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Short communication

Antimelanogenic and antioxidative effects of residual powders from *Shochu* distillation remnants

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ABSTRACT

Shochu distillation remnants (SDR) are by-products in the manufacturing process of the Japanese liquor *Shochu* and include various useful organic compounds derived from the fermentation of grains. In this study, we investigated the inhibitory effects of barley-, black rice-, rice-, and sweet potato-powder from *Shochu* distillation remnants (PSDR) on the melanogenesis of B16 cells. Barley- and black rice-PSDR showed significant decrease in the intracellular melanin content and tyrosinase activity without cytotoxicity in the concentration range of 500–1000 μg/mL. Significantly, the antioxidative capacity of PSDR correlated well with the antimelanogenesis on the basis of a radical-scavenging assay. Furthermore, some antioxidant polyphenols in PSDR were identified by HPLC and the amount of the polyphenols was in good agreement with the antimelanogenic effects of PSDR. These results indicated that the polyphenols are the active components of PSDR for the antimelanogenesis of B16 cells. This study suggests that barley- and black rice-PSDR could be novel antimelanogenic materials for skin whitening.

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1. Introduction

Skin whitening has been an important treatment for skin care and has attracted much attention in the cosmetic and aesthetic industries of Japan. In human skin, visible pigmentation results from the synthesis and distribution of melanin in the epidermis (Lin & Fisher, 2007; Simon, Peles, Wakamatsu, & Ito, 2009). Melanogenesis is catalysed by tyrosinase that acts as a rate-limiting enzyme on melanin synthesis. In addition, tyrosinase is the only enzyme absolutely required for the biosynthesis of melanin. Thus, tyrosinase is a critical target molecule for the cell biological study of melanogenesis in human skin (Khan, 2007).

Shochu is one of the traditional liquors in Japan made from grains such as barley, rice, and sweet potato. In the *Shochu* manufacturing process, a large amount of *Shochu* distillation remnants (SDR) is generated as industrial by-product. They used to be dumped into the sea as wastewater, but this is now forbidden to prevent pollution of the sea. Therefore, the development of new and effective applications of SDR would serve an important function from the viewpoint of environmental preservation.

In previous studies, we have investigated the membrane-targeted chemotherapy with hybrid liposomes composed of phospholipids and nonionic surfactants for tumour cells *in vitro*, *in vivo*, and in clinical applications (Ueoka, Matsumoto, Goto, Ichihara, & Komizu, 2011). On the basis of these studies, we have tried to develop a new processing method and medical applications for the effective utilisation of SDR. For example, we obtained useful powder (PSDR) from SDR and found that PSDR had the potency for effective growth inhibition of cancer cells *in vitro* (Funamoto et al., 2008; Kadota et al., 2005) and immunostimulatory effects *in vivo* (Funamoto et al., 2008). It has been already reported that barley-PSDR has the potency of antimelanogenesis along with the inhibition of tyrosinase activity *in vitro* (Komizu, Tomonaga, Tanoue, & Ueoka, 2007). However, the mechanistic details of antimelanogenesis induced by PSDR are still not clear. Also, recent studies reported the inhibitory effects of antioxidants on the melanogenesis of mouse melanoma B16 cells (Hanamura, Uchida, & Aoki, 2008; Sato & Toriyama, 2009). The results suggest that the antioxidative capacity of antioxidants is closely related to the antimelanogenesis in B16 cells.

In the present study, we investigated the inhibitory effects of barley-, black rice-, rice-, and sweet potato-PSDR on the melanogenesis of mouse melanoma B16 cells *in vitro*. The mechanism of antimelanogenesis in B16 cells is also discussed on the basis of the antioxidative capacity of PSDR.

2. Materials and methods

2.1. Materials

Barley-, black rice-, rice-, and sweet potato-PSDR were obtained from each SDR material according to previous reports (Funamoto et al., 2008; Kadota et al., 2005). Dulbecco's modified Eagle's

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medium (DMEM) was obtained from Invitrogen (Carlsbad, CA). Mouse melanoma B16 cells were obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). 3-(3,4-Dihydroxyphenyl)-L-alanine (DOPA), Triton X-100, dimethyl sulfoxide (DMSO), Trolox, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan).

2.2. Cell culture

B16 cells were cultured in DMEM containing 10% foetal bovine serum under the standard culture conditions (95% humidified atmosphere of 5% CO_2 at 37 °C).

2.3. Cell viability

Cell number and viability were measured with an automatic cell counter (ADAM-MC, NanoEnTek, Inc., Boston, MA) according to the manufacturer's instructions.

2.4. Melanin content

B16 cells were seeded at a density of 8.0×10^4 cells/well in 6-well plates and incubated for 24 h under standard culture conditions. The cells were treated subsequently with either phosphate buffered saline (PBS) alone or solutions of barley-, black rice-, rice-, and sweet potato-PSDR (100, 500, and 1000 μg/mL) for 72 h. Then, the cells were washed with PBS and completely lysed by 1 N NaOH containing 10% DMSO. The amount of melanin in the lysate of B16 cells was measured with a spectrophotometer (Versa Max, Molecular Device, Inc., Sunnyvale, CA) at 405 nm (Lim et al., 2009).

2.5. Tyrosinase activity

B16 cells were seeded at a density of 2.0×10^3 cells/well in 96-well plates and incubated for 24 h under standard culture conditions. The cells were treated subsequently with either PBS alone or solutions of barley-, black rice-, rice-, and sweet potato-PSDR for 72 h. Then, the cells were washed with PBS and completely lysed by PBS containing 0.1% Triton X-100. After the addition of 10 mM L-DOPA into the lysate, the plate was incubated at 37 °C for 1 h and the catalytic activity of tyrosinase was evaluated by measuring the absorbance of the lysate at 405 nm with a spectrophotometer (Kamei, Otsuka, & Abe, 2009).

2.6. Radical-scavenging activity

Eighty percent ethanol solutions of barley-, black rice-, rice-, and sweet potato-PSDR (or 80% ethanol for the control) were mixed with 80% ethanol solution of DPPH (400 μM). After the incubation of the mixed solutions at 25 °C for 2 min, DPPH radical-scavenging activity was estimated by measuring the absorbance of the mixed solutions at 520 nm with a spectrophotometer. The radical-scavenging activity of PSDR was evaluated as the amount of Trolox with an equivalent activity (Katsube, Iwashita, Tsushida, Yamaki, & Kobori, 2003).

2.7. Polyphenolic compounds analysis

Polyphenolic compounds in PSDR were analysed by HPLC (Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003; Waraska, 2006) with a model 5600A liquid chromatograph (ESA Inc., Chelmsford, MA) equipped with secondary pumps and a 16-channel CoulArray detector; column, MCM 4.6 × 150 mm (ESA Inc.); column temperature, 35 °C; mobile phase (**A**) 100 mM sodium phosphate, 10 mg/L SDS, (**B**) 30 mM sodium phosphate, 60% acetonitrile, 10% methanol, 15 mg/L SDS; flow rate, 1.0 mL/min; gradient, isocratic 6% phase **B** from 0 to 10 min, linear increase of phase **B** from 10% to 30% over 20 min, linear increase of phase **B** from 30% to 100% for 10 min, isocratic at 100% phase **B** for 5 min. Polyphenolic compounds were detected at 0–900 mV in +60 mV increments.

3. Results and discussion

With respect to the inhibitory effects of PSDR on the melanogenesis of mouse melanoma B16 cells, we investigated the melanin content of B16 cells in the presence of barley-, black rice-, rice-, and sweet potato-PSDR *in vitro*. The results are shown in Fig. 1a. The melanin content of B16 cells decreased with increasing concentration of barley- and black rice-PSDR and a significant decrease was observed at concentrations above 500 μg/mL. On the other hand,

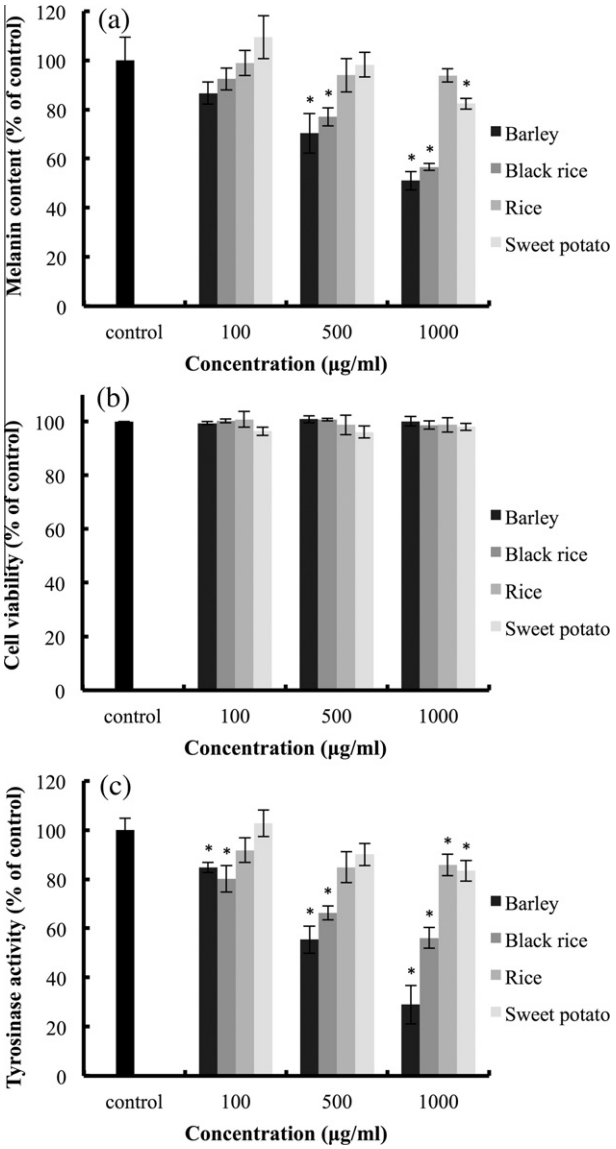

Fig. 1. Antimelanogenic effects of barley-, black rice-, rice-, and sweet potato-PSDR. (a) Melanin content of B16 cells after treatment with PSDR. (b) Viability of B16 cells after treatment with PSDR. (c) Tyrosinase activity of B16 cells after treatment with PSDR. Data are the means ± S.E. ($n = 3$). *Significant difference from the non-treated control, $p < 0.05$.

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