

Original Article (Experimental Original)

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

Kota Yamashita^{1,2}, Shigeru Tsunoda¹, Shutaro Gunji¹, Takahide Murakami^{1,2}, Takahisa Suzuki^{1,2}, Yasuhiko Tabata², Yoshiharu Sakai¹

¹ Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

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

Corresponding author

Shigeru Tsunoda, M.D., Ph.D., FACS

Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Kyoto-fu, Japan

Tel: +81 (0)75 366 7595

FAX: +81 (0)75 366 7642

E-mail: tsunoda@kuhp.kyoto-u.ac.jp

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Brief title:

Cisplatin-incorporated gelatin hydrogel for peritoneal metastases

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 accomplished good anti-tumor effect with less toxicity.

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

Results: Our new manufacturing process achieved a dramatic reduction in 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 murine abdominal cavities 7 days after intraperitoneal injection and disappeared within 3 weeks. GHG-CDDP significantly suppressed in vivo tumor growth ($p = 0.02$) and prolonged 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 reduced.

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

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. Moreover, 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 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 is still 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 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.

In this study, we report a new preparation of gelatin hydrogel granules (GHGs) incorporating CDDP (GHG-CDDP) for the sustained release of CDDP with minimal initial burst and 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) 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 containing 5% CO₂ 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⁾ Briefly, 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 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); dried GHG-CDDP was imaged by scanning electron microscopy (Model SU8000, Hitachi, Ltd., Tokyo, Japan) after platinum coating.

To evaluate 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 1 N 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) to obtain a time profile for GHG degradation.

To evaluate 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.

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) were treated by 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). 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.

Evaluation of in vivo GHG-CDDP degradability

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) 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 sacrificed 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.

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. 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 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), platelets, and serum concentrations of blood urea nitrogen (BUN) and creatinine were analyzed at FALCO Biosystems, Ltd. (Kyoto, Japan). Mice were sacrificed and the kidneys were harvested for histological evaluation on day 9, fixed in 4% (wt) paraformaldehyde, and embedded for staining with hematoxylin and eosin.

Evaluation of in vivo anti-tumor effects in a murine peritoneal metastases 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 doses of 1, 2, 3, and 5 mg CDDP/kg on days 5 and 12. To assess intraperitoneal tumor volume, macroscopic in vivo luminescence imaging was carried out using an IVIS system (IVIS spectrum, SPI, Tokyo, Japan) on day 26. Total

counts of luminescence at the abdominal sites of the mice were measured as tumor volume.

Statistical analyses

All results are expressed as the mean $\pm$ the standard error. Comparisons of values between the groups at each time point were performed using two-tailed Student's *t* tests and Tukey-Kramer method. Survival analysis was estimated 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 JMP version 12 software (SAS Institute, Cary, NC).

Results

Characterization of GHG and GHG-CDDP

Light microscopy images of GHG-CDDP in water-swollen and dry conditions are shown in Fig. 1A and 1B, 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.

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 total degradation of GHG-CDDP, cytotoxicity was lower than that of free CDDP. The cytotoxicity levels of all groups at 0.05 and 0.025 mg/ml differed significantly from each other. As expected, non-degraded GHG-CDDP had much lower cytotoxicity.

In vivo degradability of GHG-CDDP

Figure 3 shows the time profile of in vivo degradability of GHG-CDDP after injection. GHG-CDDP was 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 CDDP concentrations elevated immediately after injections 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) promoted higher blood concentrations of CDDP compared with GHG-CDDP, but 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, temporal 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 loss of 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, which was equivalent to grade 3 leukopenia in Common Terminology Criteria for Adverse Events (CTCAE) in human. (Fig. 5B)

The number of platelets was significantly lower in the free CDDP group than in the control groups, which could be graded as Grade 4 in CTCAE. (Fig. 5C) In addition, there was no severe thrombocytopenia observed in the GHG-CDDP groups.

There were no significant differences in serum BUN or creatinine concentrations between the GHG-CDDP and the free CDDP groups (data not shown). However, histological evaluation revealed patchy and diffuse denudation of renal tubular cells in the free CDDP group (Fig. 5D), which suggested acute tubular necrosis. On the contrary, there were no histological changes in the GHG-CDDP group (Fig. 5E).

In vivo anti-tumor activity of GHG-CDDP in the murine peritoneal metastases model

Figures 6A and 6B show the appearances of the tumors and IVIS images in mice 21 days after tumor inoculation. Laparotomy 2 weeks after inoculation demonstrated that many white nodules were in the greater omentum, subphrenic

area, pouch of Douglas, and hepatic portal region (Fig. 6A), and their positions were also clearly detected by IVIS analysis (Fig. 6B).

Figure 6C shows tumor volumes 21 days after the first injections of free CDDP or GHG-CDDP. Total photon counts in both GHG-CDDP groups were significantly lower than those in PBS ($P=0.02$, vs GHG-CDDP 5 mg/kg) and GHG groups. 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) didn't 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.

Discussion

Effective chemotherapy with minimal toxicity is highly desirable in order to improve the survival of patients, particularly those in advanced stages who have poor chemotolerance. In this study, our GHG-CDDP system was developed 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 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 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 with 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 these substances for the preparation of microspheres. Considering the fact that residual solvents are harmful to the human body, this alteration in the preparation method is an important improvement that could enable 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 provides anti-tumor activity. Whether the fragments of degraded gelatin molecules were still bound to the CDDP molecule was unclear, even after complete degradation of GHG-CDDP, but the antitumor activity remained.

There was no steep elevation in blood concentrations of CDDP observed after GHG-CDDP injections (Fig. 4). In other words, GHG-CDDP remained in the peritoneal cavity, maintaining anti-tumor activity for a long time, and the degrading GHG-CDDP acted on the tumor directly.

As would be expected, other platinum anticancer drugs such as oxaliplatin and carboplatin could also be incorporated in 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 point of view, the first-line treatment of advanced gastric cancer is a combination of fluorouracil (5-FU, S-1, capecitabine) and intravenous platinum (CDDP, oxaliplatin).(13, 27) Based on our results, we expect that GHG-CDDP may be a feasible replacement for intravenous platinum agents for the treatment of peritoneal metastases of gastric cancers in the future. Several studies show that the intraperitoneal use of chemotherapeutic agents could increase local drug concentrations to enhance 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 appropriate not only as a palliative treatment but also as a postoperative adjuvant chemotherapy for patients who have positive ascitic cytology during surgery or those who have tumors on the serosal surfaces. Moreover, 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 can be achieved in 54% of patients following the preoperative induction chemotherapy and lead to long-term survival.(31) Therefore, if we could improve the induction of chemotherapy by increasing the therapeutic intensity using GHG-CDDP without toxicity, it could be of substantial benefit to patients who currently have incurable Stage IV disease.

The limitation of this study needs to be acknowledged. GHG-CDDP-treated mice had prolonged survival times compared with PBS-treated mice, but the results were not robust. Note that free CDDP did not show a significant therapeutic effect at any dose including lower dose (1 mg/kg, 2 mg/kg)(Fig. 6) in contrast to GHG-CDDP, which showed therapeutic effects in this study. In other words, this study proved that GHG-CDDP had a therapeutic advantage over free CDDP.

For future clinical application, GHG-CDDP should be manufactured in larger scale following Good Manufacturing Practices (GMP) guidelines, then early-phase clinical trials would be planned to investigate the safety of GHG-CDDP treatment.

Conclusions

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

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

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Author contributions

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

Conflict of interest Statement

The authors confirm that they have no conflict of interest.

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Figure captions

Fig.1 Characterization

- (A) A light microscopic picture of gelatin hydrogel granules (GHG) incorporating CDDP dispersed in water. Scale bar: 100 μm .
- (B) SEM picture of dried gelatin hydrogel granules (GHG) incorporating CDDP. Scale bar: 100 μm .
- (C) Time profiles of the degradation of GHG prepared at various concentrations of glutaraldehyde (GA). ($\circ$) GHG1 GA 20 μL , (Δ) GHG2 GA 40 μL , ($\square$) GHG3 GA 80 μL , ($\bullet$) GHG4 GA 160 μL , ($\blacktriangle$) GHG5 GA 320 μL . (N = 5)
- (D) The time profile of CDDP released from GHG-CDDP before rinsed with water ($\circ$), and GHG-CDDP ($\bullet$) (N = 5). Collagenase was added at the time indicated by an arrow.

Fig.2

- (A) The scheme of the stage of GHG-CDDP degradation.
- (B). In vitro cytotoxicity of free CDDP and GHG-CDDP in the stage of degradation in MKN45-Luc cells. (N = 5). ($\circ$) Free CDDP, (Δ) Partially digested GHG-CDDP, ($\square$) Totally digested GHG-CDDP, and ($\bullet$) GHG-CDDP. * $p < 0.05$, significant among groups.

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

Fig.4 In vivo retention of CDDP after GHG-CDDP injection. Each groups were administered the same amount of CDDP (CDDP 100 $\mu\text{g}/\text{body}$) (N = 5). ($\circ$) Free CDDP, (Δ) Partially digested GHG-CDDP, ($\square$) Totally digested GHG-CDDP, and ($\bullet$) GHG-CDDP.

Fig.5 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 against the PBS group.
- (B) Number of WBC 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) and GHG-CDDP (5 mg/kg) (E).

Scale bars: 100 μ m.

Fig.6 In vivo anti-tumor effects of GHG-CDDP after injection in a mice model of peritoneal metastases.

(A) Tissue appearance and (B) IVIS image.

(C) Total photon counts of 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 against the PBS group (N = 6).

Figures

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

Figure 1

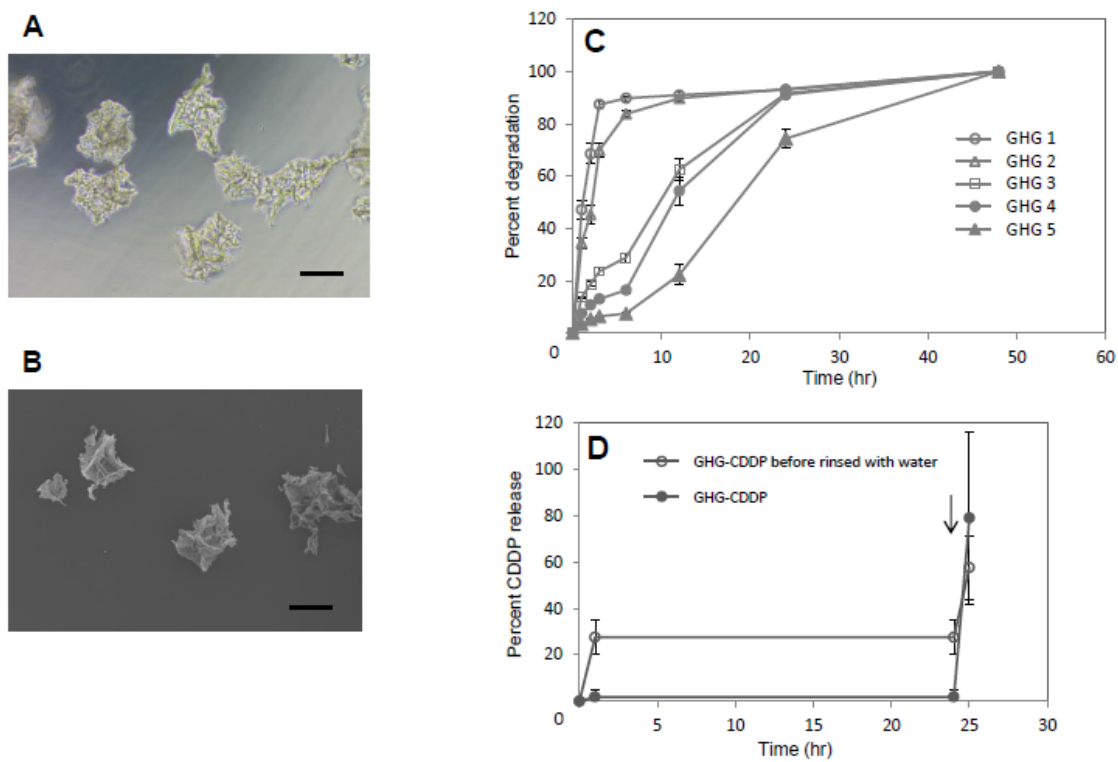
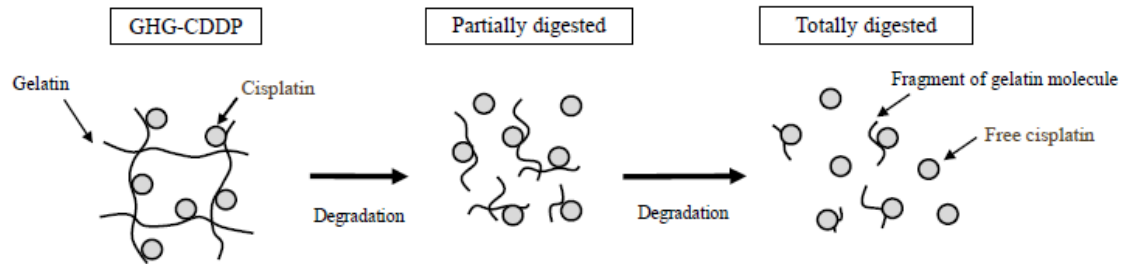


Figure 2

A



B

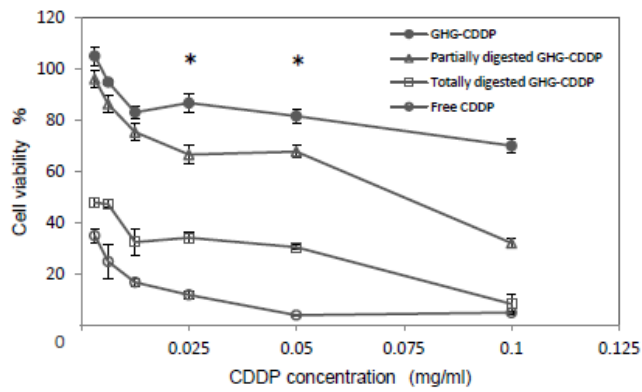


Figure 3

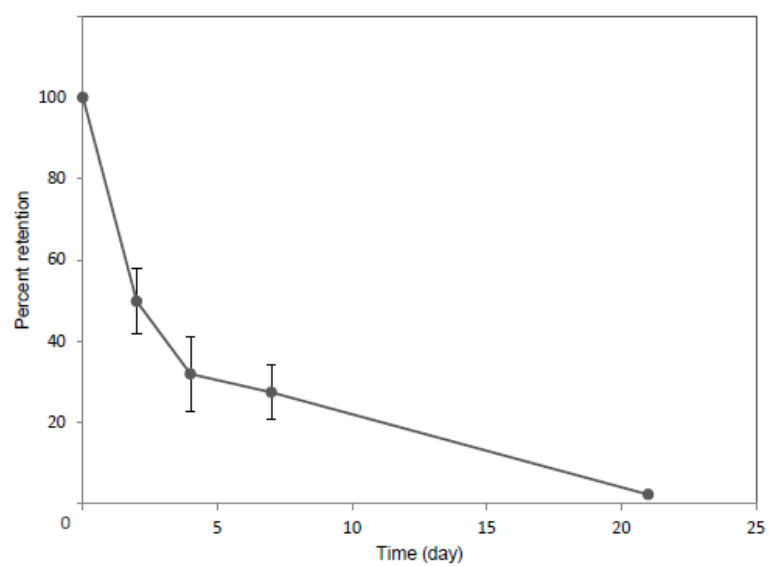


Figure 4

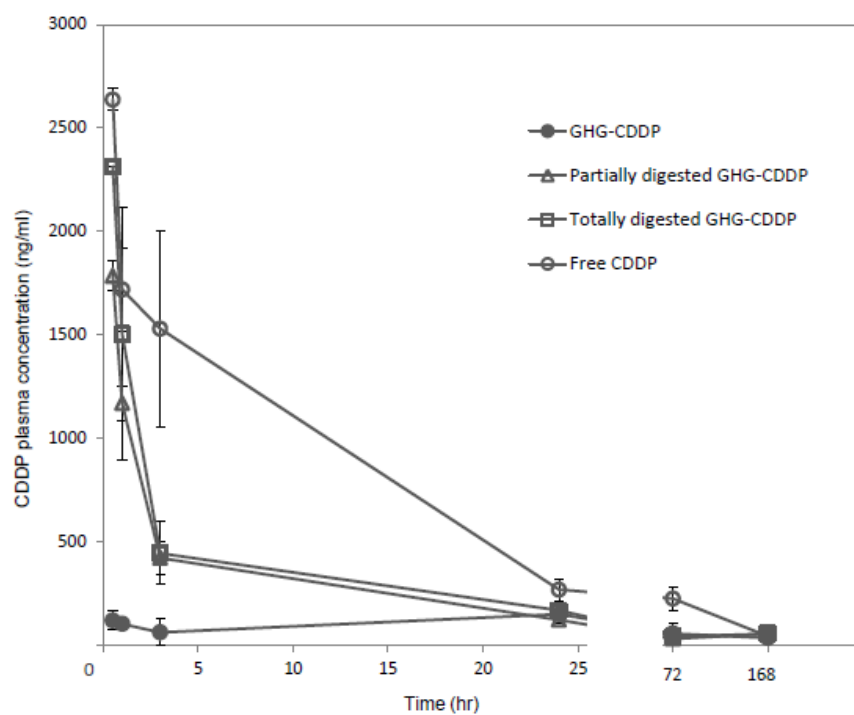


Figure 5

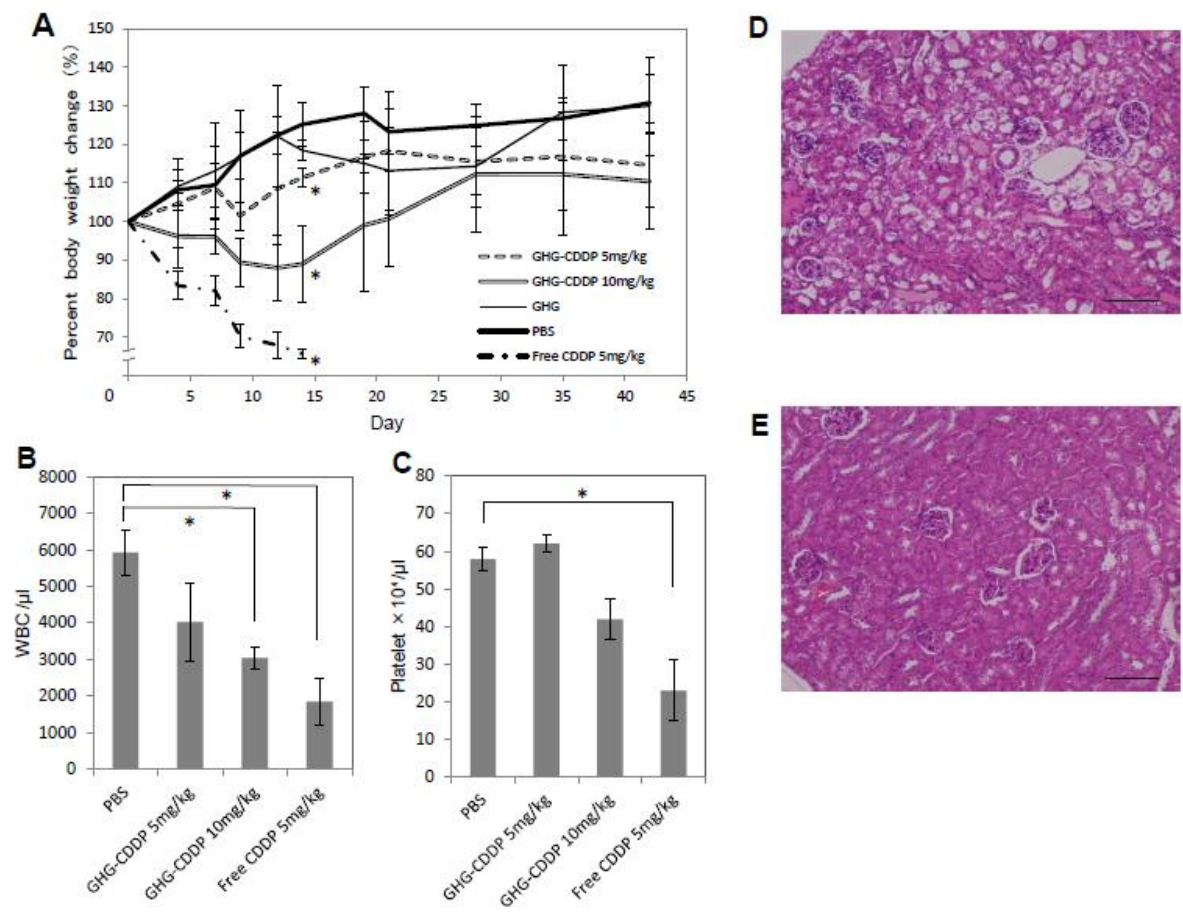


Figure 6

