가

2008 7 21 1 2008 8 18

가 가 가 , AK endogenous colony-forming unit(e-CFU) galactose 가 가

가 1. 가 protease inhibitors, 가 metallo-elements, Ca 1990 [8]. G-CSF, GM-CSF, 가 IL-1, TNF, IL-6, SCF 가 [9,10] [11], [12] 가 가 가 가 [1].

가 50 가 가 (Ethinacea) [13]. 가 [2,3]. [4-6], [14,15]. 가

[7]. 1950 가 sulphydryl

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1980

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2.
                                                      bottomed plate 2xf0ells/well
                                                                          37 °C, 5% 200cubator
                                                                          50il
                                                                                          PBS 50l
2.1
                                                             96 well-flat bottomed plate
                                                                                              Griess reagent (1%
                              (20 \pm 2g)
                                            C3H/HeN
   6-8
                Balb/c
                                                      sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride,
   (20 \pm 2g)
                                     (
                                          ) Charles
River Breeding Laboratory (Charles River Japan, Inc., 2.5% sughosphoric acid) ul 50 7
                                                              540 nm
                                                                                              (multiskan EX,
Breeding Center, Yokohama, Japan)
                                                       Thermo Labsystems).
                                                                                                   (NaNO
                   22 ±°C2.
                                  55-60%
               12
                                                                        (Nitric oxide, 2N)O
(NIH-7-open formula)
                                                      2.7 AK
2.2
                                                                                    51Cr
   6
             (300 g/pack,
                                                                 24 well plate (Corning, New York, USA) 1.5
                        4 °C
                                                      X f0cells/ml
                                                                           RPMI1640-5% FBS
             80%
                                                                        2 ml 37 °C, 5% 2CO
           12,000)
                                                                                       30 mb/ recombinant
                                                       mouse IL-2
                                                           5 X 6 dells/ml
                                                                                   . Murine leukemia cell line
2.3 PMP
                                                         YAC-1
                                                                              Na<sub>2</sub>CrO<sub>4</sub> 200 μCi 가
   Honda et a[16]
                                       (20mg) 2M-
                                                       5% CO
                                                                       60
                                                                                          5 x 4 cells/ml
HCI 가
                        2M-NaOH
                                            0.5M/L
                1001 가
PMP-MeOH
                             70 °C
                                      30
                                                       100:1, 30:1
                                                                        10:1
                                                                                               96 well round-
   . PMP(1-phenyl-3-methyl-5-pyrazolone) CHCl
                                                      bottomed microplate (Corning, New York, USA) °C,
                                 HPLC(Agilent 1100
                                                      5% CO
                                                                        4
                                                                                           2,200 rpm
HPLC System, USA)
                                                                                           - ocunter
                                                                             0.1 \, ml
D(+)-mannose (Man), L(+)-Rhamnose (Rha), D(+)-Glucose
                                                                                          (cytotoxicity)
(Glu), D(+)-Galactose (Gal), L(+)-Arabinose (Ara),
                                                      D(+)-
                                                                       (%) = (experimental release-spontaneous
xylose (Xyl), D-glucuronic acid (GluA), D(+)-Galacturonic maximum release-spontaneous release) x 100
acid (GalA), D(+)-Fucose (Fuc)
                                                  100nM
                                    PMP
                                                      2.8 Endogenous CFU-s
                                                                           100 mg/kg
                                                                                                     (5
                                                      / )
                                                                               24
                                                                                         98 cGy/min
2.4
                                                                   ^{60}\text{Co}
                                                                                          <sup>60</sup>Co theraton-780,
                                                         9 G
   RPMI1640 5% Fetal Bovine Serum, 2M2x18HEPES
                                                      Atomic Energy of Canada, Ltd., Canada).
                                                                                                                9
buffer, 2xfM glutamine, 13M10 pyruvate, 100M/penicillin
                                                                                                    Bouin
  50\mu g/ml streptomycin, 5x1^{\circ}M 2-mercaptoethanol, 1%
non-essential amino acid
                              가
GIBCO BRL (Gaithersburg, MD, USA)
                                                      2.9
                                                                     mean ± SEM
                                                                                                   one-way
2.5
                                                      ANOVA
                                                                                            (p value) 0.01
                                                         0.05
                                         96 well flat-
bottom microplate
                            1.5x10<sup>5</sup> cells/0.2 ml
     37 °C, 5% 2CO
                             3
                                             . CCK-
8(cell counting Kit-8,
                       Dojindo molecular tech., USA) well
  10 μl
               4
                                 450 nm
                                                      3.1
                        3
                                                 3
                                                                                                 가
2.6
                                                                                                       가
                      Raw 264.7
                                      96 well-round
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Table 1. Monosaccharide Composition of Panax Ginseng Extracts.

1)

(sam2),

Batch	Man	Glu	Gal	Ara	UID ¹⁾
Sam 1	2.0	64.1	26.6	5.4	2.0
Sam 2	2.8	75.9	16.1	2.1	3.1
Sam 3	1.2	86.2	9.4	2.2	1.0
Sam 4	2.4	81.8	11.7	2.2	1.9
Sam 5	2.2	70.5	24.8	0.6	1.9
Sam 6	2.3	80.0	10.4	0.9	6.4
Sam 7	2.1	79.7	15.0	2.5	0.7

가 가 [14,15] 1 Kg 7 mannose, glucose, galactose, glucose arabinose 90% galactose 1). glucose -glucan yeast, 가 [17], glucose가 mannose arabinose galactose glucose 가 galactose galactose 가 (sam1)

(sam3)

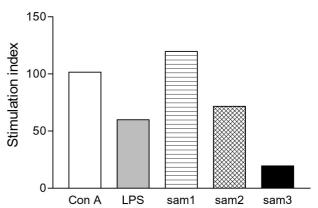


Fig. 1. Mitogenic activity of Panax ginseng extracts Murine spleen cells (NS, $1.5 \times 10^{\circ}$ cells/well) were cultured with several doses of polysaccharides on microtiter plates. After 72 h incubation, the cells were treated with $10\mu l$ of the CCK-8 solution for 4 h, and measured the absorbance at 450nm using a microplate reader. Results are presented the stimulation index (SI).

30:1 . Sam1 cell mitogen concanavalin p < 0.0013 p<0.01 A(Con A,µg5ml) B cell mitogen lipopolysaccha(ldRS, 100:1 p < 0.001 $10\mu g/ml$) sam3 sam1 sam2 2). (stimulation index, SI) Sam1 SI 119.7 con A 101.5 LPS 59.88 (p<0.001) sam2 71.69 SI sam3 19.61 1). 가 3 가 LPS($10\mu g/ml$) sam1 sam2 ⁵¹Cr-released cytotoxic assay IFN- (50U/ml) sam2 sam1 가 sam3 *p*<0.001 35%가 sam3

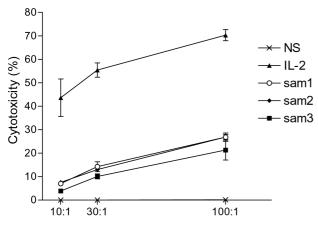


Fig. 2. The generation of AK cell by Panax ginseng extracts The splenocytes of C3H/HeN mouse $(3x10^6/ml)$ were cultured in the presence of rIL-2 (30U/ml) or each optimal concentration of polysaccharides for 5 days. Cells were collected and assayed for AK cell activity on YAC-1 tumor cells described in *Material and Methods*. The results represented the mean \pm S.E.M of values obtained from three replicated experiments.

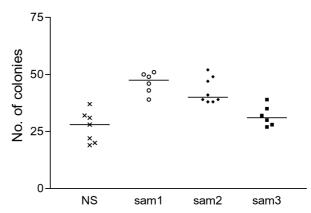


Fig. 4. Effect of Panax ginseng extracts on endogenous CFU-s Balb/c mice were *i.p* injected with *Panax ginseng* extracts 24 h before 9.0-Gy gamma irradiation. The number of colonies in spleen was macroscopically counted 9 days later, following fixation with Bouin's solution. All results are independently plotted and the bars indicate the median number.

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1.1

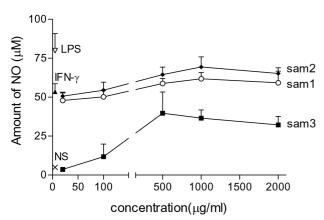
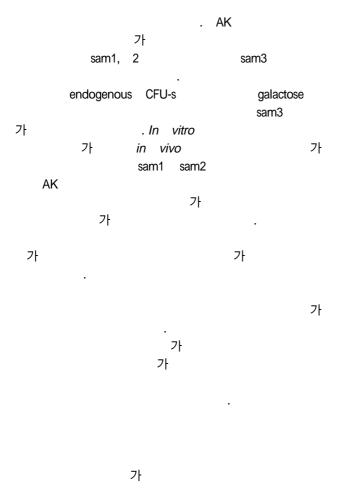


Fig. 3. Effect of Panax ginseng extracts on the production of nitric oxides Raw264.7 cells were treated with polysaccharides at serial dilutions for 48 h. Culture supernatants were collected and the levels of nitrite were measured by Griess reagent. The results are mean \pm S.E.M of at least three independent experiments.

. Sam3



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Representative Parameter of Immunostimulatory Ginseng Polysaccharide to Predict Radioprotection

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Abstract - According to the increase in the use of radiotherapy to cancer patients, many approaches have been tried to develop new agents for the protection of surrounding normal tissues. However, it is still few applied in the clinic as a radioprotector. We aim to find a representative parameter for radioprotection to easily predict the activity of *in vivo* experiment from the results of *in vitro* screening. The polysaccharide extracted from *Panax ginseng* was used in this study because the immunostimulator has been regarded as one of the radioprotective agent category and was already reported having a promising radioprotective activity through the increase of hematopoietic cells and the production of several cytokines. Mitogenic activity, AK cells activity and nitric oxide production were

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monitored for the in vitro immunological assay, and endogenous colony-forming unit (e-CFU) was measured as in vivo radioprotective parameter. The immunological activity was increased by the galactose contents of ginseng polysaccharide dependently. The result of this study suggests that mitogenic activity of splenocytes demonstrated a good correlation with in vivo radioprotective effect, and may be used as a representative parameter to screen the candidates for radioprotector.

Keywords: Immunostimulator, Mitogenic, Nitric oxide, Activated killer (AK) cells, Endogenous-colony forming unit (e-CFU)