Format for ANSWERING REVIEWERS

November 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: **5325-review**.doc).

Title: Methylsulfonylmethane suppresses hepatic tumor development through activation of apoptosis

Author: Joo-Hyun Kim, Hye-Jun Shin, Hye-Lin Ha, Young-Ho Park, Tae-Ho Kwon, Mi-Ra Jung, Hyung-Bae Moon, Eun-Sang Cho, Hwa-Young Son, Dae-Yeul Yu

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 5325

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

1 Format has been updated

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

(1) Abstract should be completely restructured according to the journal style and subheadings, e.g. aims, methods, results, etc.

● **Response**

According to the reviewer's comment, we have modified abstract.

➤ **AIM:** To investigate the effect of methylsulfonylmethane (MSM), is recently reported to have anti-cancer

effects, in liver cancer cells and transgenic mice inducing hepatic tumor.

METHODS: Three liver cancer cell lines, HepG2, Huh7-Mock and Huh7-H-*ras*^{G12V} were used. Cell growth was measured by Cell Counting Kit-8 and soft agar assay. Western blot analysis was used to detect Caspases, Poly (ADP-ribose) polymerase (PARP), and B-cell lymphoma 2 (Bcl-2) expressions. For *in vivo* study, we administrated MSM to H-*ras*^{12V} transgenic mice for 3 months.

RESULTS: MSM decreased the growth of HepG2, Huh7-Mock, and Huh7-H-*ras*^{G12V} cells in a dose - dependent manner. That was related with significantly increased apoptosis and reduced cell number in MSM treated cells. Cleaved caspase-8, cleaved caspase-3 and cleaved PARP were remarkably increased in the liver cancer cells treated with 500 mM of MSM, however Bcl-2 was slightly decreased in 500mM. Liver tumor development was greatly inhibited in the H-*ras*^{12V} transgenic mice treated with MSM compared to control by showing reduced tumor size and number. Cleaved PARP was significantly increased in non-tumor treated with MSM compared to control.

CONCLUSION: Liver injury was also significantly attenuated in the mice treated with MSM. Taken together, all the results suggest that MSM has anti-cancer effects through inducing apoptosis in liver cancer.

(2) please shorten introduction, the molecular structure of MSM is probably not needed here

● **Response**

According to the reviewer's comment, we have deleted in our revised manuscript as follows (page 5, 6).

➤ (Omission)….. Apoptosis is a physiological process for involution and atrophy of various tissues and organs during development and maintenance of tissue homeostasis. ~~It is characterized by cell shrinkage, chromatin condensation, and nuclear and cell fragmentation.~~ The apoptosis pathway is mediated by death receptor that includes tumor necrosis factor receptor (TNFR), Fas and TNF-related apoptosis-inducing ligand (TRAIL)….(Omission)

~~Methylsulfonylmethane (MSM), also known as dimethylsulfone (DMSO2) and methyl sulfone, is an organic sulfur-containing compound that occurs naturally in a variety of fruits, vegetables, grains, and animals including humans. MSM is a molar mass of 94.13g/mol. A white, odorless, slightly bitter-tasting crystalline substance containing 34 percent elemental sulfur, it is a normal oxidative metabolite product of dimethyl sulfoxide (DMSO). The effect of MSM has been reported in seasonal allergic rhinitis, osteoarthritis~~

~~pain, interstitial cystitis, colitis, and autoimmune. In addition~~ Methylsulfonylmethane (MSM) is an organic sulfur-containing compound, inhibits LPS-induced release of pro-inflammatory mediators in murine macrophages through downregulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-k B) signaling……(Omission)

(3) Statistical methods need revision, probably the use of a T-test with separate variance estimated and a two-tailed ANOVA would be more appropriate-please do not use simple two-tailed T-test or one-way ANOVA.

● **Response**

According to the reviewer's comment, we have modified statistical methods (page 11).

➤ Data were analyzed using SigmaStat 3.1 software. All data are presented as the mean ± the standard error of the mean (SEM) from at least three independent experiments. Comparisons between groups were analyzed by student *t*-test for paired and unpaired measure. *P* value < 0.001 was considered statistically significant.

(4) Please do not mix results with conclusion and in the revised results delete sentences like "These data suggest that MSM suppresses liver damage in H-ras12V transgenic mice. Taken together MSM has an effect to inhibit hepatic tumorigenesis in H-ras12V transgenic mice" these should be moved to discussion only

● **Response**

According to the reviewer's comment, we have deleted them in our revised manuscript as follows (page 14).

➤ (Omission)…..ALT levels were also lower in MSM treated group for 1 month than control group (Figure 4D). These data suggest that MSM suppresses liver damage in H-ras^{12V} transgenic mice. ~~Taken together MSM has an effect to inhibit hepatic tumorigenesis in H-ras^{12V} transgenic mice.~~

(5) Since only the highest dose was efficacious, please discuss if this dose could be feasible to be used in humans?

● **Response**

According to the reviewer's comment, we discussed in discussion if the highest dose be feasible in humans (page 16, 17).

➤ MSM was efficacious with treatment of 500 mM in inhibition of hepatic tumor cell growth. In addition, apoptosis rate was 6 - fold increased in all of liver cancer cell lines treated with 500 mM compared to control. These results indicate that MSM is efficacious with treatment of the highest dose in liver cancer cells, consistent with the result that MSM suppresses breast cancer cell growth at 300 mM [10]. MSM is an edible natural organic compound present in many food items and is not associated with any toxic effect even at higher concentration [16, 17]. MSM administration with high dose (100 μg/g) to H-ras12V transgenic mice for 3 months did not affect body weight ratio, but improved liver function by showing lowered AST and ALT levels and remarkably retarded hepatic tumor growth in MSM treated group. All the results suggest that MSM could be available for inhibition of hepatic tumor growth. Further researches are needed to be feasible in humans.

(5) Please revise discussion and discuss in details the potentials for human use.

● Response

According to the reviewer's comment, we revised discussion by discussing the potentials for human use(page 16, 17).

➤ MSM was efficacious with treatment of 500 mM in inhibition of hepatic tumor cell growth. In addition, apoptosis rate was 6 - fold increased in all of liver cancer cell lines treated with 500 mM compared to control. These results indicate that MSM is efficacious with treatment of the highest dose in liver cancer cells, consistent with the result that MSM suppresses breast cancer cell growth at 300 mM [10]. MSM is an edible natural organic compound present in many food items and is not associated with any toxic effect even at higher concentration [16, 17]. MSM administration with high dose (100 μg/g) to H-ras12V transgenic mice for 3 months did not affect body weight ratio, but improved liver function by showing lowered AST and ALT levels and remarkably retarded hepatic tumor growth in MSM treated group. All the results suggest that MSM could be available for inhibition of hepatic tumor growth. Further researches are needed to be feasible in humans.

3 References and typesetting were corrected.

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

Sincerely yours,

Dae-Yeul Yu, PhD

Aging Research Center

Korea Research Institute of Bioscience and Biotechnology

Daejeon, 305-806

South Korea

Fax: +82-42-860-4609

E-mail: dyyu10@kribb.re.kr