Study on biopolymer delivery system and effect of wound healing with polysaccharide extracted from *Agaricus blazei* Murill

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Abstract

Polysaccharide-based dressings have increasingly become viable alternatives to somewhat less biocompatible and often problematic cotton or viscose gauzes traditionally used for wound dressings. Polysaccharide which is particularly abundunt in *Agaricus blazei* Murill is known as the bioactive materials. Polysaccharide extracted from *Agaricus blazei* Murill enhances the recovery of the damaged epidermal tissue and the production of epithelial cell growth factors which are necessary to heal the burn and wound on the epidermal tissue.

In this study, the biopolymer delivery system of polysaccharide extracted from *Agaricus blazei* Murill into epidermal tissue was elucidated using the skin permeating enhancer *in vitro*. The enhancing effects of various penetration enhancers and some other enhancers on the permeation of β-glucan were evaluated using Franz diffusion cell. Permeation of polysaccharide in nomal and burn skin was compared. Polysaccharide was studied for the burn and wound healing activity in the epidermal tissue on rats.

Introduction

Wound healing is defined as the restoration of the continuity of living tissue and is an integrated response of several cell types to injury. It involves platelet aggregation and blood clotting, the formation of fibrin, an inflammatory response, alteration in the ground substance, endothelial and capillary proliferation and surface covering, regeneration of certain cell types, variable contracture and remodeling. Healing is not complete until the disrupted surfaces are firmly knit by collagen. Generally, the use of a skin substitute to provide an environment conducive to healing is necessary.

Polysaccharide extracted from mushroom has been most commonly used to enhance the immune system. β-glucan a kind of polysaccharide is particularly effective in activating white blood cells known as macrophages and neutrophils, which provide one of the immune system's first lines of defense against foreign invaders. When β-glucan activates Langerhans cells, they begin to initiate immune and reparative functions both locally and systemically just as do macrophages elsewhere. Lagerhans cells contact β-glucan they will begin to produce the cytokines, which include several powerful skin-healing substances as well as factors which prevent and fight infection. β-glucan, as a result of stimulating Langerhans cells, actually increases the skin's immune defenses in addition to acting as a sunscreen itself and reducing the painful inflammation. β-glucan is an agent for the promotion of wound healing.

Particularly *Agaricus blazei,* a novel edible mushroom, has been used as a treatment for a long time by cancer patients, and has also been reported to have antimutagenic, bactericidal, and antitumor effects. Its antitumor effect seems to be due to the restoratopm or augmentation of immunological responsiveness and to the potentiation of host-defense systems through celluar immunity. So In this study, we investigated skin permeation ability polysaccharide extracted from *Agaricus blazei* Murill in vitro and effect of dermal wound healing in rats

Materials and Methods

Skin permeation of polysaccharide *in vitro*

The fruiting body of *Agaricus blazei* extracted by hot water extraction conditions and concentrated at 60 ℃ in an evaporator. Purified powder of *Agaricus blazei* achieve by dialysis and freezing dry.

Experimental animal used hairless mouse(4~6wks, weighing 30±5g). Skin of hairless mouse were off. Optimal polysaccharide concentration determined by various concentration of polysaccharides 0 %, 0.1 %, 0.5 %, 1 %, 2 % using Franz diffusion cell. Enhancer (Oleic acid, Lauric acid Oleyl alcohol, Lauryl alcohol Tween20, Isopropyl myristate) determined by 0.1 % polysaccharide and 1 % enhancer. Enhancer concentration determined by 0.5 %, 1 %, 2 %. Finally time profile by optimal conditions carried out. Permeation aspect of normal and burn skin prepared. Total sugar measured according the phenol surfuric acid method using glucose as the standard material.

Effects of wound and burn healing *in vivo*

Sprague-Dawley male rats were anesthetized with Xylazine and Ketamine. Circular defects were made with a punch biopsy appliance. The first degree burn skin was made by pressing a stainless steel heating pad which was soaked in 100 ℃ boiling water for 3min on the back side for 5 seconds. Test substances would be applied at a different wound location in each wound and burn skin. The substances were as follow: 0.1 %, 0.5 %, 1 %, 2 % polysaccharide extracted from *Agaricus blazei* Murill, Fucidin and no treatment. Wound size measured for 7 days.

Results and Discussion

In skin permeation experiment, polysaccharide concentration of *Agaricus blazei* determined 0.1 % as permeation rate. Enhancer Tween20 was more effective than other enhancers in initial permeation rate. In enhancer concentration determination experiment was little difference among 0.5 %, 1 %, 2 %. Accordingly Tween20 concentration determined on 1 %. In fucidin treatment group, about 60 % of wound was re-epithelialized. In polysaccharides treatment groups, about 80 % of wound was re-epithelialized. Among polysaccharides treatment groups, 2 % presented the best effect, but healing effects of polysaccharides treatment groups was similar over all. So considering economical efficiency, optimal concentration of polysaccharde determined on 0.1 %.

Material delivery system of polysaccharides into epidermal tissue was developed through quantification of *Agaricus blazei* polysaccharides in/out of the hairless mouse skin. Polysaccharides extracted from *Agaricus blazei* elucidated to take effect in wound and burn healing.

Reference

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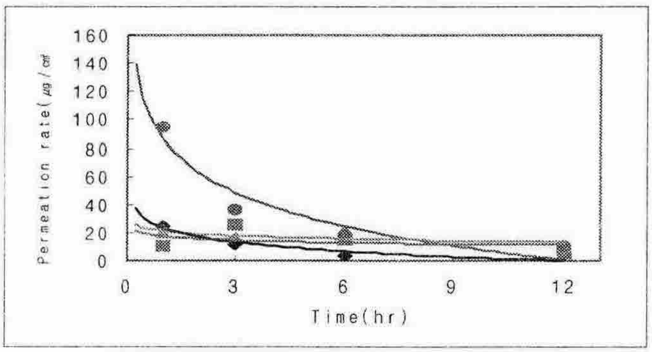

Fig. 1. Effect of *Agaricus blazei* polysaccharides concentration on the permeation rate through hairless mouse skin (◆ 0.1 %, poly, ■ 0.5 % poly, ▲ 1 % poly, ● 2 % poly)

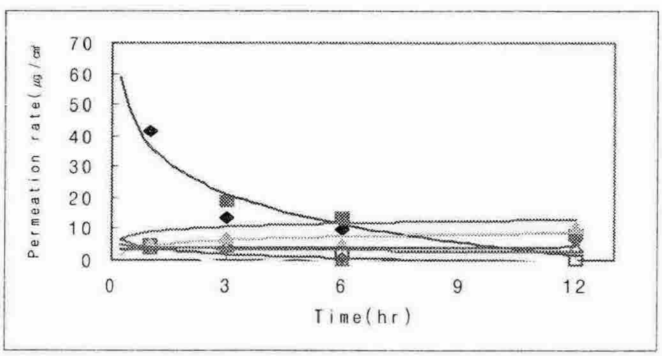

Fig. 2. Control of permeation rate from 0.1 % polysaccharides and different enhancers through hairless mouse skin at 36℃ (◆ Tween20, ■ Oleic acid, ▲ Lauryl alcohol, □ Lauric acid, △ Oleyl alcohol, ◇ Isopropyl Myristate).

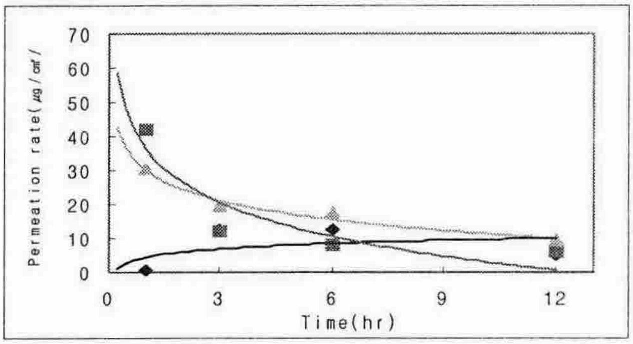

Fig. 3. Effect of permeation rate from 1% polysaccharides and different concentration of Tween 20 through hairless mouse skin at 36 ℃ (◆ 0.5 % Tween20, ■ 1 % Tween20, ▲ 2 % Tween20)

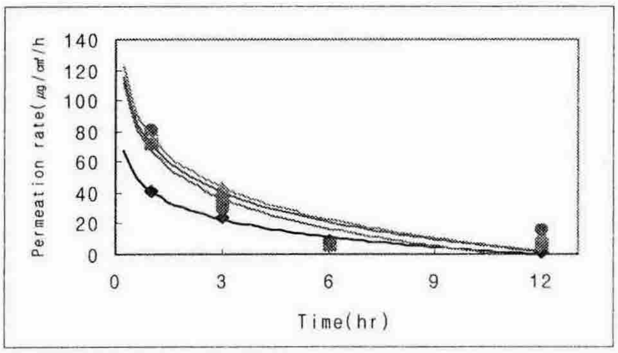

Fig. 4. Effect of permeation rate from different polysaccharides concentration and enhancer Tween20 1% through hairless mouse skin at 36 ℃ (◆ 0.1 % poly, ■ 0.5 % poly, ▲ 1 % poly, ● 2% poly)