

Peer Review File

Article information: <http://dx.doi.org/10.21037/tcr-20-2627>

Reviewer Comments

Comment 1: In cell and animal experiments, how to determine the dosage, method and course of administration of gemcitabine and Sophora japonica?

Reply 1: The dosage and course of gemcitabine is according to the manufacturer's instruction and a few published studies such as gemcitabine in PaTu8988 cell (Oncol Lett. 2013; 5 (3): 805- 812) and in in vivo nude mice experiment (Clin Cancer Res. 2000; 6 (5): 1936- 1948). The dosage and course of Huaier are also based on numerous studies in the tumor field including pancreatic cancer (Biomed Pharmacother. 2020; 127: 110126), breast cancer (Oncol Rep. 2017; 38 (6): 3455- 3464) and hepatocellular carcinoma (J Cancer. 2017; 8 (19): 4087- 4097). In these studies, the in vitro concentrations of Huaier were 0 mg/ml to 16 mg/ml and the dosage of Huaier in nude mice experiments was 50 mg or 250 mg per mouse.

Dosage: Based on these studies, we performed our experiments. As shown in the *Methods* section of "*Cell viability MTT assay*", different concentrations of gemcitabine or Huaier were prepared. Following the results (*Figure 1*), 8 μ M gemcitabine and 6 mg/ml Huaier (the dose of gemcitabine or Huaier that could inhibit approximately 50% cell viability in PaTu8988 cells) were used in further in vitro experiments. In the in vivo experiments, mice were treated with 1.5mg gemcitabine (100 mg/kg per mouse, 15g in weight, twice a week) by intraperitoneal injection or 50 mg Huaier per mouse (daily) by oral gavage.

Method and Course: In vitro experiments, the stock solution was diluted as needed to the indicated concentrations using culture medium and added into cells respectively. Then, cells were cultured for the following 24 h, 48h or 72 h. In vivo experiments, the course of drugs in the subcutaneous xenograft models and the tumor lung metastasis models was 4 weeks and 6 weeks, respectively. Moreover, when the subcutaneous tumors reached approximately 1.3 cm in length, it was approximately 4 weeks after injection.

Changes in the text: We have modified our text in the *Methods* section of "*Nude mice*" to indicate the weight of mice (see Page 8, line 181), "*Cell viability MTT assay*" to show how to determine the dosage of drugs in vitro (see Page 6, line 132 and 133) and "*In vivo animal experiments*" to show how to determine the course of drugs in vivo (see Page 8, line 200-202).

Comment 2: It is pointed out that 12 mice were randomly selected and divided into 4 groups (n = 3 in each group) for animal experiment. Is the sample size low?

Reply 2: We thank the reviewer for pointing out this issue. Because both the subcutaneous xenograft model and the tumor lung metastasis model were established in our study, we selected 12 mice in each model. Since the significant difference was observed in the experiment, we didn't expand the sample size. Thus 12 representative tumor images (n= 3 in each group) were shown in Figure 4. However, we indeed should increase the sample size in further study to make the results more convincing.

Comment 3: In addition to gemcitabine, which chemotherapy drugs are currently used in the treatment of pancreatic cancer, and what is the clinical status of these drugs?

Reply 3: In addition to gemcitabine, there are some chemotherapy drugs used in the treatment of pancreatic cancer, such as capecitabine, 5-fluorouracil (5-FU), S-1, paclitaxel for injection (albumin bound) (PAB), irinotecan and oxaliplatin. These drugs are used alone or in combination with gemcitabine for first-line or second-line treatment of pancreatic cancer. FOLFIRINOX (oxaliplatin, irinotecan, leucovorin, and fluorouracil) can improve the prognosis of advanced pancreatic cancer compared with 5-FU monotherapy. Nowadays, gemcitabine is still considered as the standard treatment and has been widely utilized as a first-line drug for advanced pancreatic cancer. According to the 2018 ASCO's recommendations on potentially curable pancreatic adenocarcinoma, adjuvant therapy with a modified FOLFIRINOX regimen brought a profoundly longer survival than gemcitabine among resected pancreatic cancer patients. However, the development of chemoresistance still leads to poor clinical outcomes of pancreatic cancer.

Comment 4: It is pointed out that in recent years, more and more studies have focused on Chinese medicine alone or in combination with chemotherapy (21-23). What is the prospect of multiple traditional Chinese medicine combined with chemotherapy in the treatment of pancreatic cancer? Recently, increasing studies have focused on the anticancer efficiency of TCM alone or combined with chemotherapy (21-23).

Reply 4: Recently, the better antitumor effects of traditional Chinese medicine combined with chemotherapy than chemotherapy alone have been found in pancreatic cancer treatment, such as Qingyihuaji formula combined with gemcitabine, *Brucea javanica* (BJO) combined with gemcitabine, melittin combined with gemcitabine, etc. They were also validated to have little toxicity such as kidney and liver injury (*Tumour Biol.* 2015; 36 (3): 1739- 1745). These results highlight the possible application of TCM in pancreatic cancer therapy. However, most research is still in an experimental stage, clinical studies should be carried out to illustrate the therapeutic potential of traditional Chinese medicine in conjunction with chemotherapy in pancreatic cancer patients.

Comment 5: Some Chinese herbal extracts combined with gemcitabine also have good antitumor effect in the treatment of pancreatic cancer. The combination of some TCM

extracts and gemcitabine are also reported to have better antitumor effects in pancreatic cancer therapy.

Reply 5: We agree. As shown in the *Discussion* section, some TCM extracts, such as Oridonin, Qingyihuaji formula (QYHJ) and curcumin, combined with gemcitabine in the treatment of pancreatic cancer, are demonstrated to have better antitumor effects than gemcitabine alone.

Comment 6: What are the advantages of *Sophora japonica* compared with other traditional Chinese medicine extracts?

Reply 6: Huaier is a type of fungus that grows on the trunk of ancient Chinese scholar trees (*Sophora japonica* L.). It has been applied as a traditional Chinese medicine (TCM) for more than 1,600 years. Huaier has a wide range of sources, convenient extraction and low price. It has several advantages that can be applied to clinical therapy, such as hemostasis, liver protection and anti-inflammation. Meanwhile, an increasing number of studies have demonstrated the anticancer effects of Huaier, such as inhibition of cell proliferation, anti-metastasis, interference with tumor angiogenesis and tumor-specific immunomodulatory effect.

Comment 7: The molecular mechanism of anti-tumor effect of *Sophora japonica* and its inhibitory effect on gemcitabine resistance of pancreatic cancer need further study. How to further study?

Reply 7: Wnt/ β -catenin signaling pathway, which plays important roles in the development of various malignancies, cell proliferation and differentiation, has been also reported to correlate with chemoresistance. Previous study (Biomed Pharmacother. 2020; 127: 110126) revealed that Huaier extract suppresses pancreatic cancer by inhibiting Wnt/ β -catenin pathway both in vitro and in vivo, as shown in the *Discussion* section of our manuscript (Reference 17). Therefore, we speculate that Huaier can reverse gemcitabine-induced Wnt/ β -catenin activation to enhance gemcitabine efficacy in treating pancreatic cancer. We should first prove that Huaier can inhibit the Wnt/ β -catenin pathway in pancreatic cancer cells. Next, we should demonstrate that Huaier can abrogate gemcitabine-induced Wnt/ β -catenin activation in pancreatic cancer cells. Immunohistochemical staining should also be carried out to show that Wnt/ β -catenin pathway were suppressed in Huaier-treated xenograft tumor tissues.