9 January 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6653-edited.doc).

Title: Melatonin attenuated cisplatin induced hepatoma (HepG2) cell death via regulation of *mTOR* and *ERCC 1* expression

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Name of Journal: *World Journal of Hepatology*

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

1. Format has been updated
2. Revision has been made according to the suggestions of the reviewer

Reviewer #1.

- The clinical applicability was elucidated more in the discussion part – last paragraph "Therefore, during cisplatin treatment of hepatocarcinoma, administration of a high dose of melatonin as an adjuvant in cancer therapy should ameliorate the adverse cisplatin effects".
- Figure 11 was referred to in the results section
- All Figure information were described briefly in the legends
- Language: English was edited and approved by Professor Dr Peter Hanna from Australia, who is an Adjunct Professor in our Anatomy Department, Mahidol University, Bangkok
- The reason of using 1mM/20mM melatonin/cisplatin was mentioned in the methods at Cell viability assay "In the combination treatment, the selected concentration was based on the minimal concentration that induced anti-proliferative effect of melatonin and the most tolerable concentration that induced cytotoxic effect of cisplatin".

Reviewer #2.

- The high concentration of melatonin used in the experiments was determined for the most appropriate concentrations as an anti-proliferative effect in HepG2 cell culture condition. Anti-tumor proliferation required higher dose than normal level in serum of an individual especially in cancer patients which may have decreased nocturnal concentration. Administration in vivo or to the patient should be optimized to reach satisfaction.
- In this paper, we have not included effects of melatonin via MT1 or MT2 receptors.
- The concentration dependent of melatonin in the cell viability result was ranged 0.5-1 mM. However, in all of the experiment we used only 1 mM concentration. Therefore, to clarified this, we added the sentence "The concentrations of melatonin and cisplatin using in the combination treatment were selected from the minimal anti-proliferative effect of melatonin which significantly decreased percent cell viability, and the most tolerable cytotoxic effect of cisplatin which induced cell death lesser than 50%. " in the results section
- The statistic ($p \leq 0.001$) on this part was added. We performed statistical analysis by one-way ANOVA test, followed by a post hoc analysis (Tukey's multiple comparison test) using Prism 5 (GraphPad Software, Inc., San Diego, CA, USA) and reconfirmed by the two-way ANOVA which significance was not changed.
- We added the information on melatonin antiproliferation effect in the introduction part as " Previous investigations, have demonstrated that melatonin also posses anti-proliferation effects, especially in cell lines derived from various malignancies such as lymphoma[14], prostate[15], melanoma[16], and hepatoma
- Reference 27 was corrected and rearranged to ref # 30

Reviewer #3.

- The rationale for the concentration of melatonin used in this study was provided as follows

  In the introduction section: "While most biological effects of melatonin are produced through activation of melatonin receptors, some are due to its role as a powerful antioxidant which plays roles in the protection of nuclear and mitochondrial DNA[18]. The antiproliferative effect of human osteosarcoma MG-63 cell line was shown to be activated by melatonin if the concentration of melatonin reached an optimal value, which was a high concentration of 4-10 mM[19].]."

  In the methods section: "In the combination treatment, the selected concentration was based on the minimal concentration that induced anti-proliferative effect of melatonin, and the most tolerable concentration that induced cytotoxic effect of cisplatin".

  In the results section: "The concentrations of melatonin and cisplatin using in the combination treatment were selected from the minimal anti-proliferative effect of melatonin which significantly decreased percent cell viability, and the most tolerable cytotoxic effect of cisplatin which induced cell death lesser than 50%. "

- The range of concentrations of melatonin (0.5-5.0 mM) and cisplatin (2.5-80.0 μM) used under Methods-Cell viability assay were added.
- In discussion & conclusion

Our presentation was mostly correlative results, but we did perform an inhibitor assay, to clarify the causative results. We found that inhibition of mTOR pathway by rapamycin did not reduce the ERCC 1 kinase effect of melatonin. So, we added these to clarify our discussion part " However, the only slight reduction of ERCC 1 in our assay using rapamycin as an inhibitor to mTOR demonstrated that melatonin effect on ERCC 1 was not directly via the mTOR activation pathway (data was not included)"

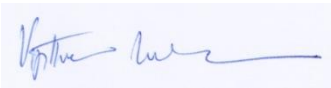
Therefore, melatonin reduced cisplatin induced DNA damage, resulting in the decrease activation of DNA repair capacity might be involved at the transcriptional regulation or an epigenetic mechanism. in the discussion part.

- The term "dose-dependent" was changed to "concentration dependent"
- abcisssa of Fig 1 was corrected
- tracking notes on Fig 4 and 8 were removed
- numbers of observation (n=3) for all the experiments were added in all figure legends and stated as triplicate. Statistic in fig 1 ($p<0.001$) is added in the figure legend.
- We did each experiment in triplicate at least, this statement was added in every part
- Fig 11 title outline was changed to **A schematic overview of major findings in the combination group.**
- The English grammar was revised extensively throughout the manuscript by Professor Dr Peter Hanna from Australia who is an Adjunct Professor in our Anatomy Department, Mahidol University, Bangkok, Thailand**.**

3. References and typesetting were corrected

Thanks you again for publishing our manuscript in *World Journal of Hepatology*.

Sincerely yours,



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