Author's response to reviews

Title: JTE-522, a selective COX-2 inhibitor, inhibits growth of pulmonary metastases of colorectal cancer in rats.

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Author’s response to reviews: see over
Dear Editor,

We appreciate your kind letter of December 3, 2004 regarding our manuscript #1014057804611616.

We are sending the revised manuscript. The manuscript was revised along with reviewer’s comments to clarify the methods used in this study, making it easy to understand for potential readers. We also corrected grammatical errors as far as we could.

Response to the reviewer and point of revisions are summarized in this cover letter.

We hope that the revised manuscript is now suitable for publication in the “BMC Cancer”.

Sincerely yours,

Hirotoshi Kobayashi, M.D.

Response to the reviewer and summary of revisions are as follows;

**Response to the reviewer**

We appreciate for your precise comments. We agreed with your suggestions and revised the manuscript in each point accordingly.

To major comments

1) According to the reviewer’s comment, we added a detailed description of the method for creating lung sections in Methods section (Pulmonary metastases of colon cancer in rats, P4, line 3 – 6). After 3-day formalin fixation, lungs were embedded in paraffin. Paraffin-embedded sections were cut into 3-micron sections, which stained with hematoxylin and eosin to evaluate the pharmacological effects of JTE-522 on pulmonary metastases. As it was mentioned by the reviewer, our description of the
criteria used for section selection might not be easy to understand. The number and the size of metastatic tumors were evaluated in the largest sagittal cross section of each specimen.

2) The reviewer mentioned that we converted distribution score to intensity score. However, we did not convert. In the present study, we evaluated the immunostaining by both distribution and intensity. The score for distribution and intensity were added and graded. To our knowledge, this is a common method for evaluation of immunostaining (Reference 16). These descriptions were mentioned in the Methods section (Evaluation of immunostaining). Moreover, we added some description to understand easily (P 4, line 38 – 39).

3) According to the reviewer’s suggestion, we added sufficient detail to figure legend of Figure 4, and used Spearman's rank test for a correlation analysis. There was no correlation between COX-2 score and VEGF score (p = 0.60). This may be attributed to the administration of JTE-522.

4) We agreed with the reviewer’s comment that colorectal metastasis occurs primarily in the liver, and that pulmonary metastasis in this study occurred as a result of the injection of tumor cells into tail vein. According to the reviewer’s suggestion, we mentioned in the background section why we chose experimental model of lung metastasis in rats. We also commented on the clinical significance of our observations in both background and discussion sections. Laparotomy is necessary for making experimental model of liver metastasis in rats. It would make them strained. On the other hand, it is less invasive to make lung metastasis by injection of tumor cells into the tail vein of rats. Thus, we considered that experimental model of pulmonary metastasis was appropriate for the evaluation of the real effect of JTE-522, a selective COX-2 inhibitor, on hematogenous metastasis of colon cancer in rats.

To minor comment

1) We corrected VEFR to VEGF in Table 1.

Summaries of revisions

Title
P 1, line 1: changed "The selective COX-2 inhibitor, JTE-522” to “JTE-522, a selective
COX-2 inhibitor

Abstract
P 2, line 18: changed “decreases” to “decreased”

Background
P 2, line 34: changed “decreases” to “decreased”.
P 3, line 4: changed “Cyclooxygenase” to “COX”.
P 3, line 12: changed “increases” to “increased”.
P 3, line 13: changed “correlate” to “correlated”.
P 3, line 16 – 23: added a description of the clinical significance of our observations

Methods
P 4, line 3 – 6: added a detailed description of the method for creating lung sections.
P 4, line 38 – 39: added a description of evaluation of immunostaining.
P 4, line 47 – 48: added a description of Spearman’s rank correlation.

Results
P 5, line 13 – 14: added the result of the correlation analysis for evaluation of the association between VEGF score and COX-2 score.

Discussion
P 5, line 20: changed “numbers” to “number”.
P 6, line 19: changed “is” to “was”.
P 6, line 20: changed “is” to “was”.
P 6, line 21: changed “is” to “was”.
P 6, line 22: changed “are” to “were”.
P 6, line 24: changed “are” to “were”.
P 6, line 25: changed “correlates” to “might correlate”.
P 6, line 27 – 28: added a description of clinical significance of this study.

Figure legends
P 9, line 42: changed “numbers” to “number”.
P 10, line 19 -21: added a detailed description on a correlation analysis for evaluation of
the association between VEGF score and COX-2 score.