

ANSWERING REVIEWERS



January 16, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8501-review.doc).

Title: RAD51 potentiates synergistic effects of chemotherapy with PCI-24781 and cis-diamminedichloroplatinum on gastric cancer

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8501

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 00077340:

Major Comments

1. What experiment did authors use gastric epithelial cell line GES-1 for? And, please mention about the background of HGC27 and AGS, such as pathological findings and information of high or low malignancy, because these two cell lines shows similar results.

Response: GES-1 was originally used for screening specificity and toxicity of drugs to normal cell or cancer cells at the beginning of this study. Unlike cancer cell lines (HGC27, AGS), no significant changes were found among viability of GES-1 upon PCI-24781 or CDDP treatment, suggesting that PCI-24781 and CDDP mainly target at cancer cells with low toxicity of normal cells. Since we didn't incorporate this data into the manuscript, we delete the information of GES-1 in revised manuscript.

The HGC-27 cell line was established by culture of the metastatic lymph node from a gastric cancer patient diagnosed histological as undifferentiated carcinoma (high malignancy) (Akagi T, Kimoto T. Acta Med Okayama 30(3):215-219, 1976). The AGS cell line was derived from fragments of a biopsy specimen of an untreated human adenocarcinoma of the stomach (Barranco SC. et al, Cancer Res 43:1703-9, 1983). Adenocarcinoma is the most common type of stomach cancer (>95%). We use these two different types of cancer cell lines to represent most of gastric cancers and explore the applicability of PCI-24781. We indeed observed their similar response to the drugs. To this point, we were expecting a novel, broad-spectrum anti-GC drug instead of histological specific drug. We added essential information of these two cell lines in the revised manuscript.

2. I have a question about the dosage of PCI-24781 and CDDP for *in vivo* therapeutic studies. About PCI-24781, what reliable informations does author have from the company? And then, how does author decide the concentration (10mg/kg/day) of CDDP?

Response: Generally speaking, it is difficult to do drug dose translation among different species though some scholar gave a clue (Reagan-Shaw. et al, FASEB J 2008; 22(3): 659-661). The information about dose of

PCI-24781 in vivo provided by the company was actually cited from the previous study (Lopez G. et al, Clin Cancer Res 2009; 15(10): 3472-3483). Dose of CDDP was also referred to previous paper which is efficient and would not cause renal dysfunction (<18mg/kg) in mice (Mathe A. et al, Lab Anim 2006; 40(3): 296-300 and Mohammad RM. et al, Cancer 2006; 106(6): 1260-1268). Thus we made modification and cited these papers in our revised manuscript.

Minor Comments

1. The title of this manuscript is grammatically difficult to understand. The author needs to revise it, such as "RAD51 potentiates synergistic effects of chemotherapy with PCI-24781 and cis-diamminedichloroplatinum on gastric cancer".

Response: We modified accordingly.

Reviewer 00069066:

Title: the title is confusing and not clear. The evaluated drug is PCI-2481. RAD51 is reduced by the drug, why was it said contrary?

Response: The aim of this study is to evaluate the efficacy of PCI-2481 on gastric cancer and explore the underlying mechanism. According to our data, RAD51 seems to play a key role in mediating the anti-cancer effect of PCI-2481 since manipulating RAD51 expression affects the cell function upon PCI-2481 or CDDP treatment (Fig.4). Thus, we modified the title as 'RAD51 potentiates synergistic effects of chemotherapy with and PCI-24781 and cis-diamminedichloroplatinum on gastric cancer'.

Abstract: State the aim of study in the background. The method should consist of place and time the study took place. You said "GC cell lines were treated with PCI-24781 and/or cis-diamminedichloroplatinum". In the text, you said that the group is (1) negative control; (2) PCI-24781; (3) CDDP. Please cross-check. It is not well written about the in vivo study in method of abstract. Statistical analysis should be described. There is no numeric data in the result. Give more data in the result besides tell the discussion. How significant did it show?

Response: We added aim into the background and modified methods and results according to your recommendation.

Introduction: Describe the aim of the study rather than give conclusion. Describe the RAD51 to introduce that before you write in the discussion. Describe how important to combine PCI-24781 with CDDP and the background you would combine it with PCI-24781 in the study.

Response: We described the aim, RAD51 and explained why we were combining CDDP and PCI-24781 in the study, as shown in the revised introduction.

Methods: Mention the protein you assayed and the gene you did the qPCR. Mention the place and time you did the study What did you do in the in vitro study? Describe it. And how do you treat the cell culture after you put in specific medium?

Response: We incorporated the detail methods into the revised manuscript.

Results: The subheadings are too long. You said the clonogenicity was impaired. How many colonies? How many percent the plating efficiency? Do not mix result and discussion. "CDDP exerts its chemotherapeutic effect mainly by causing DNA damage. However, DNA damage can be repaired through homologous recombination (HR) or through non-homologous end joining (NHEJ) [23], which can lead to chemotherapy resistance. HR usually occurs during and shortly after DNA replication during the S and G2 phases of the cell cycle, when sister chromatids are more easily available [24]." → This statement is more fit in the introduction. The therapy was initiated after the

volume 100mm³. With the observation 2x/week, how can you start with the exact 100 mm³? If not, show mean volume, when you start the treatment in each group? It would be better if you use the same measurement. First you showed the volume (100mm³) and at the termination of the study, you showed the weight. It would confuse the reader to compare.

Response: We modified the sub-title of results. For clonogenic assay, GC cells were treated in culture dishes with 0.1% DMSO (control), PCI-24781 (0.25 μM), DDP (2.5 μM) or Combination for 24 h. One hundred cells per well were replated, then allowed to grow in corresponding drug-added media for 10 d. In this way we assured the number of each group plated evenly. We also added colonies number in the results. We removed the statement to introduction. We modified the results of *in vivo* data. Volumes of tumor and tumor weights are two independent indexes to evaluate the drug effect *in vivo*, as shown in Fig.6 A and B. Comparison should be carried out between different treated groups in which combination group gained most favorable outcomes (smallest and lightest tumor burden).

Discussion: If possible, give more data about another study, e.g.: “Adimoolam et al. also confirmed that PCI-24781 could decrease RAD51 expression and suppress HR in colon tumor cells [38]” give the data about how much the decrease, so reader could objectively give opinion about it.

Response: RAD51 was reduced to 20% and the rate of HR dropped from 0.72% to 0.27% upon PCI-24781 treatment. We cited more information of this paper in discussion.

3 References and typesetting were corrected

Thank you for giving us the opportunity to revise our manuscript. We appreciate the positive and constructive comments from editor and reviewers.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely,

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