Author's response to reviews

Title: Pharmacokinetic properties and antitumor efficacy of the 5-fluorouracil loaded PEG-hydrogel

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Author's response to reviews: see over
Thank you for your message on Dec. 30, 2009. We are glad to have a chance to submit a revised version of our manuscript entitled “Effect of 5-fluorouracil/PEG-hydrogel on the pharmacokinetic properties and antitumor efficacy” (MS: 6996391423055207). I would like to express my appreciation to the reviewers for critical suggestions. We have revised our manuscript in accordance with the issues raised by reviewers. We hope that you and the reviewers will find our revisions satisfactory and that these corrections and revisions will be acceptable for publication in **BMC Cancer**.

Best regards,

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Reviewer 1.

1. Background reword; FU is administered both as bolus and infusion, does this provide any advantage?

In accordance with reviewer's comments, we have revised our background extensively as follow; “The drug 5-Fluorouracil (5-FU) is one of the most common chemotherapeutic agents used against malignant tumors [1]. However, this drug has shown some pharmacokinetic limitations including unfavorable maximum drug concentrations (Cmax) and short half lives by systemic bolus injection. Earlier reports have demonstrated that acute increases plasma 5-FU concentrations can cause severe side effects and its anti-tumor effect depends on exposure duration rather than the concentration levels [2]. In other words, 5-FU acts in a time-dependent manner than in a dose-dependent [3-5]. Therefore, a continuous infusion system may be better at the maintaining intended levels. However, this strategy has difficulties such as higher cost and patient compliance with long-term regimens. Implantable release devices have been attempted in vivo to reduce the period of hospitalization and eliminate the need for indwelling catheters [6]. Recently, we have developed PEG-hydrogel derivatives as an injectable sustained release device which can be bolused subcutaneously without any surgical implantation (US Patent 6858736; Korean Patent KR 2002-0089772 and 10-2004-0040782). We have hypothesized that this approach can provide a diffusional barrier for drug release and thereby deliver drugs for an extended period of time. In this current study, we have evaluated the drug release profiles of a 5-FU-loaded PEG-hydrogel system. To confirm the therapeutic efficacy of the designed PEG controlled-release system, we conducted a pharmacodynamic study using the A549 tumor xenograft model in nude mice.”

2. Provide reference for “We have tried to develop a PEG-hydrogel that can be injected subcutaneously without any surgical implantation.”

Since this is our first report for the PEG-hydrogel system, we have revised our manuscript and added patent informations as follow; “Recently, we have developed PEG-hydrogel derivatives as an injectable sustained release device which can be bolused subcutaneously without any surgical implantation (US Patent 6858736; Korean Patent KR 2002-0089772 and 10-2004-0040782).”

3. Provide additional details on preparation and characterization of 5-FU hydrogels. Please provide rationale for selection of 6-arm PEG-AM and PEG-
SG for hydrogel formation. Has this been previously studied, if so then provide reference.

The preparation procedure of 5-FU-loaded PEG has been described in “Materials and Methods”, in the section of “Preparation of 5-FU-loaded PEG hydrogel and in vitro Release of 5-FU-loaded PEG-hydrogel”. Further detailed information has been described in the content of US Patent 6858736 document.

4. Specify treatment duration

We treated 5-FU (or 5-FU-loaded PEG) to nude model mice once per week for 4 weeks. Therefore, the treatment occurred four times during the study.

5. Did authors measure tumor drug levels at the end of the study in treated animals.

Although we tried to analyze the drug in the tumor mass at the end of the study, we could not detect it.

6. Did authors measure antitumor activity of 5-FU and 5-FU hydrogel in the cytotoxicity assay.

Unfortunately, we did not measure in vitro antitumor activity using a cytotoxicity assay. However, we confirmed that the hydrogel itself has no cytotoxicity.

7. Other minor comments

We have revised our manuscript according to this reviewer’s suggestions.
Reviewer 2

Major comments

1. The reviewer has questioned the significance and objective of the cytotoxicity assay concerning the side effect of the hydrogel.

We agree with this reviewer’s opinion. For describing non-toxicity of hydrogel, we need further detailed data such as cytotoxicity in normal cells. Since we want to introduce just the efficacy of PEG-hydrogel loading system to antitumor drugs in this work, we have deleted the part of cytotoxicity assay in the revised version and the Figure 7 has been also removed. As the reviewer said, the assessment of safety is very important factor prior to application in the clinics. We have designed a full scale toxicity study for this GEL loading system and hope to show our data in the next report.

2. The reviewer suggested the deletion of some redundant description in Figures and Table.

In accordance with the comment, we have deleted Fig. 6 and PK parameters including AUMC, $k_a$ and $k_{el}$ in Table 1.

3. The reviewer commented “what is the difference and advantage for our systems compared to the method previously reported by Blanco et al.?”

The hydrogels used by us are multi-arm PEG derivatives including 6-arm PEG-SG and 6-arm PEG-AM which consisted of 6-arm polyethylene glycol N-hydroxy succinimidyl glutarate and 6-arm polyethylene glycol amine, respectively. The hydrogel previously reported by Blanco et al [28] was a poly 2-hydroxyethylmethacrylate-co-acrylamide hydrogel. For dosing, we employed a direct injection and mixing method of SG and AM, while the Blanco group used a surgical implantation route. We think that the differences may result from the differences in chemical structures and dosing forms. Therefore, we have simply described it in the Discussion of revised manuscript.

4. Were the injection sites separated between A549 cancer cells and 5-FU hydrogel? Is there any possibility that 5-FU released from hydrogel can directly react against the cancer cells?
In our study, the 5-FU-loaded PEG was injected into a site separated from the tumor mass. We believe that there is no possibility that 5-FU released from hydrogel can directly react against the cancer cells.

5. The authors mentioned that 5-FU-loaded PEG-hydrogel was injected at 85 mg/kg, but how did they adjust the dose of 5-FU? Did they measure the content of 5-FU in the hydrogel injected? In addition, they may need to clearly describe the solvent used for the injection of hydrogel and 5-FU alone.

We determined the dose of 85 mg/kg based on a previous paper describing the toxicity of 5-FU where the subcutaneous MTD (maximum tolerated dose) for 5-FU was 85 mg/kg in mice (Cao, D. et al., 2005. Abnormalities in uridine homeostatic regulation and pyrimidine nucleotide metabolism as a consequence of the deletion of the uridine phosphorylase gene. J. Biol. Chem. 280:21169–21175). We did not measure the drug content in the hydrogel injected since we did not designed additional animal groups for this assay. The solvents used for the injection of the hydrogel and 5-FU alone were both phosphate buffer. The PEG hydrogel consists of two liquid-SG and AM. We dissolved the 5-FU in the AM solution and PEG-SG was equally injected using a mixing syringe. The preparation procedure of 5-FU-loaded PEG has been described in the “Materials and Methods”, in the paragraph (Preparation of 5-FU-loaded PEG hydrogel and in vitro Release of 5-FU-loaded PEG-hydrogel) (page 3-4).

6. The description of exact values for each parameter would also be redundant because those are described in Table 1. The authors may alternatively need to discuss the difference between 5-FU-loaded PEG-hydrogel and 5-FU alone.

The serum concentration profiles for the free 5-FU and 5-FU-loaded PEG-hydrogel were well described by a one-compartment open pharmacokinetic model. As shown in Table 1, between the two groups there were marked differences in some parameters including the maximum serum concentrations ($C_{\text{max}}$), the elimination half-lives ($t_{1/2}$) and the area under the curves (AUC). The $C_{\text{max}}$ and $t_{1/2}$ in the free 5-FU treated group were about 68 µg/ml and 0.15 h, respectively, while those parameters in 5-FU-loaded PEG-hydrogel group were 8 µg/ml and 0.9 h, respectively. In the free 5-FU treated group, the AUC and the area under the moment curve (AUMC) were roughly 60 µg·h/ml and 33 µg·h$^2$/ml, respectively. While in the 5-FU-loaded PEG-hydrogel group, the AUC and AUMC were 14 µg·hr/ml and 112 µg·h$^2$/ml, respectively.

Minor comments
1. The release of NHS from the reaction can be written in Fig 1, and this may help the understanding by the readers.

   In accordance with reviewer’s suggestion, we have been added a sentence in the legend of Fig. 1 as follows “After displacement of the NHS (N-hydroxy succinimide) from 6-arm PEG-SG, amine residues on 6-arm PEG-AM are cross-linked with 6-arm PEG-SG.”

2. The meanings of symbols and inlet should be described for Fig 3.

   The descriptions of the symbols have been added in the legend of Fig 3.

3. Language corrections

   Revised