16th INTERNATIONAL CONGRESS OF ORIENTAL MEDICINE

PROGRAM and ABSTRACTS

The Future of Medicine, Traditional Medicine

Sep. 14(Fri)~16(Sun), 2012
COEX in Seoul Korea
Radioprotection Effect and Immune Activity by Enterococcus Facalis 2001

Yeunhwa Gu, Takenori Yamashita, Yukimitsu Sato, Masahiro Iwasa

Background & Aim:
Although it has been reported that Enterococcus Facalis 2001 (EF 2001) shows immunoenhancement activity and antioxidant activity, its protective effects against radiation have not been investigated.

Materials & Methods:
It is unknown what mechanisms are involved in the protective effect against radiation and the anti-radiation activity in these substances. Blood cells are one of the indices for examining protective effects against radiation. Radioprotective activities of peroxidases and free radicals are highly sensitive to radiation, and a decrease of immunity caused by a decrease in white blood cells and peroxidases were remarkable after exposure to radiation. Furthermore, measurement of Lymphocyte subsets, SOD-like activity, an antioxidant activity, and scavenging activity based on immunoenhancement methods, and absolute amounts of free radicals based on ESR were carried out and indeed scavenging activity with a scavenger of free radicals was measured.

Results:
In addition, we examined antioxidant activity and immunoenhancement activity, both of which are closely related to tumor suppression, and further investigated effects on suppressive effects on tumor growth and anti-tumor effects of tumors provoked by EF 2001.

Conclusion:
We found that a combination of immunotherapy using natural materials such as EF 2001 with immunoenhancement activity may increase the potency of peroxidase scavenge.

Keywords:
Radioprotection effect, immune activity, SOD, Anti-oxidant activity, Radical scavenging activity.
Radioprotection effect and immune activity by *Enterococcus Facalis* 2001

Yeun-Hwa GU¹, Takenori Yamashita², Yukimitsu Sato¹ and Masahiro Iwasa³

¹Department of Radiological Science, Faculty of Health Science, Junshin Gakuen University, 1-1-1 Chikushigaoka, Minami-ku, Fukuoka 815-8510 Japan
²Department of Radiological Science, Faculty of Health Science, Suzuka University of Medical Science, 1001-1 Kishioka-cho, Suzuka-shi, Mie 510-0293 Japan
³Nihon BRM Research Center, Akasaka Tokyu Bilding 9F, 2-14-3, Nagata-cho, Tsidoda-ku, Tokyo 100-0014 Japan
Purpose

- We study mechanism of anticancer effect and immunological enhancement effect for *EF 2001*.
- SOD-like activity and radical scavenging ability by *EF 2001*.
- We investigated in CD 4 and CD 8 activity for EF 2001

End point: Examination to radiation protection effect and antitumor effect
Enterococcus Faecalis

Enterococcus Faecalis under microscope (left) x 20, (right) x 12900
Material & Methods

- Anti-cancer effects
- Seven-weeks-male ICR (Crj) mice
- Cancer cells: SCC-7 (2 x 10^6)
- EF 2001 200, 400mg/kg of heat-killed EF2001 (EF2001) were administrated in oral each for 2 weeks every other day
- Statistical methods: t-test, Dunnett's-test
Material and Methods

Blood corpuscle cell: ICR mouse (five week of age, ♂): 
Use machinery; a roentgen radiation device (product made in Philips Corporation, MG226/4.5); all; automatically; an erythrocytometer surveying instrument (Nihon Kohden celltac- α)

Medication method; EF 2001 o.p. at 200, 400mg/kg for every day

Irradiation condition; 6Gy/ (0.355Gy/min): three times division local irradiation

Experimental group: Control group, 200mg/kg group, 400mg/kg, 800mg/kg, 6Gy group, EF 2001 +6Gy irradiation group
Anti-tumor Activity: The effect that radioprotector gives a tumor

A purpose on the occasion of radiotherapy is breakdown and annihilation of tumor cell, annihilation.

It is important that we consider the influence that protective agent gives a tumor.

We measure a longer axis (A) and a minor axis (B) in micrometer calipers every other day and convert it into volume (V) in the next formula:

\[ V = \frac{(A \times B^2)}{2} \text{ [mm}^3\text{]} \]
Analysis of T lymphocyte by flow cytometry

- Animal: C57BL (five weeks of age, ♂)
- Analysis device: flow cytometer (BD Co, Ltd.)
- Antibody: CD3/CD4/CD8 and negative control
- Medication method: EF 2001 200mg/kg
  (administered in o.p. every day)
- Drawing blood: After 2Gy radiation 7 days and 10 days
- Experimental group:
  - Control group
  - EF 2001 group
  - 2Gy group
  - EF 2001 + 2Gy group
Experiment Schedule

Measure every other day since tumor size measurement —①
7th days
Enucleation of tumor ———①(35th days)
Roentgen radiation ———①—③(15,18,21 days judgment)
Blood count ———①—⑦(14,16,20,22,24,28,36 day judgment)

Extra breeding
Sample administration
A tumor inoculation
Leukocyte counts on different days after irradiation in mice of different groups. The number of leukocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.
Lymphocyte counts on different days after irradiation in mice of different groups. The number of lymphocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.
Repeated dose effect of EF 2001 on the NK activity in mice. Groups of ten mice each were subjected to each treatment. Results represent means ± S.D. * Statistically significant ($P < 0.05$) from the control group.
Effect of EF2001 on the tumor growth in mice inoculated with SSC-7 carcinoma cells. Groups of ten mice each were subjected to each treatment. Results represent means ± S.D. * Statistically significant (P<0.05) from the control group.
The increased percentage of CD4+, CD8+ and CD16+ T-lymphocytes in PBLs compared to the experimental data baselines of the groups. The unit is in percentage (%). Significantly different from *P<0.05 Control group vs. EF2001 groups by Dunnett test.
Conclusion

- Anti-cancer effects: EF 2001 administration group: positive
- Radiation protection effect: EF 2001 administration group: precision
- Immune activity: EF 2001 administration group: positive
Conclusion

- Leukocyte: Immunological enhancement action was accepted by *EF 2001* administration in leukocyte, lymphocyte by *EF 2001* treated group in comparison with irradiation group.
- Radiation protection effect was suggested by decrease of a blood cell count by irradiation was restrained, and early convalescence having been accepted.

\[\beta-D\text{-glucan and heteroglucan of } EF2001 \text{ contribute to immunological enhancement action.}\]
\[\beta-D\text{-glucan mainly acted as radical scavenger, and we prevented stromatolysis of a blood corpuscle by the oxidation, and radiation protection effect was obtained.}\]
Antitumor effect

Tumor growth inhibition was accepted by a $EF\ 2001$ treated group in comparison with control group, and increase of NK-cell was accepted.

It is thought that the $\beta$-D-glucan which is an active principle of $EF\ 2001$, heteroglucan immunisation enhancement by melanin, antioxidation, antiinflammatory action, and antitumor effect was obtained by self-cure indirectly.

In addition, it is thought that $\beta$-1,3-Dglucan having loud antitumor effect of $EF\ 2001$ acts on a indirect tumor, and antitumor effect was obtained.
3. Notice

- If you have to withdraw the abstract after the acceptance notice. If you need to cancel your abstract after the acceptance notice, please do not forget to inform the secretariat.

We cordially announce that all speakers are requested to check notice and the venue where presentation will be took place.

If you have any questions, please contact the 16th ICOM secretariat by phone (+82-2-2152-5024) or e-mail (akom@icom2012.org).

We are looking forward to your participation in the congress and seeing you in September.

Thank you.

[Signature]

Jeong-Gon, KIM OMD, PhD
President of 16th ICOM
President of AKOM
ABSTRACT ACCEPTANCE LETTER

July 20, 2012

Dear Yeunhwa Gu:

On behalf of the 16th ICOM committee, we are pleased to inform you that your thesis has been approved for the presentation of the congress to be held from Sept. 14 to 16, 2012.

Please confirm us the following information.

- Name of the applicant: Yeunhwa Gu
- Affiliation: Faculty of Health Science, Junshin Gakuen University
- Country: Japan
- Registration number of the abstract: 2012-0028
- Title of the abstract: "RADIOPROTECTION EFFECT AND IMMUNE ACTIVITY BY ENTEROCOCCUS FACALIS 2001"
- Date of presentation: September 16
- Type of presentation: Poster
- Allocated session: Current Status of Medical Devices Industry in Traditional Medicine
- Allocated Q&A time: 15:30-16:00

Rules of presentation

1. All speakers shall be registered with the congress.
   - Pre-registration for poster presenter is by August 20.

2. Composition and presentation
   - Please refer to the guideline of poster presentation posted at ICOM website.

16th ICOM Organizing Committee
26-27, Gayang-dong, Gangseo gu, Seoul, 157-200, Republic of Korea
Phone: +82-2-2152-5024  Fax: +82-2-3401-2572  E-mail: akom@icom2012.org