The 13th International Congress of Oriental Medicine

ABSTRACTS

Subject  Ways to a Better Life in an Ageing Society
Period  October 20-23, 2005
Venue  EXCO DAEGU, KOREA

Sponsored By
- World Health Organization (WHO)
- Ministry of Health & Welfare (MOHW), R.O.K.
- Ministry of Culture & Tourism (MCT), R.O.K
- Daegu Metropolitan City
- Korean National Tourism Organization (KNTO)
Anti-cancer effect, Radioprotection effect and Anti-allergy for Enterococcus Faecalis (EF 2001)

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The preparation reduced to powder, which removed fungi mainly from Enterococcus / Fekaris 2001 (BeRMKAIN), which was Enterococcus / Fekaris 2001, was used in this study. Because L/P activity was recognized from Enterococcus / Fekaris (BeRMKAIN), it was examined about the anti-neoplasm effects that it faces Math/A fibrosarcome in the mice, and the analgesic action by the preceding research. They are inoculated 1 x 10^6 cells and 2 x 10^5 directly in the right and the left low abdomen region the intratumorale as for the anti-neoplasm simulation by the way of the administration meth A fibrosarcome to each of the BALB/c mouse. The dimension of the tumor enhancement was measured more than the implantation posticus the 3rd day from a total 10 times, the top of the cutaneous even in the 2nd de die in diem the every other day, and the total was examined, and gradation was seen at the time as the sutra. It was dissolved with the fraction of 1mg/0.1ml/mouse in the isotonic sodium chloride solution, and specimen substantial administered neoplasm vicinal rear 3, 4, the 5th-day total 3 times for the right intratumorale. And, pain relief simulation was done with the acetac acid lasing method. The L/P activity had statistical significant difference with the 200ug/mouse dose rate (p<0.05). Catalatic determination was judged by the antitumor action the tumor enhancement in both low abdominal regions. Then, catalatic determination was recognized with 1000mg/kg by the analgesic action to the control group.
Immune Activity and radioprotection effect for Enterococcus Faecalis (EF 2001)

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Anti-cancer effect, Radioprotection effect and Anti-allergy for *Enterococcus Faecalis* (EF 2001)

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Abstract

1. Immunisation effect is accepted in *Enterococcus Faecalis* (EF2001), and, as for the action mechanism, EF 2001 by macrophage activity is clarified by preliminary research.

2. Presence of antibody production by *Enterococcus Faecalis* and mechanism of immunostimulation stuck and investigated it by immunoglobulins IgG, the IgM measurement and reviewed it.

3. By the immunoglobulins of IgE measurement by *Enterococcus Faecalis*, we examined about allergic inhibitory reaction.
Material and Methods

We transduced it in C3H and BALC mouse (♀ 0.6 week of age), and 250mg/kg gave an abdominal cavity Enterococcus Faecalis which did a mist in saline after breeding preliminarily of one week every every other day.

We obtained mouse serum after three weeks from an administration start and measured total IgE, IgG and IgM of the whole serum by ELISA method.

It is C3H and six BALC mouse (♀) week of age extra breeding one week animal used for experiment

It is each ten groups an experiment group

Control group (saline administration)]

IgE group (Enterococcus Faecalis administration)

IgG group (Enterococcus Faecalis administration)

IgM group (Enterococcus Faecalis administration)
Immunochemical measurement methods

- Gel diffusion
  (single radial immunodiffusion method, electroimmunodiffusion)

- Immunisation turbidimetric method

  - Solid phase immunoassay
    - Radioimmunoassay
      (RIA method)
    - An enzymatic immunisation assay
      (EIA method)
Use machinery and chemical reagent

- ELISA (Enzyme-linked immunosorbent assay) kit (an antimagous IgE antibody) of BIOTAK of Pharmacia
- Mouse IgG ELISA fixed-quantity kit made in BT company
- Antimouse IgG solid phase antibody, mouse IgG normal serum (7.3mg/ml)
- HRP mark antimouse IgG antibody
- Mouse IgM ELISA fixed-quantity kit made in BT company
- Antimouse IgM solid phase antibody, mouse IgM normal serum (0.21mg/ml)
- HRP mark antimouse IgM antibody
- 0.05M Sodium Carbonate, pH 9.6 Coating Buffer
- 50mM Tris, 0.14M NaCl, 20, 0.05% Tween pH 8.0s Wash Solution
- 50mM Tris, 0.14M NaCl, 1% BSA, pH 8.0 Postcoat Solution
- 50mM Tris, 0.14M NaCl, 1% BSA, 0.05% Tween, pH 8.0 Sample/Conjugate Diluent
- TMB Enzyme Substrate
- 2M H2SO4 Stopping Solution
- Microtiter tray (a product made in ベッテル company)
- Leader microtiter tray MPR A4 (450nm) made in Orient soda water company
Measurement procedure

1. Coat with Capture Antibody
2. Postcoat
3. Standard and Sample
4. Enzyme Conjugate
5. Enzyme Substrate
6. Stop Reaction
7. Read Absorbance (450nm)
Fig. 1. BLB/C mice of IgE in the blood. Each histogram represents the mean value ±SE for 10 mice IgE (M). Significantly different *p<0.05 Control vs. EF 2001.
Fig. 2. C3H mice of IgM in the blood. Each histogram represents the mean value ±SE for 10 mice IgM (M) significantly different *p<0.05 Control vs. EF 2001.
Fig. 3. C3H mice of IgG in the blood. Each histogram represents the mean value ± SE for 10 mice IgG (M). Significantly different *p<0.05 Control vs. EF 2001.
Discussion (the measurement of IgG and IgM)

There was production of the antibody which *Enterococcus Faecalis (EF2001)* administration could put and compared it with control group, and *Enterococcus Faecalis (EF2001)* treated groups increased the level of total IgM of the whole serum. However, the level of total IgG in serum rather decreased slightly.

Promotion of humoral immunity antiaction is thought about without immunostimulation action to have of *Enterococcus Faecalis (EF2001)* lets cell-mediated immunity such as a macrophage and NK cell, T cell activate, and becoming it.
Discussion (the measurement of IgE)

There is production of *Enterococcus Faecalis* (EF2001) administration antibodies to be able to put; in control group; competition, an *Enterococcus Faecalis* treated group; of the whole serum; the level of IgE decreased all-out.

By administration of *Enterococcus Faecalis* (EF2001), a blood IL-2 level increases, and it is thought that lienal cytokine production changed from Th2 type into Th1 type. Therefore, we decrease IgG and an IgE level, and it is suggested that we showed allergic inhibitory effect.
Immune Activity and radioprotection effect for *Enterococcus Faecalis* (EF 2001)

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Material & Methods

- Anti-cancer effects
  - Seven-weeks-old male ICR (Crj) mice
  - Cancer cells: sarcoma 180 ($2 \times 10^6$)
  - EF 2001 5mg/kg of heat-killed EF2001 (EF2001) were injected interpretational (endoceliac) each for 2 weeks every other day
  - Statistical methods: t-test
Enterococcus Faecalis under microscope (left) × 20, (right) × 12900
1. Radiation protection effects

Seven-weeks-old male C3H mice

12mg/Kg 24mg/Kg of heat-killed EF2001 (EF2001) were injected interpretational each for 2 weeks every other day

8Gy of whole body irradiation (Philips co. 200kV)

Change of body weight Survival after irradiation

Sections of the large and small intestines with a microscope
2. Assay of NK cell activity by $^{51}$Cr label YAC-1 cells

Injection of the EF2001, in the same condition using the examination of radiation protection effect.

The mice was scarified and the spleen was extracted.

The spleen was smashed with stainless steel mesh, and then mixed PBS and the suspect ion was centrifuged three times.

The concentration of the spleen cells was adjusted to be $2 \times 10^7$ cells/ ml. Then, $1.25 \times 10^6$, $2.5 \times 10^6$, $5.0 \times 10^6$, $10.0 \times 10^6$, $20.0 \times 10^6$ of the spleen cells were added to $1 \times 10^4$ of YAC-1 cell which labeled $^{51}$Cr of 1mCi and incubated 96 hole plate for 6 hours.

Only liquid component in each hole measured with liquid scintillation.
Survival after irradiation
Surviving fraction was increased after injection of EF2001.
Small intestines with a microscope

Control

8Gy

8Gy + 12mg/kg

8Gy + 12mg/kg
NK cells activity
Activities of NK cells are enhanced 1.46 and 1.94 times in EF 12mg and EF 24mg groups respectively.
Anti-tumor effect of BRM on Meth A fibrosarcoma (solid type) in BALB/c mice

* p<0.05 vs Control
Anti-tumor effect of EF-2001 on S-180 in ICR mice

**P<0.01  *P<0.05 vs Control group**
BLB/C mice of IgE in the blood. Each histogram represents the mean value ±SE for 10 mice IgE (M). Significantly different *p<0.05 Control vs. EF 2001.
C3H mice of IgM in the blood. Each histogram represents the mean value ±SE for 10 mice IgM (M) Significantly different *p<0.05 Control vs. EF 2001.
C3H mice of IgG in the blood. Each histogram represents the mean value ±SE for 10 mice IgG (M). Significantly different *p<0.05 Control vs. EF2001.
Conclusion 1

- Anti-cancer effects: EF 2001 administration group: positive
- EF 2001 to the radiation protection effect: precision
- Immune activity effect: EF 2001 administration group: positive
- Anti-aging effect: EF 2001 administration group: positive
- Long life effect: EF 2001 administration group: positive
- It put the *Enterococcus Faecalis* dosage, and the level of total IgE in serum glutamic-oxaloacetic fell.

- The level of total IgM in serum glutamic-oxaloacetic increased the *Enterococcus Faecalis* dosage group. However, the level of total IgG in serum glutamic-oxaloacetic rather fell slightly.
Conclusion 2

1. It let cell-mediated immunity such as a macrophage and natural killer T cell activate, and the immunization activation action that *Enterococcus Faecalis* has does not become it, and promotion of humeral immunity anti-action is thought about.

2. In the dosage of *Enterococcus Faecalis*, an IL-2 level in blood rises, and it think that cytokine of the spleen changed from Th2 type into Th1 type. Therefore, it decrease IgG and an IgE level, and it is suggested that it showed an allergic restraint effect.