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TITLE: Experimental Treatment of Prostate Cancer Models with Rh2, An Isolated Ginsenoside Compound

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Fort Detrick, Maryland 21702-5012

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Experimental Treatment of Prostate Cancer Models with Rh2, An Isolated Ginsenoside Compound

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Ginseng is commonly used in herbal preparations for traditional Chinese medicine. Rh2, one of the ginsenosides, has been shown to suppress growth and induce apoptosis in a number of cancer cell lines both in vitro and in vivo. To evaluate the combined efficacy of Rh2 and two chemotherapeutic agents, Paclitaxel and mitoxantrone, mice bearing the LNCaP or PC3 prostate tumor xenograft were treated with corn oil (po) and saline (iv), Rh2 (50mg/kg po daily), paclitaxel (6 mg/kg iv on day 1, 4, 15, and 18), mitoxantrone (2.5 mg/kg iv on day 1, 4, 15, and 18), Rh2 + paclitaxel and Rh2 + mitoxantrone. Tumor volumes were measured twice weekly for 4 weeks. Serum PSA were tested using ELISA for LNCaP models. Results showed 1) For the LNCaP models, student t-test was performed on the data acquired and results showed statistically significant differences exist between the tumor growth ratio of control group and Rh2+ paclitaxel treatment group (P<0.05) from day 9. No statistical significant differences existed between the control group and the Rh2, paclitaxel or mitoxantrone monotreatment groups. Paclitaxel monotherapy and paclitaxel + Rh2 combination showed significant (P<0.05) and very significant (P<0.01) inhibitory effect on serum PSA levels. 2) There was no statistically significant differences exist between PC-3 model groups treated with placebo, Rh2, Paclitaxel, mitoxantrone or combination. Overall, our results suggest that oral administration of Rh2 can sensitize low dose of Paclitaxel in the treatment of mice bearing subcutaneous LNCaP prostate tumors and exhibits potential as a chemosensitizer of paclitaxel for treatment of androgen-dependent prostate cancer.

Prostate cancer, tumor xenografts, ginsenoside Rh2, chemotherapy

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Ginsenosides, the major active component in Panax ginseng, contains a series of derivatives of the triterpene dammarane attached by sugar moieties. Ginsenoside Rh2 has been reported to induce cell proliferation [1] and induce apoptosis [2-4], inhibit proliferation [5] in various human tumour cells. Previous study conducted in our group also demonstrated that Rh2 could inhibit tumour growth in LNCaP prostate cancer models, when used as a monotherapy. In the research reported here, we studied the combination therapy effect of Rh2 and other chemotherapy reagents in prostate cancer models, both androgen dependent and androgen independent.

1) Original Statement of Work (Copied from the grant proposal):

Task 1. To study the toxicity of Rh2 co-administered with Paclitaxel or mitoxantrone in nude mice. (months 1-3)

Although our previous study showed that Rh2 is well tolerated, it is unknown whether toxicity would occur upon combination of Rh2 with conventional chemotherapeutic agents. Thirty nude mice will be divided into 6 groups, and vehicle solution, Rh2, Paclitaxel, mitoxantrone, Rh2 + Paclitaxel, Rh2 + mitoxantrone will be administered. A report will be generated by the end of the toxicity study describing any side effect(s)/toxicity. Daily observational and body weight records will be attached.

Task 2. To compare tumor inhibitory effect of Rh2 in vivo (months 3-19)

The efficacy of treatment with Rh2 alone and in combination with conventional therapeutic agents will be examined in the LNCaP and PC-3 prostate tumor model. To limit treatment groups to a manageable size, this part of study will be divided into two sections: efficacy study in androgen-dependent prostate tumor models and efficacy study in androgen-independent prostate tumor models. Sixty nude mice will be used in each study. These will be divided into 6 groups to which vehicle solution, Rh2, Paclitaxel, mitoxantrone, Rh2 + Paclitaxel, Rh2 + mitoxantrone will be administered. Tumor volume and PSA will be recorded weekly. At the end of each study, tumor tissue will be harvested and stored at -80°C until further gene expression analysis.

Task 3. To study the mechanism of action of the tumor inhibitory effects of Rh2. (months 6-24)

a) The RNA of LNCaP tumors from different treatment/control groups will be prepared using TriZol (LifeTech).

b) Gene array analysis will be carried out using RNA from treated and untreated tumors in vivo. Human EST gene microarray slides will be purchased from OCI, Ontario, CA. Gene array analysis will be carried out in the array facility at The Prostate Centre (see letter of collaboration, Nelson). (months 6-19)

c) The results of gene array analysis will be interpreted using an online database. A report will be generated following cluster analysis to determine differences in gene expression patterns in tumor tissue following treatment, comparing with untreated tumor tissue. Genes involved in apoptosis and cell survival will be examined to specifically detect any change in their expression levels. (months 20-24)

2) Experiment accomplished:

a. Toxicology study:

i. The first toxicology study was started on Jun 24, 2002 and terminated on July 10, 2002, due to the severe toxicity showed in mitoxantrone treatment group (Fig. 1). The mice in both mitoxantrone alone and mitoxantrone + Rh2 group lost >15% body weight in 1 week. Treatment was stopped and mice in both groups regained body weight.
ii. The second toxicology study was started on July 8, 2002 and terminated on August 5, 2002. Due to the mitoxantrone toxicity showed in the first toxicology study, the mitoxantrone dosage was reduced to 2.5 mg/kg i.v. twice weekly, every other week which is similar to the optimal dosage of mitoxantrone in nude mice reported by Miyake et al [6]. Accordingly, Paclitaxel dosage was reduced to 6 mg/kg i.v. twice weekly, every other week.

Based on the findings of the acute toxicity study, we determined that the dosing regime of 6 mg Paclitaxel/kg body weight twice weekly and 2.5 mg mitoxantrone/kg body weight twice weekly are safe in nude mice. The change of dosing regime had been sent to Ms. Cockerham c/o Dr. Nrusingha C. Mishra on 20 June 2002 (see attached copies of email and letter).

b. Efficacy study in nude mice bearing LNCaP prostate tumour xenografts.
Sixty nude mice were purchased and inoculated with LNCaP cells. The tumours achieved 100~150 mm$^3$ in size by 2nd week of October 2002. The treatment started on 18 October 2002. Mice were divided randomly into 6 groups and dosed for 4 weeks as outlined in Table 1.
Table 1. Dose regime for efficacy studies in nude mice bearing LNCaP or PC-3 tumour xenografts.

<table>
<thead>
<tr>
<th>Group</th>
<th>Corn Oil p.o. (5 days/week)</th>
<th>Rh2 50 mg/kg p.o. (5 days/week)</th>
<th>Saline i.v. (twice/week)</th>
<th>Paclitaxel 6 mg/kg i.v. (twice/week)</th>
<th>Mitoxantrone 2.5 mg/kg i.v. (twice/week)</th>
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For LNCaP models, tumour sizes were measured twice weekly and 100 µl of blood was collected via saphenous vein puncture bleeding. The serum PSA were measured using an ELISA kit (Clinpro, Union City, CA, USA).

![Graph](image)

Fig. 3. Tumour growth ratio for Rh2/Paclitaxel combination therapy. From day 9, the group treated with Rh2+Paclitaxel showed significant suppression with control group (Student t-test, p<0.05*).
c. Efficacy study in nude mice bearing PC-3 prostate tumour xenografts.
Sixty nude mice were purchased and inoculated with PC-3 cells. The tumours achieved 100–130 mm³ in size by 3rd week of April 2004. The treatment started on 24 April 2004. Mice were divided randomly into 6 groups and dosed for 4 weeks as outlined in Table 1. Although mitoxantrone and mitoxantrone/Rh2 combination groups showed the trend of tumour growth inhibition (Fig. 7), there was no significant difference observed.
d. Tissue Micro Array (TMA) construction and histochemistry staining studies. Specific aim 3 (gene array analysis for the gene expression pattern change for the tumour cells in the treatment groups) was suggested to be removed by the grant reviewer and thus the fund has not been provided for such study. Alternatively, the novel TMA technology (provided by the pathology core of Prostate Centre at Vancouver General Hospital, headed by Dr. Martin Gleave) was used to analyze the possible cancer related proteins/antigens expression pattern change. In brief, the nude mice bearing LNCaP tumour xenografts from the efficacy study were sacrificed at the end of the study and tumour tissue were collected and fixed. A 312-core TMA was constructed and 100 slides were produced from the tissue block. Various antibodies had been used for the histochemistry staining. An image database had been generated using both manual microscope image capture and Bliss System. Due to the novelty of this technology, the automatic histochemistry score system is not
available yet. All the results are pending score by in-house pathologist. Fig 8 demonstrates one of the TMA slides stained using H&E staining.

Key Research Accomplishments

1) Acute toxicity study showed that 4-week treatment with Rh2 (50 mg/kg p.o. 5 days/week) + Paclitaxel (6 mg/kg i.v. twice weekly) or mitoxantrone (2.5 mg/kg i.v. twice weekly) is safe for nude mice;

2) Efficacy study conducted in nude mice bearing LNCaP xenografts showed that Paclitaxel+Rh2 treatment significantly inhibits tumour growth in vivo (Student t-test, p<0.05) and significantly inhibits serum total PSA levels (Student t-test, p<0.01).

3) Though the efficacy study conducted in nude mice bearing PC-3 xenografts showed no significant differences between different treatment regimes.

Reportable Outcomes


A manuscript is under revision and will be submitted to Molecular Cancer Therapeutics by the end of May 2004.

Multiple pharmacokinetics and efficacy study in different tumour models is undergoing, based on the results reported here. After the summary of all the studies, the possibility of clinical trial using the combination of Rh2 and chemotherapeutic agents (e.g. paclitaxel/docetaxel) will be reviewed.

Conclusion

Low dose Paclitaxel (6 mg/kg i.v. twice weekly) combined with ginsenoside Rh2 (50 mg/kg p.o. 5 days per week) has been proved to be safe and effective therapeutic regime in LNCaP prostate tumour models. Ginsenoside Rh2 sensitizes the tumour inhibitory effects of low dose of Paclitaxel in this androgen-dependent prostate tumour model.
REFERENCE


APPENDICES

1. U.S. Army Medical Research and Materiel Command Animal Use Report for fiscal year 2002;

# U.S. Army Medical Research and Materiel Command Animal Use Report

**Facility Name:** Jack Bell Research Centre  
**Address:** 2660 Oak Street  
Vancouver, BC V6H 3Z6  
Canada  
**Contract Number:** DAMD17-02-1-0260  
**Principal Investigator:**  
(Signature)  
**Principal Investigator:** Xiaowei S. Xie  
(Typed/Printed Name)  
**E-mail Address:** sxie@vanhosp.bc.ca  
**Phone Number:** 604-875-4111 x 63439  
**Fax Number:** 604-875-5654  

This Report is for Fiscal Year 2003 (01 October 2002 - 30 September 2003)

### Definitions of Column Headings on Back of Form

<table>
<thead>
<tr>
<th>A. Animal</th>
<th>B. Number of animals purchased, bred, or housed but not yet used</th>
<th>C. Number of animals used involving no pain or distress</th>
<th>D. Number of animals used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to alleviate pain</th>
<th>E. Number of animals used in which pain or distress was not alleviated</th>
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Column E: Number of animals used in painful procedures in which pain relieving compounds were not administered.

Column F: Sum of columns C, D, and E.

Forward this report by DECEMBER 01 of each year to:

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U.S. Army Medical Research and Materiel Command
ATTN: MCMR-RCQ-AR
504 Scott Street
Fort Detrick, MD 21702-5012

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