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Short communication

Effect of 15-hydroxyprostaglandin dehydrogenase inhibitor on wound healing

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ABSTRACT

PGE_2 is an important mediator of wound healing. It is degraded and inactivated by 15-hydroxyprostaglandin dehydrogenase (15-PGDH). Various growth factors, type IV collagen, TIMP-2 and PGE_2 are important mediators of inflammation involving wound healing. Overproduction of TGF-β and suppression of PGE_2 are found in excessive wound scarring. If we make the condition downregulating growth factors and upregulating PGE_2, the wound will have a positive effect which results in little scar formation after healing. TD88 is a 15-PGDH inhibitor based on thiazolinedione structure. We evaluated the effect of TD88 on wound healing. In 10 guinea pigs (4 control and 6 experimental groups), we made four 1 cm diameter-sized circular skin defects on each back. TD88 and vehicle were applicated on the wound twice a day for 4 days in the experimental and control groups, respectively. Tissue samples were harvested for qPCR and histomorphometric analyses on the 2nd and 4th day after treatment. Histomorphometric analysis showed significant reepithelization in the experimental group. qPCR analysis showed significant decrease of PDGF, CTGF and TIMP-2, but significant increase of type IV collagen in the experimental group. Taken together TD88 could be a good effector on wound healing, especially in the aspects of prevention of scarring.

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1. Introduction

Hypertrophic scar formation is a problematic and is resulted from imbalance of cytokines in wound healing. Even a normal wound healing process necessarily involves inflammatory responses and the poor wound healing conditions, such as infection, may cause an abnormal wound healing process in some cases. Excessive proliferation of fibroblasts during such diverse wound healing processes produces an excessive amount of extracellular matrix (ECM). The excessive amount of ECM produced by fibroblasts include collagens, which remain even after the wound has been healed, forming a hypertrophic scar that causes an esthetic problem with thickening of the healed region or, in worse cases, causing dysfunctions, such as restricted motions, due to contracture [1–3].

The inflammatory mediators involved in a normal wound healing process include growth factors and cytokines such as prostaglandin (PG). Reportedly, wound healing mainly involves growth factors, such as transforming growth factor-β (TGF-β), vascular endothelial growth factors (VEGFs), connective tissue growth factors (CTGFs), and platelet derived growth factors (PDGFs), and PG, such as PGE_2. ECM, especially type IV collagen known as its principal component, which forms the basement membrane, and a tissue inhibitor of metalloproteinase (TIMP), which controls degradation of some components of ECM, are known to be deeply involved in a wound healing process [4].

It is important to note that the above-mentioned growth factors and PGE_2 have been found to play a major role during an abnormal wound healing process that produces an excessive amount of ECM. That is, a larger number of growth factors and a lower level of PGE_2 formation compared with a normal wound healing process can actually generate a more serious scar [4]. This is opposed to the existing general paradigm of wound healing that prescribes non-steroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase-2 (COX-2) inhibitors to relieve pain. In particular, administration of these agents in the later proliferative phase of wound healing can prevent PGE_2 generation and aggravate a scar [4]. Therefore, the best way to heal a wound is to create an environment that can reduce growth factors and enhance PGE_2.

Recently, some attempts are continuously made to develop compounds involved in PG metabolism to keep the concentration of

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PGE_2 high; for example, 15-hydroxyprostaglandin dehydrogenase (15-PGDH) inhibitors are used to control degradation of PGE_2 and keep its concentration high [5–8]. 15-PGDH is an enzyme in charge of PGE_2 degradation, and previous research found that a 15-PGDH inhibitor could strongly inhibit degradation of PGE_2 and increase its concentration effectively [5].

Using guinea pigs, we compared the changes of wound healing status, some growth factors within the wound, collagen, and collagen modulators to determine how the 15-PGDH inhibitor affected wound healing.

2. Materials and methods

2.1. Manufacturing animal model

Animals were subjected to skin wounding as previously described [9,10]. The experiment involved 10 adult male guinea pigs (250–350 g) to identify the experimental effects more accurately by minimizing the hormonal effects. The status of trauma and disease was examined visually to screen healthy animals; the temperature in the breeding room was kept at 21 to 23 °C and the animals were permitted to drink water and have special feed freely while light alternated with shade in a 12-h interval to minimize environmental stressors. The animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals. The Ethics Committee for Animal Experiment of the Chosun University approved the protocol for this study.

Each animal was placed in ventral recumbency after anesthesia using an inhalation anesthetic, sevoflurane, and its back was shaved with standard animal clippers. The dorsal skin was scrubbed with betadine and ethanol, and four circular wounds to penetrate the skin horizontally to remove the epidermis and the dermis were made per animal by lifting the skin above the spine. A kind of punch composed of the circular blade of 1 cm in diameter and a case was used to make circular wounds of uniform size in the animals. All wounds were sprayed with a gentamycin containing wound-spray immediately. After surgery all guinea pigs were fitted with “Elizabethan” type collars to prevent them from scratching their wounds.

Four of the wounded guinea pigs were classified into the control group and the remaining six into the experimental group.

2.2. Drug preparation and administration

The 15-PGDH inhibitor in this study is 5-(2-chloro-3- (3-phenylpropoxy) benzylidene) thiazolidine-2,4-dione (compound 17, TD88) (Fig. 1); TD88 was dissolved into polyethylene glycol to get the concentration of 25 mM and was diluted with 20% Tween 20 in the ratio of 1:4 to get the final concentration of 5 mM for the experimental group and the 1:4 dillution of polyethylene glycol and 20% Tween 20 was used as a vehicle for the control group.

2.3. Methods

Right after hemostasis, we dripped two drops of the vehicle for the control group and TD88 for the experimental group on the wound from a serum separator; then, we continued to drip them in the same way in every twelve hours until the animals were sacrificed at the second or fourth day. Two animals in the control group and three in the experimental group were sacrificed by paralyzing the cervical spinal cord at the second day to get quadrilateral flaps including normal skin tissues around the wound. Of the four circular wound flaps obtained from each animal, one flap, which was a tissue of about 1.5 cm in diameter surrounding the wound, was kept fresh in a deep freezer and the remaining three were pinned on a cork board in formalin. At the fourth day, the remaining animals were sacrificed to obtain tissues in the same way.

2.4. Histological assessment

The tissues in formalin were washed twelve hours later and were manufactured into paraffin blocks by using an automatic tissue processor. These blocks were sectioned into 4 μm thickness, were stained with hematoxylin and eosin (H&E), and were manufactured into slides for microscopic observation and analysis. Then, they were stained with Masson's trichrome to examine the intra-tissue collagen distribution.

Tissue slides stained with H&E were used to assess the recovery of the wounded surface of 1 cm in diameter through microscopic observation. Reepithelization of the epidermis removed by experimental manipulation was measured to compare the recovery of the wounded surface. In measuring reepithelization, MagnaFire digital camera system (Optronics, Goleta, CA, USA) was used to get images from H&E slides and Visus Image Analysis System (Image & Microscope Technology, Daejon, Korea) was used to measure the wound defect precisely.

The wound healing rate—intra-wound reepithelization—was calculated in the following way:

$$\text{rate of reepithelization}(\%) = \text{total length of reepithelization} / \text{length of skin defect} \times 100$$

Masson's trichrome staining was performed to determine the level of collagen formation in the region of wound restoration. Since Masson's trichrome dyes collagen blue, richness of the color was compared to analyze the level of collagen formation in each group.

2.5. Molecular biological assessment

To determine if TD88 made favorable changes in some growth factors, ECM components, and their degradating factors involved in a wound restoration process, comparative analysis was made with the control group through real-time PCR. Such growth factors as TGF-β, VEGF, CTGF, and PDGF-D, type IV collagen, which is a component of ECM, and TIMP-2, which affects their degradation, were analyzed by the following method: the tissue kept in a deep

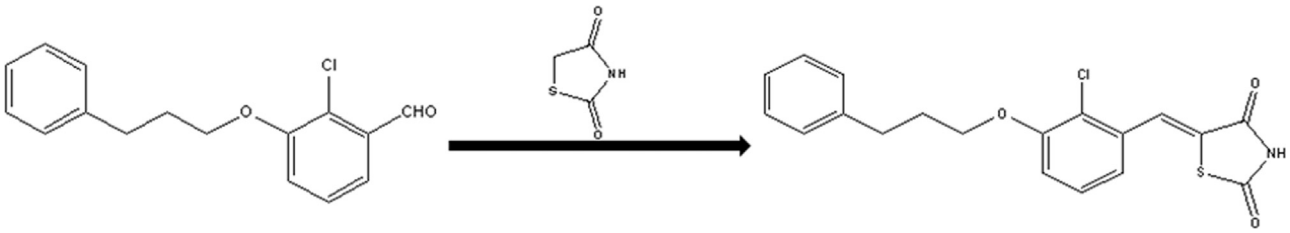

Fig. 1. Structure of the 5-(2-chloro-3-(3-phenylpropoxy) benzylidene)thiazolidine-2,4-dione (TD88).

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