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Evaluation of cisplatin-hydrogel for improving localized antitumor efficacy in gastric cancer

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Abstract

Gastric cancer, one of the most common disease, has become a major public health problem worldwide. Cisplatin (DDP) has been a widely used drug for the treatment of cancer, also usually applied in gastric cancer in clinic. However, the side effects including toxicity and drug-resistance restricted the usage of DDP in clinic, so we prepared a DDP-complexed hydrogel (DDP-Gel) and investigated its efficacy in gastric cancer. For in vivo studies, MKN45-Luc cells were injected into BLAB/C node mice subcutaneously to establish gastric cancer with orthotopically grown tumors. Mice bearing tumors were treated with normal saline, DDP and DDP-Gel. Body weight and survival condition were observed and recorded. The treatment efficacy in vivo was

detected by luciferase imaging and histological evaluation was performed by H&E staining of different organs. Additionally, normal ICR mice were treated with different doses of DDP/DDP-Gel to calculate their LD_{50} in vivo. The results showed that DDP-Gel prolonged survival time and ameliorated body weight changes of mice bearing tumors. DDP-Gel exhibited higher efficacy to inhibit tumor growth and metastasis, compared to DDP. Besides, LD_{50} of DDP-Gel was 166.0mg/kg, 13.2 folds higher than DDP. As a conclusion, DDP-Gel showed a more effective and safer function than DDP in gastric cancer, which indicating that DDP-Gel might be a novel strategy for gastric cancer therapy.

Key words: orthotopic; gastric cancer; cisplatin; hydrogel; toxicity

Introduction

Gastric cancer (GC) is one of the most common disease and has become a major public health problem worldwide [1]. In China, gastric cancer is the most common malignant tumor and the second leading cause of cancer-associated death, resulting in great burden for people and society [2]. The current standard treatments for GC patients include surgery combined with chemotherapy and/or radiation [3]. Cisplatin (DDP), a common cell-cycle nonspecific antineoplastic agent in clinical, has been widely utilized for the treatments of various solid tumors including GC[4].

However, DDP has a short plasma half-life (25-49 min), rapid binding with protein, oxidative damages and severe toxicity in normal tissues such as hepatotoxicity, neurotoxicity and nephrotoxicity, which restricted its clinical application [5, 6]. Therefore, to reduce these side effects of DDP, improve its therapeutic effects and delivery medicine to tumor tissues selectively, controlled and targeting or localized release technology has been replacing the systemic administration and is highly desired for postsurgical treatment.

This is where hydrogel comes into play. Hydrogels are three-dimensional polymeric networks cross-linked by physical, chemical interactions or a combination of both. Due to the hydrophilicity of the polymer chains in network, hydrogels can absorb and retain lots of water while immersed in aqueous solutions [7]. The advantages of hydrogel include well biocompatibility, biodegradability and controlled drug-release

ability, hence hydrogel has been always used as drug delivery and immensely progressed in recent years to supply significant advances in treatments of cancers [8, 9]. Compared to free DDP, a hydrogel sustained release agent containing DDP could improve tumor inhibition and life extension of tumor bearing mice [10]. It is reported that encapsulating BA-TPQ (7-(benzylamino)-1,3,4,8-tetrahydropyrrolo[4,3,2-de]quinolin-8(1H)-one), a highly potent anticancer drug, into hydrogels could significantly increase its transport via cell monolayers where the apparent permeability of BA-TPQ-hydrogel cubes was 2-fold higher than that of BA-TPQ. Besides, BA-TPQ-hydrogel exhibited better anticancer activity and amplified the potency of the drug compared to BA-TPQ [11]. Hence the combination of drug and hydrogel is deserved to research and it is expected to be a trendency in the future.

In the present study, we developed a hydrogel for local GC treatment. A DDP-complexed hydrogel (DDP-Gel) was established and its toxicity and antitumor efficacy in tumor bearing mice were investigated, providing a novel strategy for GC therapy to reduce side-effects and improve the efficacy of DDP.

Material and methods

Preparation of DDP-Gel

Sodium carboxymethylcellulose (SCMC; Sunhere Pharmaceutical Excipients Co., Ltd, China) was mixed into normal saline for 5 min to be a 6% Gel suspension. A DDP injection (QiLu Pharmaceutical Co., Ltd, China) was added into the Gel suspension and mixed for 1 h at 20 °C, then the DDP-Gel at 0.4 mg/ml was formed.

Cell culture

Human gastric cancer cell line MKN45-Luc were purchased from the Genechem Co., Ltd, Shanghai, China. Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Lonza, Switzerland), 100U/ml penicillin and 100μg/ml streptomycin (Gibco, USA). All cells were maintained at 37 °C under 5% CO_2 in a humanized atmosphere.

Animals

5-6 week-old female BLAB/C nude mice were purchased from Vital River Laboratory Animal Technology Co., Ltd, Beijing, China. 5-6 week-old ICR mice were

also obtained from Vital River Laboratory Animal Technology Co., Ltd, Beijing, China. Mice were housed under pathogen-free conditions and fed a diet of animal chow and water ad libitum through the experiment. All experiments were approved by the Animal Care Committee at Nanjing Drum Tower Hospital, China and performed in compliance with the animal experimental guidelines set by the committee.

Subcutaneous and orthotopic gastric cancer model and animal treatment

MKN45-Luc cells were implanted into BLAB/C mice by inoculating subcutaneously with a total of 2.0×10^6 cells in their right axillary side to develop implantation tumor. Tumors were measured every other day when they became visible and palpable. When the tumor diameter exceeded 1 cm, the mice were sacrificed and the subcutaneous tumors were excised, dissolved and suspended in normal saline. Then the tumor cell suspension was injected into other mice to develop subcutaneous tumor which was regarded as the second generation tumor. Repeat one more time and the third generation tumor was taken out for use. After necrotic tissue and fibrous tissue were removed, the remaining cancerous tissue was divided into small pieces of about 1 mm^3 and put into normal saline for preparation.

BLAB/C nude mice were anesthetized with pentobarbital (6mg/kg). The abdomen was sterilized with alcohol swabs and iodine. A small midline incision was made through peritoneum and enterocoelia. The stomach wall was carefully exposed, and a part of serosal membrane was pricked with an injection near the pylorus and formed to be capsular using smooth forceps. A tumor piece of 1 mm^3 was then fixed into the capsule of gastric serosa with an Organism Glue Paste. Then the stomach was returned to the peritoneal cavity, and the abdominal wall and skin were sutured.

After 14 days of tumor implantation, the mice bearing tumors were randomly separated into 3 groups (n=7): control group, administrated with normal saline by tail vein injection; DDP group, administrated with DDP (6mg/kg) by tail vein injection; DDP-Gel group, administrated with DDP-Gel (6mg/kg) orally.

Imaging of tumor in mice

Tumor imaging analysis was performed every other week after the tumor bearing mice received treatments. An aqueous solution of D-luciferin potassium (Promega,

USA) was injected into the peritoneal cavity of mice 5 minutes before imaging (150mg/kg). Prior to imaging, mice were sedated with inhalation of a mixture of oxygen with 5% isoflurane. Then the in vivo imaging were acquired on a Perkin Elmer animal in imaging system (IVIS Lumina XRMS Series III, USA). The ROI (regions of interest) of the same size and shape was used for all mice throughout the study. The ROI analysis was conducted with the assistance of Perkin Elmer Image software.

Histologic evaluation

The mice were sacrificed at the 28th day after establishment of tumor xenograft model. Then the heart, spleen, liver, lung, kidney and tumor were collected and fixed into 10% formalin for 48h. Then these samples were embedded in paraffin and sectioned at 5 μm. Finally, tissue samples were stained with hematoxylin-eosin (H&E, Sigma Aldrich, USA) and examined under a light microscope (Invitrogen™ EVOS™ FL Auto, USA).

In vivo toxicity study of DDP-Gel

The evaluation of acute toxic effects was performed by the determination of median lethal dose (LD_{50}). The ICR mice were divided into three groups: control group (n=21), administrated with normal saline; DDP group (n=21), receiving different concentrations of DDP (5mg, 10mg, 15mg, 22.5mg, 33.8mg, 50.7mg, 76mg) by tail vein injection; DDP-Gel group (n=21), orally administrated with different concentrations of DDP (32.7mg, 49.05mg, 73.6mg, 110.4mg, 165.5mg, 259mg, 370mg). All animals were then maintained in cages with chow and water ad libitum and observed for 72h. During this period, the number of deaths of every group was observed and recorded. Calculation of LD_{50} was performed by semi-logarithmic interpolation, where concentrations of DDP/DDP-Gel were plotted on the abscissa axis and values corresponding to the probit percentage of deaths on the ordinate axis.

Statistical analysis

All statistical data were analyzed by SPSS 18.0 software (SPSS, Chicago, USA). All error bars used in this study are mean ± SD of at least three independent experiments. Two-tailed Student's t-test and one-way AVONA test were used for significance testing. Statistically significant p values are indicated in figures as *, $P<0.05$.

Results

DDP-Gel treatment prolongs the survival time of the mice bearing gastric tumors

To observe the efficacy of DDP-Gel on the survival condition of mice visually, the survival time of mice in different groups were investigated. Kaplan-Meier curves (Figure 1) indicated that the control group has a lowest survival rate in the earlier stage, and after 14 days, mice in DDP group exhibited a larger death rate, which might be caused by the side-effect of DDP. The survival time of the DDP-Gel treatment group was significantly longer than that of control group and DDP group.

DDP-Gel ameliorated body weight change of mice

During the experiments for determining the efficacy of DDP-Gel against gastric cancer, we monitored the body weight of mice. As shown in Figure 2, body weight of all mice presented a decline. In particular, the weight of mice that treated with DDP decreased most, which might be a cause of the toxicity of DDP, consistent with the low survival of DDP group. There was a similar decrease in weight between the control group and DDP-Gel group in the earlier stage, and the weight of mice administrated with DDP-Gel rose after 21 days, inflecting that DDP-Gel could slightly remitted the weight loss caused by cancer.

DDP-Gel treatment inhibits tumor metastasis

Luciferase imaging detected luciferase signals of the tumor area, indicating tumor metastasis and severity. As can be seen from Figure 3, luciferase signals in the mice were apparent and strong at the first day that mice bearing tumors received different treatment after 14 days of tumor xenograft model establishment. Tumor signals in control group increased over the next 21-day period. Tumor signals in DDP and DDP-Gel group were also stronger in the next 7 days. However, since day 7, the signal intensity presented a decline both in DDP and DDP-Gel group. Luciferase signal intensity of DDP-Gel group decreased most and the area of luciferase signal was smallest, which indicated that treatment of DDP and DDP-Gel could relief tumor metastasis and severity, and DDP-Gel was more therapeutic than DDP.

Furthermore, histological examination was used to further evaluate the therapeutic efficacy of DDP-Gel. The H&E staining images of different tissues reflected the

condition of tumor metastasis. As seen from Figure 4, there was no significant difference between control, DDP and DDP-Gel groups of heart, kidney, lung and spleen. In terms of tumor, cells structure collapsed in control group, while cells with structural integrity arranged well in DDP-Gel group. It was the similar change of histological evaluation in liver.

Overall, the above results suggested that localized drug delivery system based on DDP-Gel led to enhanced antitumor efficacy with low systemic toxicity and less tumor metastasis in vivo.

Toxic effect of DDP and DDP-Gel.

As shown in Table 1, no death occurred in mice treated with doses of DDP below or equal to 5 mg/kg or and doses of DDP-Gel below or equal to 49.05 mg/kg. However, at doses above 10 mg/kg of DDP or 73.6 mg/kg of DDP-Gel, the death in mice appeared and toxic signs such as convulsion and diarrhea were observed when mice were administrated with higher doses. The calculated LD_{50} of DDP was 12.6 mg/kg and LD_{50} of DDP-Gel was 166.0mg/kg, 13.2 folds higher than DDP alone.

Discussion

As one of the first line chemotherapy regimens, DDP is widely applied in postoperative chemotherapy. It has been proved that DDP could combat different types of cancers including bladder, head and neck, ovarian, lung and testicular cancer and so on through suppressing DNA synthesis and mitosis, damaging DNA, and inducing apoptotic cell death [12]. DDP interacts with DNA, and forms covalent adduct with purine DNA bases, which is responsible for cytotoxic effect of DDP contrarily [13]. DDP therapy has been linked with some toxic side effects including nephrotoxicity, cardiotoxicity, hepatotoxicity and other organ toxicity [12]. Hence, side effects of DDP in gastric cancer is a great challenge in clinic, and there is an urgent need to develop novel treatment strategy. Of note, various drug delivery systems have been developed to change the delivery strategy of drugs, which effectively improved the efficacy and safety of drugs [14]. Herein, we developed a novel DDP-complexed hydrogel based on SCMC gel for improving the localized antitumor efficacy of DDP. SCMC, a cellulose ether with a carboxymethyl radical introduced into the hydroxyl, featured as strong

hydroscopicity and well biodegradability. We utilized the SCMC gel to prepare DDP-Gel and then the antitumor efficiency of DDP-Gel was investigated in vivo to verify the importance of controlling drug release in local drug delivery system.

Tumor burden is still the key reason for animal death. Although systemic treatment of mice with DDP reduced the tumor burden and metastatic spread, a better response was observed for DDP-Gel. In addition to reduced tumor growth, we found that DDP-Gel has a lower metastatic tumor spread in mice than DDP, which might directly result in a higher survival rate in mice suffering from cancer. It is apparent that the well locoregional control accomplished in this research, which plays a good foundation for human cancer therapy in clinical.

From histological evaluation of different organs, we could see that tumor metastasis appeared and the treatment efficacy was most obvious in liver despite of tumor itself. Tumor metastasis is a complex and multistep process and is closely involved in the progress of tumor exacerbation. Notably, liver, the organ responsible for hematopoiesis during fetal life, is also a target organ of metastasis of cancers [15]. Thus, with the development of gastric cancer, tumor metastasis, especially hepatic metastasis, came out and became an important hallmark. Similarly, liver exhibited a more obvious change when receiving effective therapy, indicating that liver played a vital role in the development or treatment of cancer, and the protection of liver might be a strategy for the prevention and treatment of other cancers indirectly.

As intravenous infusion usually caused poor lymph node and tumor penetration, oral administration of hydrogel system created the opportunity to deliver potent anti-tumor drugs more effectively and targeted to tumor [16]. Compared with DDP alone, the hydrogel system may be safer and more effective for the treatment of gastric cancer, with a reduction of side effects, as the results demonstrated that the calculated LD_{50} for DDP-Gel, given orally, was 166.0 mg/kg, 13.2 folds higher than DDP, which indicated that DDP-Gel broke through the limitation of DDP activity in stomach and made high dosage treatment to local gastric lesions possible.

However, this study still had some limitations. First, this study focused on the anti-tumor efficacy of DDP-Gel, while the physical property of DDP-Gel itself was lacking,

such as stability, delivery and release rate. Next, whether DDP-Gel is fully released localizing the gastric tumor in mice and the bioavailability of DDP-Gel are also necessary to be clarified. The problems above are deserved to be further investigated and also would be the next research targets for us in the future, and the present study, revealing the more effective efficacy and lower toxicity of DDP-Gel in the therapy of gastric cancer, play an important basis for further investigation.

Conclusion

In summary, we have demonstrated that DDP-Gel inhibits growth and metastasis of orthotopically xenografted gastric cancer and prolongs the survival time of mice bearing tumors. Additionally, DDP-Gel significantly decreases the toxic effect induced by DDP itself during the treatment of cancer. The enhanced localized antitumor efficacy and low systemic toxicity in vivo provide strong evidence that DDP-Gel may be a potential anticancer agent in the therapy of human cancer.

Acknowledgments

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Conflicting of interests

The authors declared no conflicts of interest.

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Figure legends

Figure 1. DDP-Gel treatment prolongs the survival time of mice with gastric tumors.

Kaplan-Meier curves for mice treated without or with DDP and DDP-Gel are shown. As the time went by, the survival condition of mice in different groups changed, and at the 28th day, all mice were sacrificed.

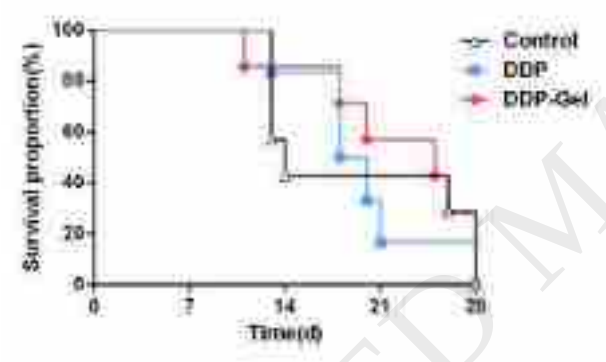

Figure 2. Body weight change of mice in different groups

With or without the administration of DDP/DDP-Gel, the body weight of mice presented different changes.

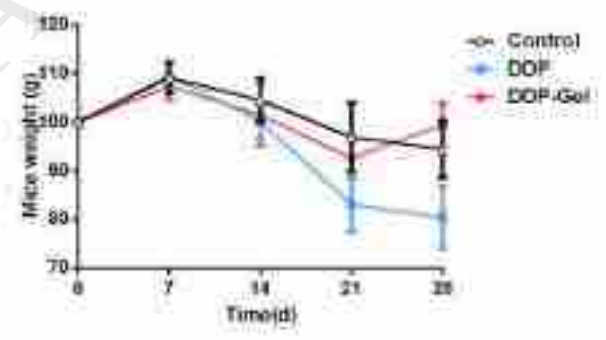

Figure 3. Fluorescence monitoring of orthotopic xenograft gastric cancer in nude mice.

(A) Representative luciferase images of the anesthetized mice from the control and DDP/DDP-Gel treated groups are shown. The days show the time of luciferase imaging beginning from therapeutic treatments. (B) Tumor burden following treatment are measured by luciferase activity. Errors are reported as SD (n=5). Statistical analysis was performed by two-way analysis of variance, **, $P<0.01$.

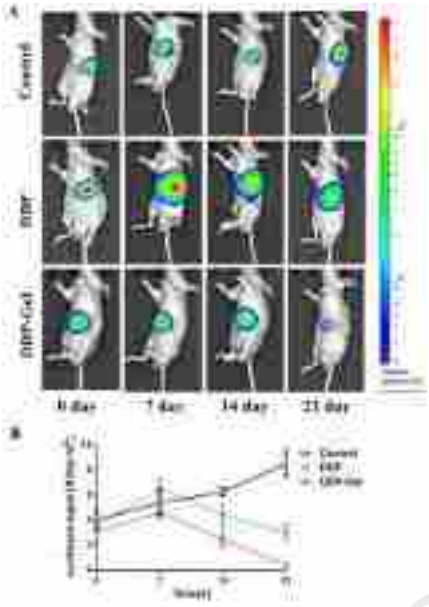

Figure 4. Histological section and H&E staining of organs.

Tumor, heart, kidney, liver, lung and spleen were separated and stained with H&E, and then histological examination was evaluated. Scale bar is 100 μm.

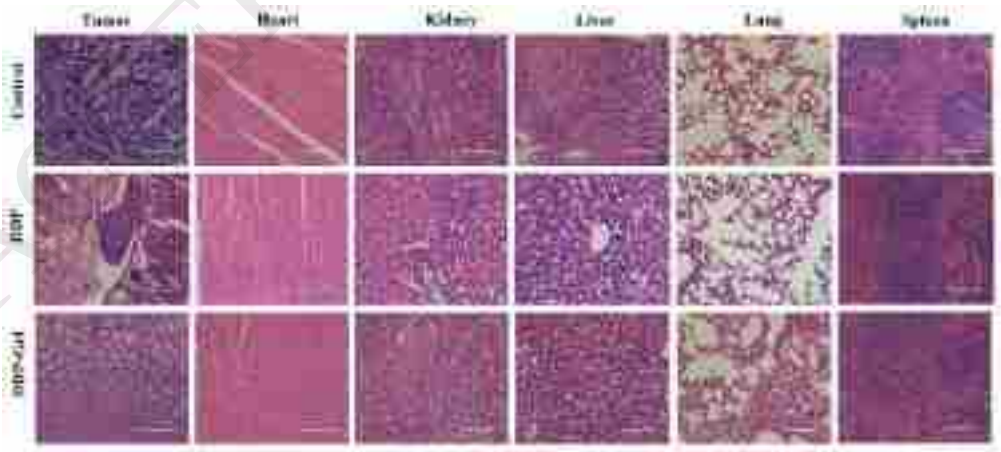

Table 1. Symptoms and deaths of animals in different groups.

Group	Concentration(mg/kg)	Deaths	Symptoms
Control	-	0	None
DDP	5	0	None
	10	1	Death
	15	3	Death
	22.5	3	Convulsion and death
	33.8	2	Convulsion and death
	50.7	3	Diarrhea
	76	3	Diarrhea
DDP-Gel	32.7	0	None
	49.05	0	None
	73.6	1	Death
	110.4	1	Death
	165.5	2	Death
	259	2	Death
	370	3	Diarrhea and death