The Evaluation of Potential Skin Sensitization and Collagen Synthesis of Rats Receiving Proteoglycan Serum

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Abstract

OBJECTIVE: The aim of this study was to evaluate potential skin sensitization and collagen synthesis of rats receiving proteoglycan (PG) serum.

MATERIAL AND METHODS: Eighteen rats were randomly divided into 3 groups equally and assigned to receive 1 ml of serum base (control), 1% PG and 2% PG applied to skin. Histological analysis was evaluated for potential skin sensitization and collagen synthesis on days 7, 14, 21 and 28.

RESULTS: Histological analysis showed that 1% PG and 2% PG accelerated collagen synthesis. The evaluation of potential skin sensitization, on day 7, 14, 21 and 28 of rat applied with 1% PG and 2% PG were not significantly different when compared to the control group.

CONCLUSION: This study demonstrated that PG serum accelerated collagen synthesis and low potential skin sensitization.

Keywords: proteoglycans, collagen synthesis, potential skin sensitization, Trichopoduspectoralis

Proteoglycan (PG) is a hybrid molecule composed of a central core protein by bonding it with glycosaminoglycans (GAGs) with a covalent bond. PGs are essential components of cartilage, and make up approximately 90% of dry weight. PGs play an important role in collagen synthesis and wound healing because the molecular structure is similar to fibroblast growth factors (FGFs) and to an epidermal growth factor (EGF)-like domain. The bioactive mechanism of PGs is also similar to FGFs and EGF with regard to collagen synthesis and to promoting tissue repair. PG can also bind to several types of growth factor receptors, including FGF-2, Transforming growth factor-beta (TGF-β), Platelet-derived growth factor (PDGF) and Vascular endothelial growth factor (VEGF) family receptors, to promote cell proliferation, cell division and activation of neovascularization.

In 1992, Japanese researchers had first discovered and developed PG extraction from nasal tip cartilage of salmon (Oncorhynchuska) for commercial uses and named it “Proteoglycan-IPC” (IchimaruPharcos Co, LTD., Gifu, Japan). This material has been added to cosmetics to promote collagen synthesis and wrinkle relief.

Fish bone head is composed of cartilage, which contains a high amount of PGs. In general, this part of the fish (Trichopoduspectoralis) would be discarded or used as animal feed after fish processing such as sunny fish. Therefore, it would be beneficial to use fish cartilage for the extraction of PG for cosmetic use such as anti-aging serum or to promote collagen synthesis and increase the value of agricultural waste as well.

The aim of this study is to evaluate potential skin sensitization and collagen synthesis on the skin of rats receiving PG serum extracted from fish (Trichopoduspectoralis) in vivo model.
Materials and Methods

Animals: In this study, we used male Wistar rats because they were convenient to obtain, the results in experiments are similar to those found in rabbits, and the price of Wistar rats is cheaper than rabbits. Male Wistar rats (age 8 weeks, weighing 250-300 grams) were purchased from the national Laboratory Animal Centre, Mahidol University, Salaya, Thailand. They were housed in the Laboratory Animal Unit under standard conditions of temperature 25 ± 2 °C, 50 - 60 % humidity, and a 12 hours/12 hours light/dark cycle. The rats were kept under laboratory conditions for one week prior to the start of the experiments and allowed food and water ad libitum. At the end of each experiment, the rats were sacrificed with carbon dioxide asphyxiation. Animal experiments in this study were carried out in accordance with the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Serum base preparation: Glyceryl monostearate, Propylene glycol, PVM/MA Copolymer, Glycerine, Butylene glycol, Carbomer, Polysorbate 20, Disodium EDTA (2NA EDTA), DMDM Hydantoin, and Iodopropynyl butylcarbamate were dissolved in warm water and then mixed with other ingredients during the cream-forming process.

PG serum preparation: PG solution, Glyceryl monostearate, Propylene glycol, PVM/MA Copolymer, Glycerine, Butylene glycol, Carbomer, Polysorbate 20, Disodium EDTA (2NA EDTA), DMDM Hydantoin, and Iodopropynyl butylcarbamate were used to formulate PG serum. PG solution were dissolved in warm water and then mixed with other ingredients during the serum-forming process.

Animal preparation: The Male Wistar rats were randomly divided into 3 groups of 6 animals, and assigned to receive 1 ml of serum base (control), 1% PG and 2% PG applied to the skin. This PG concentrations were the same as previous studies. Histological analysis evaluated skin sensitization potential and collagen synthesis on days 7, 14, 21 and 28.

Histological analysis

Potential skin sensitization was proved by skin stained with hematoxylin and eosin dye (H&E). At the end of experiment, six Wistar rats in each group were sacrificed and skin samples were taken. Tissues were fixed in 10 % buffered formalin and embedded in paraffin. Thin sections (5 μm) were prepared and stained with hematoxylin and eosin dye. Potential skin sensitization and collagen synthesis were examined histologically using a light microscope (Nikon 516609, Japan) with 40× and 100× objective lenses. The sample was sectioned into 3 pieces and 3 fields per sample were randomly evaluated for collagen synthesis, number of cells/field (including mast cells and macrophages) compared with control group.

Masson’s trichrome examination

Collagen synthesis was proved by skin stained with masson’s trichrome. Skin tissue sections were deparaffinized in xylene, hydrated, and stained in Weigert’s iron hematoxylin solution for 10 min. They were washed and stained in acid fuchsin solution for 3 min, rinsed in distilled water, treated with phosphomolybdic-phosphotungstic acid solution for 15 min, immediately submerged into aniline blue solution for 10 min, rinsed in distilled water, treated with acetic solution for 10 min, dehydrated in 95% alcohol, cleared twice in xylene, mounted with a cover slip, and observed under a light microscope.

Statistical analysis: Results are expressed as means ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA), followed by a Bonferroni post hoc test using SPSS for Windows, ver. 22. Values of \( p < 0.05 \).

Results

Potential skin sensitization

Number of mast cells: On day 7, 14, 21 and 28 post applied, number of mast cells/field of the skins applied with 1% PG and 2% PG showed no significant difference when compared to the control group (Figure 1).
**Masson’s trichrome examination**

The results from Masson’s trichrome staining can clearly differentiate important morphological keys for collagen synthesis assessment. Hemoglobin, muscle fiber and keratin are stained red color. Adipose tissue and cytoplasm are stained pink or light red. Cell nuclei show dark brown to black and collagen fiber is stained aniline blue. On day 14, 21 and 28 post applied with 1% PG and 2% PG showed higher levels of collagen fiber when compared to the control group (Figure 3).

![Figure 2: Number of macrophages/field on day 7, 14 21 and 28 post applied. Data are shown as means ± SE, N = 6 for all groups.](image)

**Number of macrophages:** On day 7, 14, 21 and 28 post applied, number of macrophages/field of the skins applied with 1% PG and 2% PG showed no significant difference when compared to the control group (Figure 2).

![Figure 3: Histological observation of skin sections on day 28 post applied, stained with Masson’s trichrome, (A,C) Serum base (control), (B) 1 % PG serum, (D) 2 % PG serum, Bar = 50 µm, n = 6 for all groups.](image)
Discussion

Dermal contact to skin sensitizing substances (contact allergens) may result in contact allergy (sensitization) or an allergic contact dermatitis (ACD). Repeated contact with a potent sensitizer may be sufficient to induce sensitization (contact allergy), and the re-exposure of a sensitized individual to that contact allergen can then result in an allergic reaction. For skin sensitization, skin rash, redness and edema are typical responses (adverse outcome).

Skin sensitization is involved with mast cells and macrophages. Mast cells are hematopoietic cells that originate from progenitor cells in the bone marrow. Mast cells play as the major effector cells in hypersensitivity or ACD and release pro-inflammatory mediators such as histamine, which causes rash, itching, vasodilation and local inflammation. The number of mast cells increase in the presence of allergy, irritation, or rash, itching, vasodilation and local inflammation. The number of pro-inflammatory mediators such as histamine, which causes rash, itching, vasodilation and local inflammation. The number of mast cells in all groups on days 7, 14, 21 and 28 was similar to those in the control group, indicating that the ingredients of the PG serum did not cause an inflammatory reaction.

From the results, the number of mast cells in all groups on days 7, 14, 21 and 28 was higher than the control group. Therefore, PG serum maybe plays a role in fibroblasts proliferation, which increases collagen synthesis in skin.

Conclusion

The results from this study demonstrate that PG serum extract from Trichopoduspectoralis is an accelerate collagen synthesis with low potential skin sensitization. These results provide scientific information for the use of PG serum to accelerate collagen synthesis for cosmetic use.

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