

ANSWERING REVIEWERS



March 21, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2069-review.doc).

Title: Intraperitoneal perfusion of cytokine-induced killer cells with local hyperthermia for advanced hepatocellular carcinoma

Author: Xiao-Pu Wang, Meng Xu, Hong-Fei Gao, Jian-Fu Zhao, Ke-Cheng Xu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2069

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

(1) Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).

Answer: Please find enclosed the language certificate letter, which provided by Jing-Yun Ma Office for SCI Biomedical Editing and Publishing. The edited paper has reached grade A as defined by WJG language evaluation.

(2) The title page carries the title, the authors, the authors' affiliations, and footnotes. Title: The title must be informative, specific, and brief (Title should be no more than 10~12 words/60 bytes. Please revise it). Words should be chosen carefully for retrieval purposes.

Answer: The title is (Intraperitoneal perfusion of cytokine-induced killer cells with local hyperthermia for advanced hepatocellular carcinoma)

(3) Please provide the author contributions.

(4) Family name should be put first in full, followed by middle names and first name in abbreviation with first letter in capital

Answer: Author contributions: Wang XP and Xu M contributed equally to this work. Xu M and Xu KC designed research; Wang XP and Xu M performed research; Zhao JF provided new reagents or analytic tools; Gao HF analyzed data; Wang XP and Gao HF wrote the paper.

(5) Please provide the fax number.

Answer: Fax: +86-20-38688000

(6) It's too short. Please add it to 80 words. Thank you!

Answer: METHODS: Patients with advanced primary HCC were included in this study. CIK cells were perfused intraperitoneal twice a week, using 3.2×10^9 to 3.6×10^9 cells each session. Local RF hyperthermia was performed 2 h after intraperitoneal perfusion. Following an interval of one month, the next course of treatment was administered. Patients received treatment until disease progression. Tumor size, immune indices (CD3+, CD4+, CD3+CD8+, CD3+CD56+), alpha-fetoprotein (AFP) level, abdominal circumference and adverse events were recorded. Time to progression and overall survival

were calculated.

(7) 请按此格式修改其他数据。

(8) Authors should present exact P value where necessary and must provide relevant data to illustrate how it is obtained.

Answer: The data and exact P value has been provided in this essay. For example, following combination treatment, CD4+, CD3+CD8+ and CD3+CD56+ cells increased from 35.78% \pm 3.51%, 24.61 \pm 4.19% and 5.94 \pm 0.87% to 45.83 \pm 2.48% (P=0.016), 39.67 \pm 3.38% (P=0.008) and 10.72 \pm 0.67% (P=0.001), respectively.

(9) The description of results should not simply reiterate data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. The results section should be concise and follow a logical sequence. If the paper describes a complex series of experiments, it is permissible to explain the protocol/experimental design before presenting the results. Do not discuss the results or draw any conclusions in this section. This section may be divided into subheadings to assist the reader. Large datasets or other cumbersome data pertinent to the manuscript may be submitted as supplementary information.

Answer: Table 2 has been deleted and the content of results has been modified (page 7-8).

(10) A decomposable figure is required. It means that the fonts and lines can be edited or moved.

Answer: The figure has been edited as excel format.

(11) The colour figures should be saved in TIFF format at a resolution of 300 dpi; each figures in a separate file, and no smaller than 86 mm \times 50mm.

Answer: All of the figures were edited again according to the regulation.

(12) Do not recapitulate the results, but discuss their significance against the background of existing knowledge, and identify clearly those aspects that are novel. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take. This section may be divided into subheadings to assist the reader.

Answer: The section of result has been divided into four subheadings. They are Tumor killing ability of CIK cells, Intraperitoneal perfusion with local hyperthermia, Adverse events and Conclusion. This part have also been expressed logically and the final paragraph provided some indication of the direction future research. (page11-14)

COMMENTS

Answer: Comments have been written following the requires.

Background

HCC is one of the most common malignant tumors worldwide. Recently, integrative therapy has become a useful treatment for patients with advanced HCC. Due to the close relationship between the pathogenesis of HCC and the autoimmune system, cellular immunotherapy has been used in the clinical treatment of advanced HCC. In addition, local hyperthermia increases the role of CIK cells in killing tumor tissues.

Research frontiers

Intraperitoneal perfusion of CIK cells in combination with local RF hyperthermia can improve immune function in HCC patients, shrink the tumor, and relieve discomfort such as debilitation and abdominal distention.

Innovations and breakthroughs

Recently, intravenous infusion of CIK cells has been widely used in the treatment of HCC. However, the concentration of CIK cells is low in tumor tissues following intravenous infusion, and the anti-tumor effect is low. In our research, intraperitoneal perfusion of CIK cells in combination with local RF hyperthermia resulted in a high concentration of CIK cells in the abdominal cavity and effective killing of tumor tissues.

Applications

The authors have been using this treatment strategy for more than 1 year. Forty-two patients accepted this treatment, of whom 31 were selected for this study. There were no serious adverse events following this treatment. A few patients showed low-grade fever or slight chest distress which resolved after symptomatic treatment. Following intraperitoneal perfusion of CIK cells in combination with local RF hyperthermia, abdominal distention resolved, tumor size diminished, and survival time was prolonged in some patients. This treatment strategy is worthy of further investigation.

Terminology

CIK cells are alloplasmic cells with non-MHC restriction anti-tumor activity which is acquired by multiple-cell factor-cultivation in vitro from human PBMCs. CD3+CD56+ T is the main effector cell of the CIK cell group, and is also called NK cell-like T lympholeukocyte. These cells have powerful anti-tumor activity and MHC restriction of T lympholeukocytes.

Local RF hyperthermia is used in the non-invasive treatment of malignant tissues. The difference between the complex dielectric constant (complex impedance) of malignant and healthy tissues makes it possible to select malignant tissues.

Peer review

In this study, 31 patients with advanced HCC who could not be treated by surgical treatments or interventional therapies, were treated by CIK cell intraperitoneal perfusion in combination with RF local hyperthermia. The result showed that this treatment is safe and reliable, and worthy of further clinical studies of a larger sample. It is a new option of treatment for patients with advanced HCC.

(13) Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

Answer: References and typesetting were corrected. PMID and DOI were added.

3 References and typesetting were corrected

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

Sincerely yours,
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