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Rice protein prolamin promotes anti-leukemia immunity and inhibits leukemia growth in vivo

Chien-Kuo Liu [a, b], Chien-An Chen [c], Tzung-Yan Lee [d], Hen-Hong Chang [e, f], Hui-Fen Liao [g, **], Yu-Jen Chen [f, h, *]

[a] Division of Colon and Rectal Surgery, Department of Surgery, Mackay Memorial Hospital, Taipei 10449, Taiwan
[b] Mackay Junior College of Medicine, Nursing and Management, Taipei 11260, Taiwan
[c] Department of Radiation Oncology in Zhongxing Branch, Taipei City Hospital, Taipei 10341, Taiwan
[d] Graduate Institute of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan
[e] School of Post-Baccalaureate Traditional Chinese Medicine, and Research Center for Chinese Medicine and Acupuncture, China Medical University, Taichung 40402, Taiwan
[f] Department of Chinese Medicine, China Medical University Hospital, Taichung 40447, Taiwan
[g] Department of Biochemical Science and Technology, National Chiayi University, Chiayi 60004, Taiwan
[h] Department of Radiation Oncology, Mackay Memorial Hospital, Taipei 10449, Taiwan

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ABSTRACT

Prolamin is a heat-stable storage protein of rice (*Oryza sativa*). This study aimed to examine the effect of prolamin on anti-tumor immune response *in vitro* and leukemia growth in vivo. The prolamin-enriched rice fractions were prepared to stimulate peripheral blood mononuclear cells (MNC) from mice spleen. The MNC-conditioned medium (MNC-CM) was collected to treat leukemia L1210 cells. Human MNC-CM was prepared to treat Jurkat acute T cell leukemia cells. Purified prolamin was orally administered to syngeneic L1210-bearing DBA/2 mice to assess weights of tumor, liver and spleen, liver histopathology, peripheral blood neutrophil count and cytokine levels. Prolamin-prepared MNC-CM inhibited the viability of murine leukemia L1210 cells and human leukemia Jurkat cells, indicating an immunomodulatory effect. In syngeneic L1210-bearing DBA/2 mice, oral administration of purified prolamin dose-dependently decreased the tumor weight and attenuated the leukemia-induced reduction of liver and spleen weights. Prolamin inhibited the increase of peripheral blood leukocyte count. The levels of tumor necrosis factor-α and interferon-γ in MNC-CM and mice serum were significantly increased by prolamin treatment. No significant change in body weight, serum alanine aminotransferase and creatinine levels was noted by prolamin treatment. Rice prolamin could effectively promote anti-tumor immunity and inhibit leukemia growth without significant toxicity.

1. Introduction

Rice (*Oryza sativa*) is one of the important cereals and is a staple food worldwide. It provides both energy and nutrition beneficial to human health with advantages including fewer allergenic properties and easier digestion (Moron et al., 2008). A significant portion of calories ingested is from rice in many countries, especially in Asia (Khush, 1997). There are many ingredients isolated and derived from rice which possess various pharmacological and biological activities. For example, the water unextractable arabinoxylans of defatted rice containing phenolic compounds exhibited higher diphenyl-1-picrylhydrazyl radical scavenging and ferric iron reducing ability (Yuwang et al., 2017). In pigs infected with human rotavirus to induce diarrhea, rice bran administration significantly promoted the growth of both probiotic strains in the gut and increased the body-weight-gain compared to control group (Yang et al., 2015).

In composition of rice, the starch and protein are the two major components (Marshall and Wadsworth, 1994). Rice seeds contain

* Corresponding author. Department of Radiation Oncology, Mackay Memorial Hospital, Taipei, Taiwan, 92, Section 2, Chung Shan North Road, Taipei, 10449, Taiwan.
** Corresponding author. Department of Biochemical Science and Technology, National Chiayi University, 300 University Road, Chiayi, 60004, Taiwan.
E-mail addresses: liao.huifen@gmail.com (H.-F. Liao), chenmdphd@gmail.com (Y.-J. Chen).

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approximate 9% protein and contribute 28%–54% of the protein in the diet of Asian people (Duff, 1991). Four important fractions of rice proteins with differential solvent solubility are identified. Among these proteins, rice contain 5–10% alcohol soluble proteins (prolamin), 4–10% salt-soluble proteins (globulin and albumin), and 80–90% alkali soluble proteins (glutelin) (Cagampang et al., 1966). By using an experimental model of anti-leukemia immunity, we previously reported that water extracts of a rice cultivar, Japonica rice milled Taiwan 9 (MT9), could stimulate cytokine release from human mononuclear cells to inhibit growth and induce differentiation of human leukemic U937 cells towards macrophages (Liao et al., 2006). In the rice extracts with anti-leukemia immunity, we further identified that the main active component is prolamin (Chen et al., 2010).

Leukemia is the most common cancer in children (Shi et al., 2009). Although chemotherapeutic agents have extensive progress in the treatment of leukemia, drug-related toxicity remains an unsolved problem. The strategy of stimulating anti-leukemia immune reactions through enhancing endogenous cytokine release is more tolerable, especially when it comes from food, such as rice, with safety. According to *in vitro* results from anti-leukemic immunity model, we proposed the in vivo validation for effect of rice prolamin in experimental animals.

2. Materials and methods

2.1. Preparation of rice storage protein prolamin

Rice prolamin was effectively extracted by using appropriate solvents, as previous reported (Chen et al., 2010). Briefly, rice endosperm was defatted with hexane and was dried in a hood at ambient temperature for 24 h. The dried flour was then extracted with various solvents for albumin, globulin and glutelin followed by prolamin extraction with 70% ethanol for further 4 h with subsequent lyophilisation. The prolamin-enriched rice fractions were prepared to stimulate peripheral blood mononuclear cells (MNC).

2.2. Preparation of mononuclear cell-conditioned medium (MNC-CM) from mouse spleen and human peripheral blood

The spleens were removed from the mice, washed with PBS, homogenized into single cells and dispersed into suspension using a 1-mm metal sieve. Mononuclear cells (MNC) from the spleen cells were separated by centrifugation on a density gradient (Ficoll-Hypaque, 1.083 g/ml, Pharmacia Fine Chemicals). A concentration of 10^7 MNC/ml were cultured in 10% FCS-containing RPMI 1640 medium with or without various concentrations of prolamin (0, 30, and 100 μg/ml) at 37°C in a fully humidified atmosphere of 5% CO_2. MNC-conditioned media (MNC-CM) were collected after 1 day, sterilized by filtration, and then stored as 1-ml aliquots at −70°C until use. Human MNC from the peripheral blood of healthy donors were collected by centrifugation on a density gradient (Ficoll-Hypaque, 1.077 g/ml, Pharmacia Fine Chemicals). Cells were cultured at a similar condition and the MNC-CM was harvested after 24 h for further treatment to Jurkat cells.

2.3. L1210 and Jurkat cell culture and viability assay

The DBA/2 mouse lymphocytic leukemia L1210 cell line (ATCC Number CCL-219) was cultured in high glucose DMEM medium (Promocell, Heidelberg, Germany) supplemented with 15% certified FBS, 100 Unit/ml penicillin and 100 μg/ml streptomycin, and 0.1% mycoplasma removal agent at 37 °C in a humidified 5% CO_2 incubator. The human acute T cell leukemia cells, Jurkat, from ATCC (ATCC number TIB-152) were cultured in RPMI 1640 medium supplemented with 10% FCS and maintained at 37 °C in a humidified 5% CO_2 incubator. We cultured 10^5 cells/ml in the presence or absence of 30% (v/v) of normal MNC-CM (N-MNC-CM), or Prolamin (30 and 100 μg/ml)-MNC-CMs. Phytohemagglutinin P (PHA, 10 μg/ml; Difco Lab., Detroit, MI) was also used to prepare MNC-CM (PHA-MNC-CM) for a positive control. After 3 days of incubation, cells were collected by gently rubbing the dishes with a rubber policeman, and the number of viable cells was counted using the trypan blue dye exclusion test.

2.4. Syngeneic mouse leukemia model

The DBA/2 mice (approximate 7 weeks old, 18–22 g) were obtained from BioLASCO Taiwan Co., Ltd. All mice were housed in specific pathogen-free condition. Syngeneic mouse model was used to evaluate the effect of prolamin on L1210 leukemia cells. L1210 cells (1×10^6 cells per mouse) were injected into the mice through subcutaneous (*s.c.*) route. After 4 days, prolamin (diluted in normal saline) was administered by oral feeding (*p.o.*) every day for 5 days. All mice were divided into 4 groups with 6 animals for each group: (1) normal mice which were treated with normal saline, (2) mice which were injected with L1210 cells by *s.c.*, (3) prolamin 10 mg/kg *p.o.* per day in L1210-bearung mice, (4) prolamin 30 mg/kg *p.o.* per day in L1210-bearung mice. For leukemia assay, the weights of tumor, liver and spleen were recorded after removal at the day of sacrifice. Moreover, the peripheral blood was collected for 5 days to record the leukocyte count in the blood. The leukocyte counts of the blood samples were analyzed by an automatic Coulter counter (Model Z1, Beckman Coulter Electronics, Fullerton, CA).

2.5. Toxicity assessment

The body weight was recorded every day. On day 5, the mice were sacrificed and the blood was collected for measurement of hepatic and renal functions. Hematoxylin and eosin stain of liver specimen was performed for histopathological examination. Plasma levels of alanine aminotransferase (ALT) and creatinine (Cr) were measured by a standard colorimetric method using a Synchron LX20 spectrophotometer (Ortho-Clinical Diagnostics, 1859685, 8327462).

2.6. Serum cytokine analysis

Serum collected at day 5 from blood samples were frozen at −80 °C and thawed to room temperature. The levels of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in the serum were measured using enzyme-linked immunosorbent assay (ELISA) (R&D Systems).

2.7. Statistical analysis

Results were expressed as means ± standard errors (SEM) from at least three independent experiments. Statistical comparisons were performed using one-way analysis of variance. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Inhibition of leukemia L1210 and Jurkat cell viability by prolamin via stimulating soluble factors release from MNCs

Prolamin-prepared MNC-CM, but not prolamin *per se* (2.6 ± 1.1% growth inhibition), inhibited the viability of leukemia L1210 cells, indicating an immune modulation effect. In comparison to normal MNC-CM with baseline growth inhibitory rate 9.6 ± 2.7%, the

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