP/kg and with free CDDP doses of 5 mg CDDP/kg on days 0 and 7.

For hematological evaluations, mice were injected intraperitoneally with GHG–CDDP and free CDDP at doses of 5 mg CDDP/kg on days 0 and 7, and the blood was taken directly by cardiac puncture on day 9. The numbers of white blood cells (WBCs) and platelets and the serum concentrations of blood urea nitrogen (BUN) and creatinine were analyzed at FALCO Biosystems, Ltd. (Kyoto, Japan). Mice were killed, and the kidneys were harvested for histological evaluation on day 9, fixed in 4% (wt) paraformaldehyde, and embedded for staining with hematoxylin and eosin.

The evaluation of the in vivo anti-tumor effects in a murine peritoneal metastasis model

MKN45 Luc cells (5×10^6/mouse) were inoculated into the peritoneal cavities of 6-week-old female BALB/c nude mice on day 0. Subsequently, the mice were injected intraperitoneally with GHG–CDDP at doses of 5 and 10 mg CDDP/kg or free CDDP at doses of 1, 2, 3, and 5 mg CDDP/kg on days 5 and 12. To assess the intraperitoneal tumor volume, macroscopic in vivo luminescence imaging was carried out using an IVIS system (In vivo imaging system) (IVIS spectrum; SPI, Tokyo, Japan) on day 26. Total counts of luminescence at the abdominal sites of the mice were measured as the tumor volume.

Statistical analyses

All results are expressed as the mean ± the standard error. Comparisons of values between the groups at each time point were performed using two-tailed Student's *t* test and

the Tukey–Kramer method. A survival analysis was performed using the Kaplan–Meier method, and differences were compared by the log rank test. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the JMP software program, version 12 (SAS Institute, Cary, NC, USA).

Results

Characterization of GHG and GHG–CDDP

Light microscopy images of GHG–CDDP under water-swollen and dry conditions are shown in Fig. 1a, b, respectively. GHG–CDDP particles had irregular shapes of 80–120 μm under both conditions. Figure 1c shows the in vitro degradation profiles of GHGs prepared at different GA concentrations. GHGs degraded faster as the GA concentration decreased. GHG4s showed the same in vitro degradation profiles as the gelatin microspheres reported previously [20]. Based on these findings, GHG4 was used for the subsequent experiments.

Figure 1d shows the in vitro time profiles of CDDP release from the GHG4-CDDP. The original GHG–CDDP showed an initial burst of 30% CDDP, while no further release was observed thereafter. However, when the original GHG–CDDP was rinsed (to remove non-bound free CDDP), the initial burst was significantly reduced to approximately 2%. When collagenase was added to force GHG to degrade, CDDP was rapidly and completely released.

The in vitro anti-tumor activity of GHG–CDDP

As stated previously, GHG–CDDP was prepared at the stage of degradation (Fig. 2a). Figure 2b shows the in vitro cytotoxicity of free CDDP and GHG–CDDP at the degradation stage in MKN45-Luc cells. As GHG–CDDP degraded, the therapeutic effect increased proportionally. However, even after the total degradation of GHG–CDDP, the cytotoxicity was lower than that of free CDDP. The

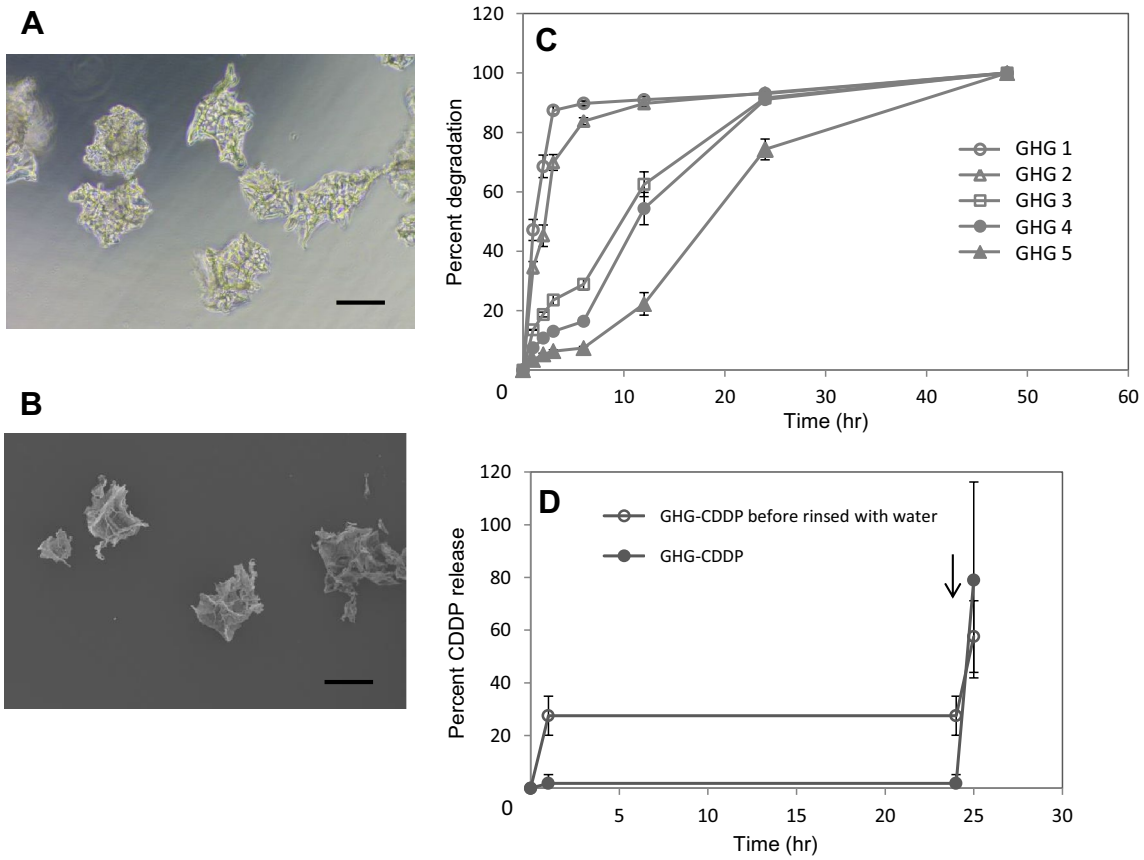

Fig. 1 Characterization. **a** A light microscopic picture of gelatin hydrogel granules (GHGs) incorporating CDDP dispersed in water. Scale bar: 100 μm. **b** SEM picture of dried GHGs incorporating CDDP. Scale bar: 100 μm. **c** Time profiles of the degradation of GHGs prepared at various concentrations of glutaraldehyde (GA). (○) GHG1 GA 20 μL, (△) GHG2 GA 40 μL, (□) GHG3 GA 80 μL, (●) GHG4 GA 160 μL, (▲) GHG5 GA 320 μL. ($N = 5$). **d** The time profile of CDDP released from GHG–CDDP before being rinsed with water (○) and GHG–CDDP (●) ($N = 5$). Collagenase was added at the time indicated by an arrow

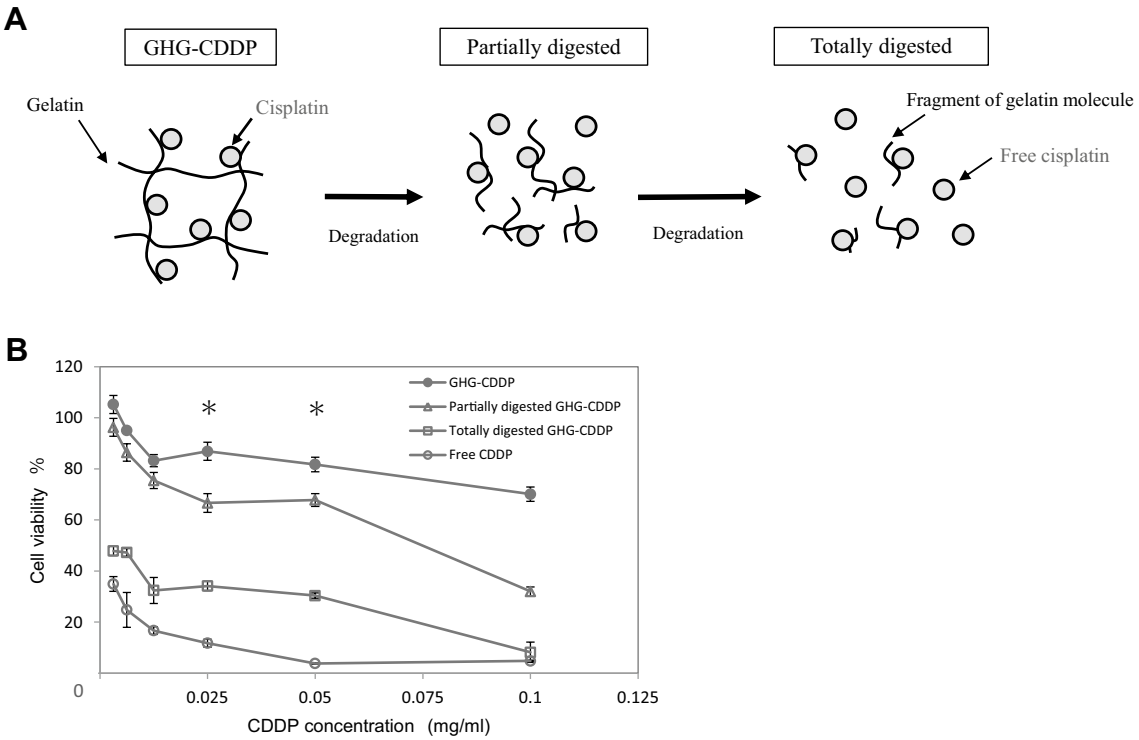

Fig. 2 **a** Schematic illustration of the stage of GHG–CDDP degradation. **b** In vitro cytotoxicity of free CDDP and GHG–CDDP at different stages of degradation in MKN45-Luc cells (N = 5). (○) Free CDDP, (Δ) partially digested GHG–CDDP, (□) totally digested GHG–CDDP, and (●) GHG–CDDP. *$p < 0.05$, significant among groups

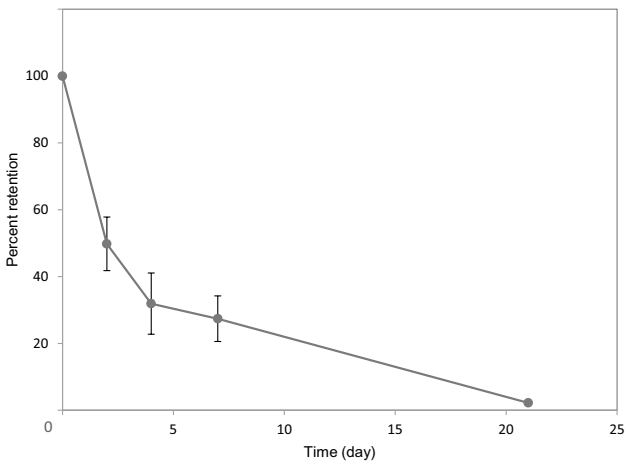

Fig. 3 Time profile of the in vivo degradability of GHG–CDDP after implantation ($N = 5$)

cytotoxicity levels of all groups at 0.05 and 0.025 mg/ml differed significantly from each other. As expected, the non-degraded GHG–CDDP had much lower cytotoxicity than the totally degraded GHG–CDDP.

In vivo degradability of GHG–CDDP

Figure 3 shows the time profile of the in vivo degradability of GHG–CDDP after injection. GHG–CDDP degraded in the abdominal cavity over time to 27% at 7 days after injection and disappeared within 3 weeks.

In vivo retention of CDDP after GHG–CDDP injection

While the CDDP concentrations increased immediately after the injection of free CDDP, the concentrations were slightly elevated after GHG–CDDP injections and much lower over the following days (Fig. 4). Interestingly, collagenase-digested GHG–CDDP (Fig. 2a) provided higher blood concentrations of CDDP than GHG–CDDP, but the levels were still lower than those after free CDDP injections.

In vivo toxicity

Figure 5a shows the in vivo toxicity in normal mice. After two intraperitoneal injections of GHG–CDDP at a 1-week interval, temporary dose-dependent weight loss was observed. However, it was considerably less than that following free CDDP and recovered within 4 weeks to a level equal to that of the control (PBS injected) group. The weight

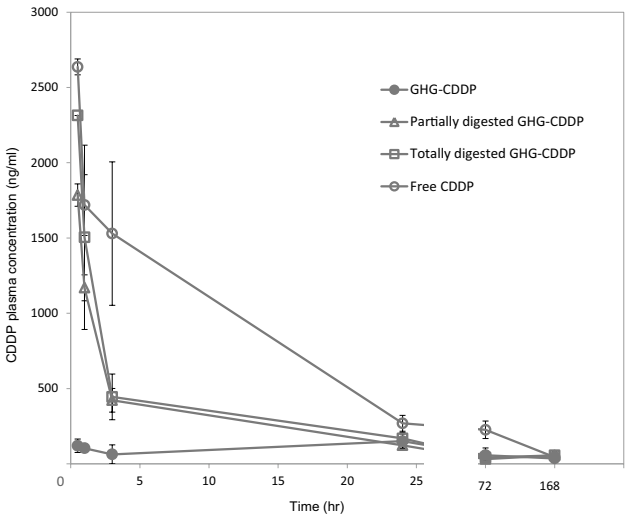

Fig. 4 In vivo retention of CDDP after GHG–CDDP injection. Each group was administered the same amount of CDDP (CDDP 100 μg/body) ($N=5$). ○, free CDDP, Δ, partially digested GHG–CDDP, □, totally digested GHG–CDDP, and ●, GHG–CDDP

loss in the free CDDP group was severe and significantly lower than that of the GHG–CDDP group (5 mg/kg). Free CDDP injections resulted in the death of all mice within 16 days after the first injection. In contrast, no deaths were observed, even at 10 mg CDDP/kg, in the GHG–CDDP group, in which the preparation contained more CDDP than that of the free CDDP group (5 mg/kg).

The number of WBCs was significantly lower in the free CDDP group than in the control PBS-injected group, being equivalent to Grade 3 leukopenia according to the Common Terminology Criteria for Adverse Events (CTCAE) in humans (Fig. 5b). The number of platelets was significantly lower in the free CDDP group than in the control groups, being equivalent to Grade 4 according to the CTCAE (Fig. 5c). In addition, no severe thrombocytopenia was observed in the GHG–CDDP groups.

There were no significant differences in the serum BUN and creatinine concentrations between the GHG–CDDP and the free CDDP groups (data not shown). However, a histological evaluation revealed patchy and diffuse denudation of

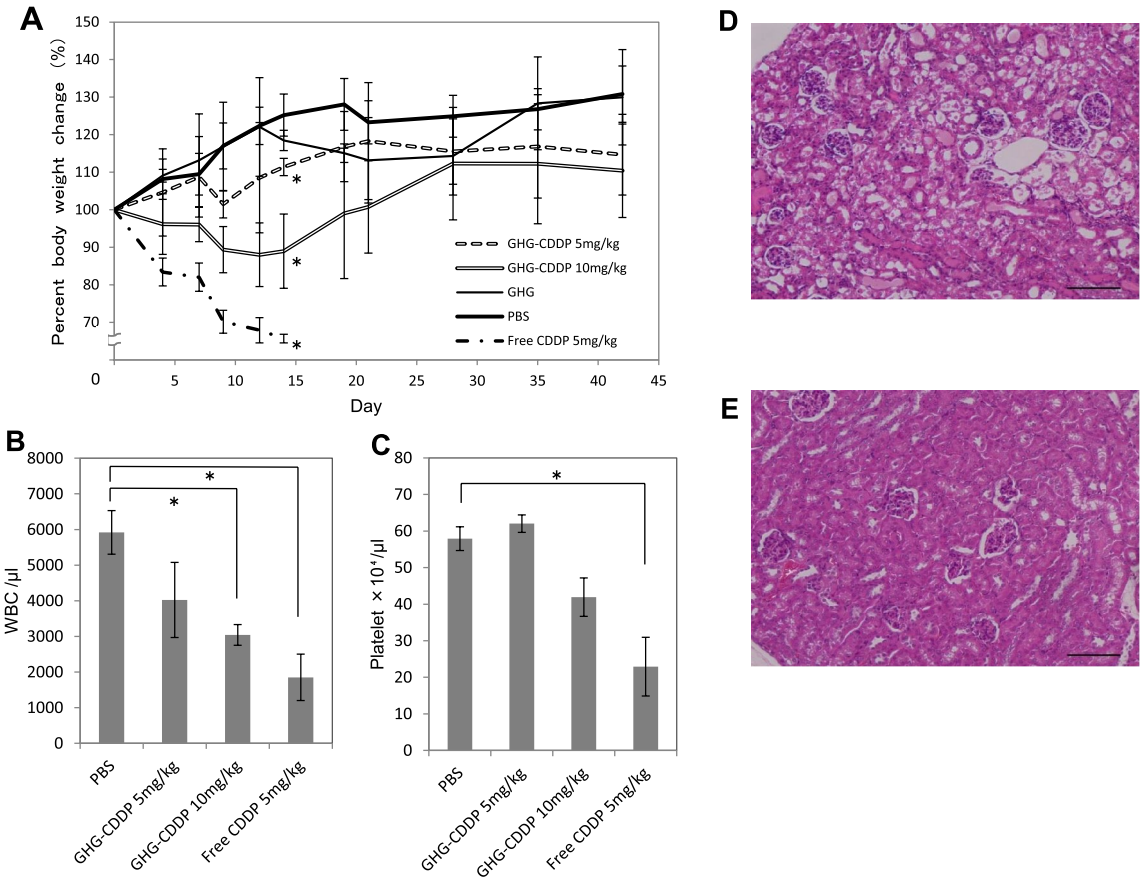

Fig. 5 An in vivo toxicity evaluation of normal mice after GHG–CDDP injection. **a** Time profiles of body weight changes in normal mice after intraperitoneal injection of PBS (thick solid line). GHG (thin solid line), free CDDP 5 mg/Kg (dot-dash line), GHG–CDDP 5 mg/kg (double dashed line), and GHG–CDDP 10 mg/kg (double line) ($N=4$). *$p<0.05$, significant difference versus the PBS group. **b** Number of WBCs or **c** platelets 9 days after injection ($N=5$). *$p<0.05$, significant between the two groups. **d, e** Histological sections of kidney 9 days after injection of free CDDP (5 mg/kg) (**d**) or GHG–CDDP (5 mg/kg) (**e**). Scale bars: 100 μm

renal tubular cells in the free CDDP group (Fig. 5d), which suggested acute tubular necrosis. In contrast, there were no histological changes in the GHG–CDDP group (Fig. 5e).

The in vivo anti-tumor activity of GHG–CDDP in the murine peritoneal metastasis model

Figure 6a, b shows the appearances of the tumors and IVIS images in mice 21 days after tumor inoculation. Laparotomy 2 weeks after inoculation revealed many white nodules in the greater omentum, subphrenic area, pouch of Douglas, and hepatic portal region (Fig. 6a), and their positions were also clearly detected by IVIS (Fig. 6b).

Figure 6c shows the tumor volumes 21 days after the first injections of free CDDP or GHG–CDDP. The total photon counts in both GHG–CDDP groups were significantly lower than those in the PBS ($p = 0.02$, vs. GHG–CDDP 5 mg/kg) and GHG groups. The evaluation of the free CDDP group was impossible because all mice died beforehand.

Survival times in both GHG–CDDP groups (5 and 10 mg/kg) were significantly longer than in the PBS and GHG groups (Fig. 6d, $p = 0.0012$ and $p = 0.0012$, respectively). The lower dose CDDP (1 mg/kg, 2 mg/kg) did not prolong the survival time. All mice in the free CDDP group (5 mg/kg and 3 mg/kg) died within 2 weeks of the injections because of chemotoxicity. In contrast, all mice in the GHG–CDDP groups survived longer without CDDP-induced death, even at CDDP concentrations two times higher than that of the free CDDP group.

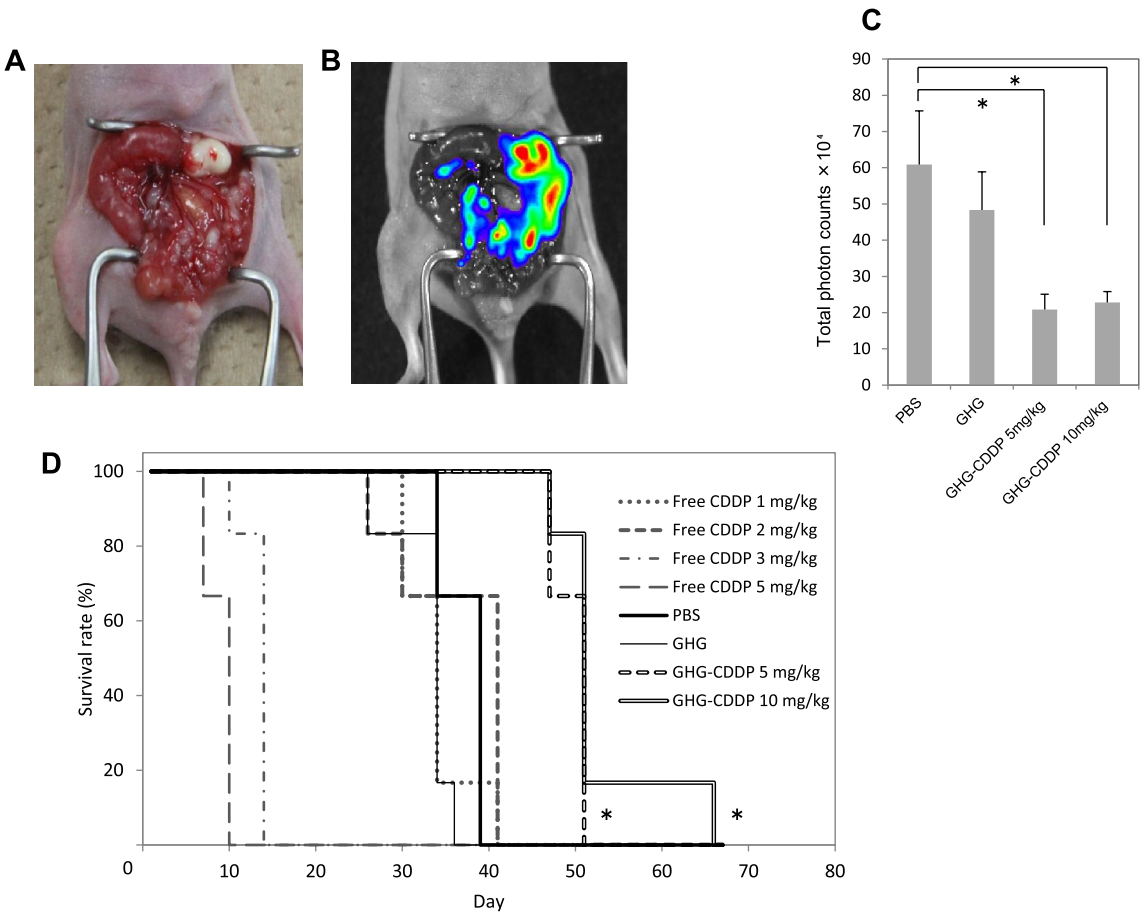

Fig. 6 In vivo anti-tumor effects of GHG–CDDP after injection in a mouse model of peritoneal metastases. **a** Tissue appearance and **b** IVIS image. **c** Total photon counts on IVIS for mice 21 days after tumor inoculation ($N = 6$). *$p < 0.05$, significant between the two groups. **d** Survival time profile. PBS (thick solid line), GHG (thin solid line), free CDDP 1 mg/Kg (dotted line), free CDDP 2 mg/Kg (dashed line), free CDDP 3 mg/Kg (dot–dash line), free CDDP 5 mg/Kg (thick dashed line), GHG–CDDP 5 mg/kg (double dashed line), and GHG–CDDP 10 mg/kg (double line). Drugs were injected at the time indicated by arrows. *$p < 0.05$, significant difference versus the PBS group ($N = 6$)

Discussion

Effective chemotherapy with minimal toxicity is highly desirable to improve the survival of patients, particularly those in advanced stages who have poor chemotolerance. In this study, we developed a GHG–CDDP system to dramatically reduce the notorious toxicity of CDDP while maintaining well-established antitumor effects due to its almost complete sustained release properties.

Initial bursts are phenomena that are often observed in sustained release formulations. However, it is often challenging to develop drug formulations that reduce the initial release of drugs [22, 23]. Our previous gelatin hydrogel system for the sustained release of CDDP [20] showed an initial burst of 30% of the total amount of CDDP, which caused a rapid increase in the intraperitoneal concentration of CDDP and a subsequent steep elevation in the blood concentration. In this study, GHGs were rinsed to allow non-gelatin-bound CDDP molecules to be removed from GHG–CDDP. This process was effective in significantly reducing the initial burst release (Fig. 1d) and diminished the steep elevation in the blood concentrations of CDDP after GHG–CDDP injections (Fig. 4). Accordingly, no major systemic adverse effects, such as body weight loss, bone marrow suppression, or renal dysfunction, were observed. Injections of 5 mg/kg free CDDP resulted in the deaths of all mice in that group within 16 days of the injections. In contrast, no deaths were observed in the GHG–CDDP groups, even at the higher dose of 10 mg/kg (Fig. 5). In addition, GHG–CDDP was prepared without the use of organic solvents, while our previous gelatin hydrogel systems required such substances for the preparation of microspheres. Given that residual solvents are harmful to the human body, this change in the preparation method is an important improvement that may facilitate its future clinical application.

The binding mechanism between CDDP and gelatin molecules has been previously explained as a coordination bond between the Pt atom and the carboxylic groups of gelatin side chains [24]. The ligand exchange of Pt from chlorides to the carboxylic acid of gelatin takes place readily in aqueous solution. When injected into the peritoneal cavity, GHGs are gradually digested via enzymatic biodegradation by MMPs, collagenases, proteases, and so on [25, 26]. Our in vitro cytotoxicity assay showed that CDDP released as a result of GHG degradation had antitumor effects (Fig. 2). This means that, as GHGs are degraded, CDDP bound to gelatin molecules is released and exerts anti-tumor activity. Whether or not the fragments of degraded gelatin molecules were still bound to the CDDP molecule was unclear, even after the complete degradation of GHG–CDDP, but the antitumor activity remained.

No steep elevation in the blood concentrations of CDDP was noted after GHG–CDDP injections (Fig. 4). This indicates that GHG–CDDP remained in the peritoneal cavity, maintaining its anti-tumor activity for a long time, and the degrading GHG–CDDP acted on the tumor directly.

As expected, other platinum anticancer drugs, such as oxaliplatin and carboplatin, were also able to be incorporated into gelatin hydrogels. They were released in a sustained fashion, although the release pattern differed from that of CDDP (data not shown). Therefore, gelatin hydrogels may be potential carriers of anticancer agents for treatments targeting various kinds of peritoneal metastases.

From a clinical perspective, the first-line treatment for advanced gastric cancer is a combination of fluorouracil (5-FU, S-1, capecitabine) and intravenous platinum (CDDP, oxaliplatin) [13, 27]. The present findings suggest that GHG–CDDP may be a feasible replacement for intravenous platinum agents for the treatment of peritoneal metastases of gastric cancer in the future. Several studies have shown that the intraperitoneal use of chemotherapeutic agents increases the local drug concentrations, thereby enhancing the antitumor effects not only against tumor nodules but also against micro-metastases or invisible free tumor cells in the abdominal cavity [28–30]. This suggests that GHG–CDDP may be appropriate not only as a palliative treatment but also as a postoperative adjuvant chemotherapy for patients with positive ascitic cytology during surgery or who have tumors on the serosal surfaces. Furthermore, we previously reported that patients with limited peritoneal metastases of gastric cancer may be potential surgical candidates if the metastases reach complete remission through preoperative induction chemotherapy using S-1 and CDDP [31–33]. Indeed, even in patients with stage IV disease and peritoneal metastases in the initial evaluations, curative surgery was able to be achieved in 54% of patients following preoperative induction chemotherapy, leading to a long-term survival [31]. Therefore, if we could improve the induction of chemotherapy by increasing the therapeutic intensity using GHG–CDDP without toxicity, it would be of substantial benefit to patients who currently have incurable stage IV disease.

Several limitations associated with the present study warrant mention. GHG–CDDP-treated mice had a longer survival than PBS-treated mice, but the results were not robust. Of note, free CDDP did not show a significant therapeutic effect at any dose, including lower doses (1 mg/kg, 2 mg/kg) (Fig. 6), in contrast to GHG–CDDP, which showed therapeutic effects in this study. These findings indicate that GHG–CDDP had a therapeutic advantage over free CDDP.

For future clinical applications, GHG–CDDP should be manufactured on a larger scale following Good Manufacturing Practices (GMP) guidelines, and early-phase clinical trials should be conducted to assess the safety of this treatment.

Conclusions

We developed GHG–CDDP to enable the sustained release of CDDP with a minimal initial burst and suppression of the in vivo tumor growth in a mouse model of peritoneal metastasis. The formulation prolonged the survival time significantly and showed less systemic toxicity than free CDDP. GHG–CDDP showed almost complete sustained release properties with therapeutic efficacy and overcame the systemic adverse effects of CDDP.

GHG–CDDP appears to be a promising novel treatment option for peritoneal metastases. Further studies to evaluate the optimal GHG–CDDP dose, number of injections, time interval between injections, and appropriate combinations with other anticancer drugs are needed for its future clinical application.

Acknowledgements The authors would like to thank Dr. Takaki Sakurai for providing veterinary pathology opinions. This work was supported in part by a JSPS Grant-in-Aid for Scientific Research (C) Grant number JP15K08586.

Author contributions KY, ST, YT, and YS designed the studies. KY, TM, and TS performed the experiments. KY and ST conducted the experiments and analyzed the data. SG assisted with the design of the experiments. KY and ST wrote the manuscript. YT and YS supervised and edited the manuscript. All authors contributed to the research.

Compliance with ethical standards

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

References

1. Sugarbaker PH. Peritoneal metastases from gastrointestinal cancer. Curr Oncol Rep 2018;20:62
2. Huo YR, Richards A, Liauw W Morris DL. Hyperthermic intraperitoneal chemotherapy (HIPEC) and cytoreductive surgery (CRS) in ovarian cancer: a systematic review and meta-analysis. Eur J Surg Oncol 2015;41:1578–1589.
3. Yan TD, Black D, Savady R. Sugarbaker PH Systematic review on the efficacy of cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal carcinoma. J Clin Oncol 2006;24:4011–4019.
4. Esquivel J. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for colorectal cancer: Survival outcomes and patient selection. J Gastrointest Oncol 2016;7:72–78.
5. Helm JH, Miura JT, Glenn JA, Marcus RK, Larrieux G, et al. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for malignant peritoneal mesothelioma: a systematic review and meta-analysis. Ann Surg Oncol 2015;22:1686–1693.
6. Elias D, Goéré D, Dumont F, Honoré C, Dartigues P, et al. Role of hyperthermic intraoperative peritoneal chemotherapy in the management of peritoneal metastases. Eur J Cancer 2014;50:332–340.
7. Alexander HR, Li CY, Kennedy TJ. Current management and future opportunities for peritoneal metastases: peritoneal mesothelioma. Ann Surg Oncol 2018;25:2159–2164.
8. Choudry HA, Pai RK. Management of mucinous appendiceal tumors. Ann Surg Oncol 2018;25:2135–2144.
9. Elias D, Gilly F, Quenet F, Bereder JM, Sidéris L, et al. Pseudomyxoma peritonei: a French multicentric study of 301 patients treated with cytoreductive surgery and intraperitoneal chemotherapy. Eur J Surg Oncol 2010;36:456–462.
10. Baratti D, Kusamura S, Pietrantonio F, Guaglio M, Niger M, et al. Progress in treatments for colorectal cancer peritoneal metastases during the years 2010–2015. A systematic review. Crit Rev Oncol Hematol 2016;100:209–222.
11. Sugarbaker PH. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the management of gastrointestinal cancers with peritoneal metastases: progress toward a new standard of care. Cancer Treat Rev 2016;48:42–49.
12. Lokich J, Anderson N. Carboplatin versus cisplatin in solid tumors: an analysis of the literature. Ann Oncol 1998;9:13–21.
13. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol. 2008;9:215–21.
14. Sautner T, Hofbauer F, Depisch D, Schiessel R, Jakesz R. Adjuvant intraperitoneal cisplatin chemotherapy does not improve long-term survival after surgery for advanced gastric cancer. J Clin Oncol 1994;12:970–974.
15. Miyashiro I, Furukawa H, Sasako M, Yamamoto S, Nashimoto A, et al. Randomized clinical trial of adjuvant chemotherapy with intraperitoneal and intravenous cisplatin followed by oral fluorouracil (UFT) in serosa-positive gastric cancer versus curative resection alone: final results of the Japan Clinical Oncology Group trial JCOG9206-2. Gastric Cancer 2011;14:212–218.
16. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, et al. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2:751–60.
17. Yokoyama M, Okano T, Sakurai Y, Suwa S, Kataoka K. Introduction of cisplatin into polymeric micelle. J Controll Release 1996;39:351–356.
18. Uchino H, Matsumura Y, Negishi T, Koizumi F, Hayashi T, et al. Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats. Br J Cancer 2005;93:678–687.
19. Iinuma H, Maruyama K, Okinaga K, Sasaki K, Sekine T, et al. Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. Int J Cancer 2002;99:130–137.
20. Gunji S, Obama K, Matsui M, Tabata Y, Sakai Y. A novel drug delivery system of intraperitoneal chemotherapy for peritoneal carcinomatosis using gelatin microspheres incorporating cisplatin. Surgery 2013;154:991–999.
21. Matsui M, Tabata Y. Enhanced angiogenesis by multiple release of platelet-rich plasma contents and basic fibroblast growth factor from gelatin hydrogels. Acta Biomater 2012;8:1792–1801.
22. Ahmed T, Review. Approaches to develop PLGA based in situ gelling system with low initial burst. Pak J Pharm Sci 2015;28:657–665.
23. Yeo Y, Park K. Control of encapsulation efficiency and initial burst in polymeric microparticle systems. Arch Pharmacal Res 2004;27:1–12.
24. Nguyen MH, Tran TT, Hadinoto K. Controlling the burst release of amorphous drug-polysaccharide nanoparticle complex via crosslinking of the polysaccharide chains. Eur J Pharm Biopharm 2016;104:156–163.

25. Young S, Wong M, Tabata Y, Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J Control Release 2005;109:256–274.
26. Tabata Y, Ikada Y. Vascularization effect of basic fibroblast growth factor released from gelatin hydrogels with different biodegradabilities. Biomaterials. 1999;20:2169–75.
27. Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, et al. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27:v38
28. Markman M, Intraperitoneal chemotherapy. Crit Rev Oncol Hematol. 1999;31:239–46.
29. Markman M. Intraperitoneal antineoplastic drug delivery: rationale and results. Lancet Oncol 2003;4:277–283.
30. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 2006;354:34–43.
31. Okabe H, Ueda S, Obama K, Hosogi H, Sakai Y. Induction chemotherapy with S-1 plus cisplatin followed by surgery for treatment of gastric cancer with peritoneal dissemination. Ann Surg Oncol 2009;16:3227–3236.
32. Satoh S, Hasegawa S, Ozaki N, Okabe H, Watanabe G, et al. Retrospective analysis of 45 consecutive patients with advanced gastric cancer treated with neoadjuvant chemotherapy using an S-1/CDDP combination. Gastric Cancer. 2006;9:129–35.
33. Satoh S, Okabe H, Teramukai S, Hasegawa S, Ozaki N, et al. Phase II trial of combined treatment consisting of preoperative S-1 plus cisplatin followed by gastrectomy and postoperative S-1 for stage IV gastric cancer. Gastric Cancer 2012;15:61–69.