EFFECTS OF *CHANNA STRIATA* EXTRACT ON ANGIOGENESIS IN THE PROCESS OF WOUND HEALING IN TYPE-2 DIABETIC MICE

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Background

Starting from the advantages provided by Channa striata, and concerns about the existence of wounds that are difficult to heal in DM patients.

Interesting and important to study how the impact of ECS on wound healing in DM . patients

Background

Diabetes mellitus: prevalence

Uncontrolled hyperglycemia stimulates ROS hyperproduction oxidative stress endothelial dysfunction resulting in DNA damage and apoptosis and no formation of Nitric Oxide (NO) in the endothelium complications of vascular disease in DM

One of the vascular failures that occur in hyperglycemic conditions is wound healing

Background

Channa striata (CS) is one type of freshwater fish that has higher protein and several mineral levels than some other fish. >50% of the total protein content is albumin.

Channa Striata

(Gum et al 2005, Suryaningrum dkk 2010)

CS has been known to be a potential food for "natural medicine"

It has higher levels of protein and some minerals than some other fish.

Reduce pain

Wound healing

Background

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Several studies have shown that CS can increase serum albumin (Soemarko, 1998; Nilasanti, 2003; Agung 2005; Etik, 2007; Sutami, 2008).

Background

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Able to form new tissue in wound healing (Soemarko, 1998; Asikin, 1999; Eddy et al, 2003; Endang, 2006). The potential of CS in tissue synthesis is similar to the remodeling phase of normal wounds.

Background

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The presence of tissue granulation will further increase the tensile strength of the wound in the skin (Baie and Shekh, 2000; McLenan et. al., 2006).

Background

- Although several studies on the effect of CS on wound healing have been carried out, studies on the effect of Channa striata extract (ECS) given as food orally on wound healing in diabetes have not been carried out.

Background

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  It is necessary to conduct in vivo research with a molecular mechanism approach to the role of amino acids as the largest content in Channa striata extract in diabetic wound healing.

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Amino acids contained in protein are indispensable for normal cell function and for wound repair (Harding et al., 2002), CS extract is known to have a potential role in wound healing. the presence of growth factor stimulation supports diabetic wound healing by increasing angiogenesis (Romano et. al., 2002).

Scientific name	*Channa Striata*
English name	Snakehead murrel
Japanese name	Raigyo

fresh water fish

Materials and Methods

Animals Model

Type 2 diabetes model mice（16 head）
C57BL/6JHamSlc-ob/ob mouse
leptin defective mice）
control mice（4 head）
C57BL/6JHamSlc-+/+ mouse

左：+/+ 非肥満　　右：ob/ob 肥満

Materials and Methods

Secondary antibody

DyLight 488 ® –conjugated goat anti-rabbit IgG

Cy3-conjugated donkey anti-rat IgG

VECTASTAIN ABC kit (VECTOR LAB)

Experimental Procedure

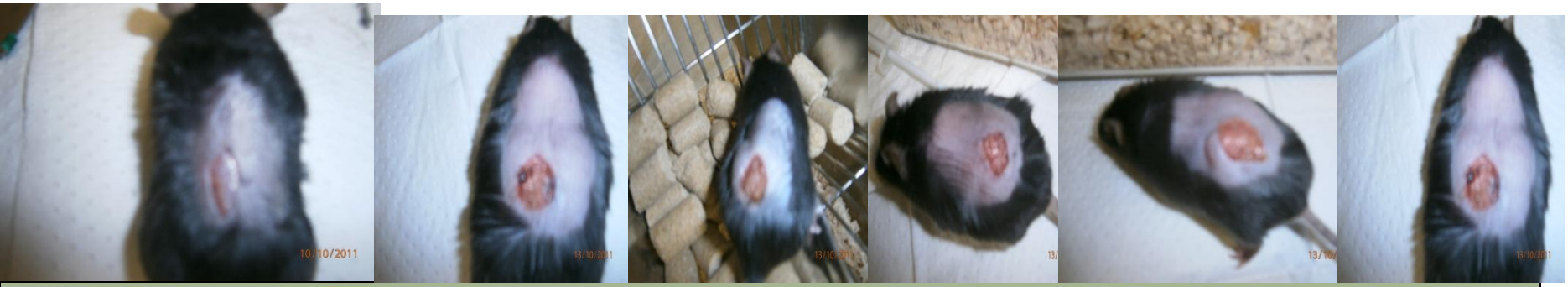
We made a cut (diameter 8~10mm) in mice back 5 days after normal feeding

Experimental feeding periods:

3 days, 1 week, 2 week

Feeding place: Yamanashi Institute of Environmental Sciences

Result



Experimental procedure

Control mice

Group A: normal feeding

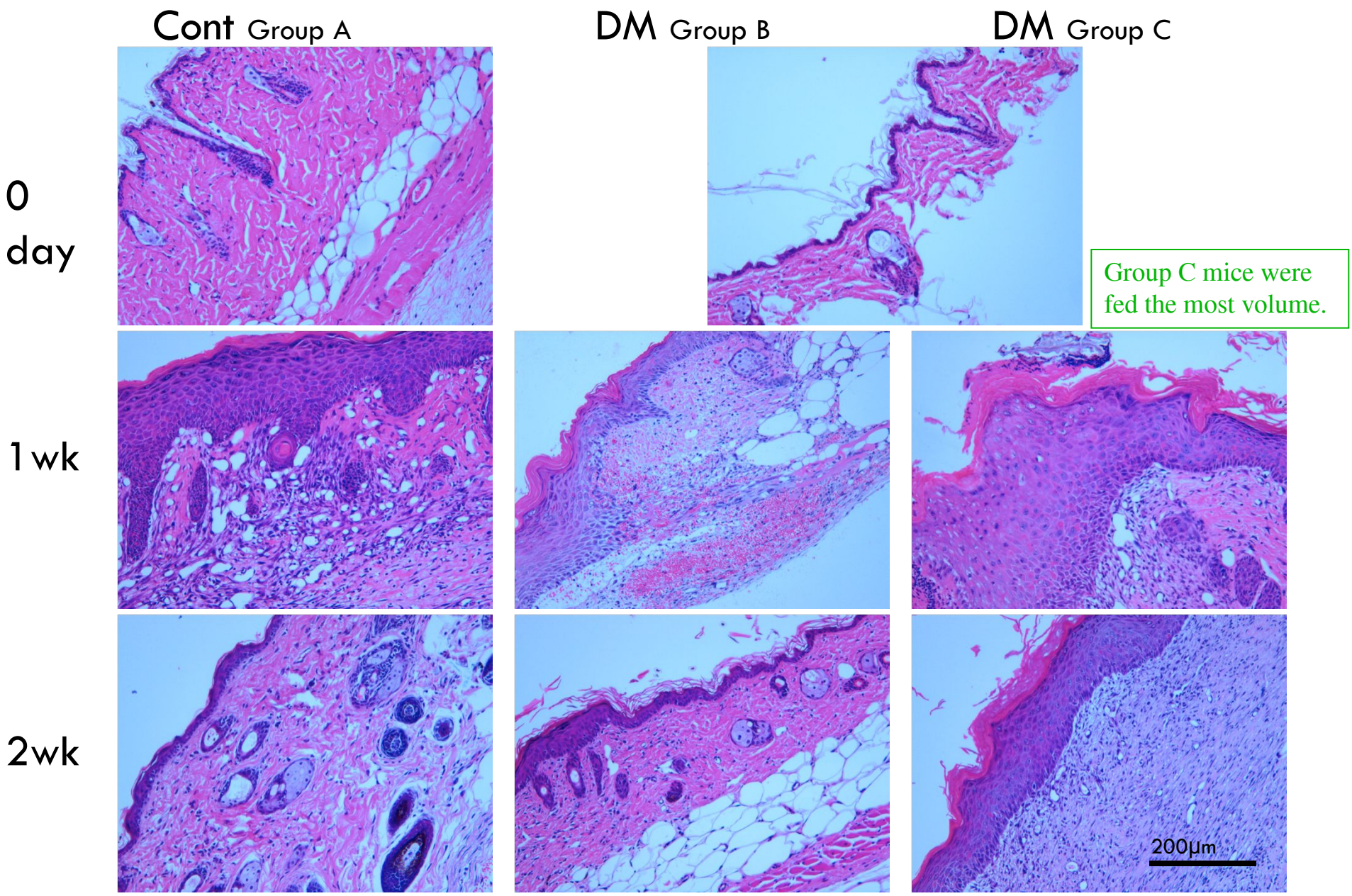
Diabetes mellitus mice

Group B: normal feeding (no administration of CSE)

Group C: administration of CSE in oral every day except Saturday and Sunday.

(This group were divided into 3 groups by applied dose, mice administrated the 3, 6, 9 ml dose of CSE.)

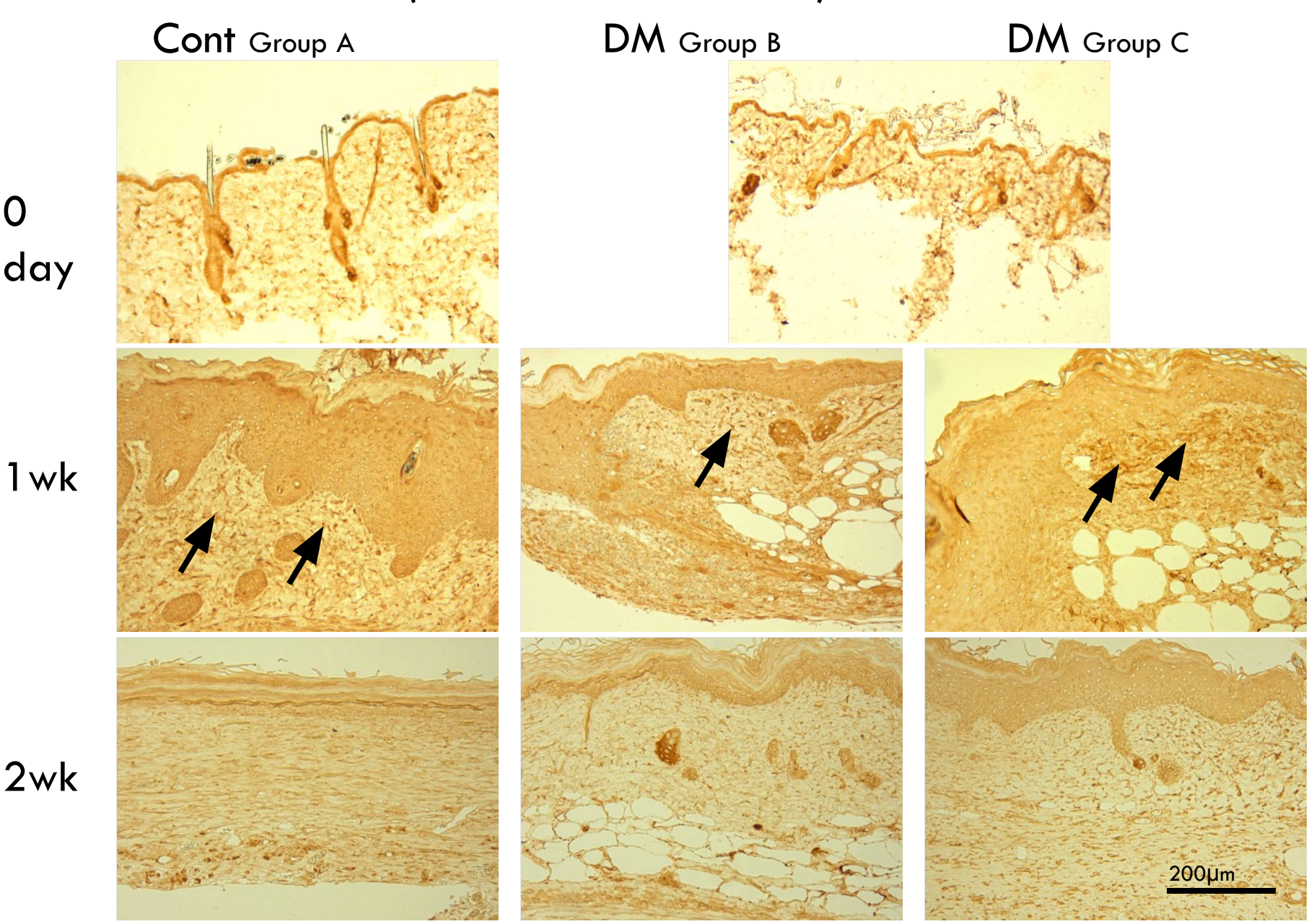
Results HE staining



From Hematoxylin-Eosin staining:

DM mice skins were thinner than control mice skins, especially in subcutaneous tissue. The epidermis and granulation of DM mice (GC) underwent more pronounced hyperplasia compared with those of DM control mice (GB). The keratinocytes of the epidermis in DM mice (GC) were biggest in size among three groups, and the keratinocytes made the stacked layers.

ABC method: CD34 (arrows show EPC cells)

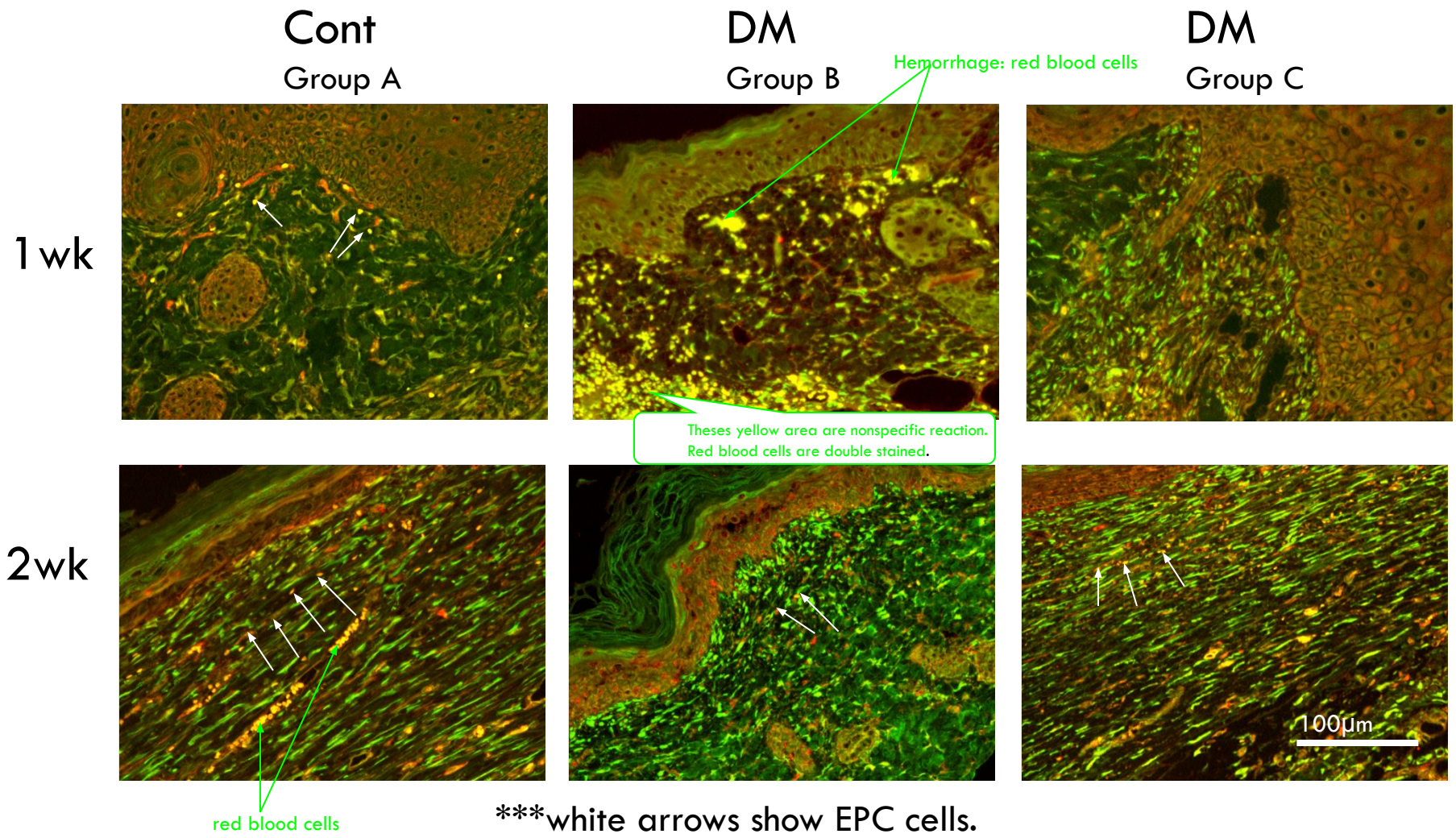


CD34 localization in subcutanous area around the wound using ABC method:

Anti-CD34 antibody reaction products were localized in endothelial progenitor cell (EPC), interstitial cells, fibroblasts and keratinocyte. EPC cells and hair follicle were stained strongly.

Double staining of immunofluorescence

red : CD34 green : VEGF yellow : VEGF+CD34



***white arrows show EPC cells.

Double staining with fluorescent dyes:

Anti-VEGF antibody labeled with DyLight 488 ®–conjugated goat anti-rabbit IgG was shown in green color, and anti-CD34 antibody labeled with Cy3-conjugated donkey anti-rat IgG was shown in red color. Thin lines or small dots colored in yellow that coexisted VEGF and CD34 show EPCs (white arrows in figures). (Some areas stained in yellow were red blood cells.)

Results and conclusion

General observation of symptoms:

The activities of DM mice (group C (GC) that were administrated CSE were higher than DM control mice (group B (GB), and those of DM mice (GC) and control mice (group A (GA) were equally likely (I did not do a quantification, only visual check.) Few differences of the blood glucose level between DM mice (GC) and DM control mice (GA) were found. Wound healing period showed few differences when we compared control mice (GA), DM control mice (GB) and DM mice (GC).

Reference for discussion

Our goal is to show that feeding CSE to DM mice is effective to activate angiogenesis for wound healing. Actually, we observe some good data at competitive with GA, GB and GC, about angiogenesis in the subcutaneous area around a wound and in the granulation. These areas of angiogenesis were shown by the existence of EPC cells, stained both VEGF and CD34.

Reference for discussion

The EPCs of GA (normal control mice group) and GC (DM mice fed CSE) have increased than those of GB (DM control mice). (but could not count the number of EPCs, only visual effects.) Consequently we thought CSE feeding could promote wound healing. However, regeneration of the epidermis and granulation under the wound in CSE fed DM mice (GC) have displayed differences with normal control mice (GA).

Reference for discussion

The size of keratinocytes in GC mice were larger than them in GA mice, also a stack layer of the keratinocytes was wider than that in GA. In addition the granulation of the GC mice has densely-packed fibroblasts than that of GA. Finally, the granulation tissue is replaced by normal tissue for skin regeneration.

Reference for discussion

We should think influence about the nutrition of CSE. CSE includes some protein rich and mineral, especially zinc is needed for protein synthesis or dissolution and improves immune function. Zinc oxide ointment is a popular medicine for wound repairing from old times.

BACKGROUND

- Protein deficiency will cause the inflammatory phase to be longer due to inhibition of fibroblast formation, collagen and proteoglycan synthesis and neoangiogenesis and inhibition of wound remodeling (Ruberg, 1984; Haydock et. al., 1988).

- Adequacy of amino acids is needed to bind mRNA as a material for albumin synthesis so that the adequacy of albumin is met (De Feo et al., 1991)

Diabetic rats (without ECS) had significantly lower serum amino acid, arginine, and leucine levels than the normal rat group.

- Arginine and Leucine are amino acids that play a role in neutralizing blood glucose levels and stimulating wound healing (McKay and Miller, 2003).

- Low concentrations of amino acids in diabetics cause excessive protein glycation and modification, resulting in oxidative damage and a decrease in the status of tissue proteins and amino acids as their constituent elements (He and Carter, 1992; Oldfield et al., 2001).

Further research results

1. Channa striata extract is known to contain complete and balanced essential and non-essential amino acids, and 2,913±0.23 or 52.7% of the total protein in this extract is albumin.

2. . Administration of CSE to diabetic Wistar rats with wounds significantly accelerated wound healing, by increasing the levels of total amino acids, arginine, leucine and serum albumin (P = 0.000; = 0.01)

3. The increased concentration of arginine in serum, strongly correlated and contributed greatly to the formation of vascularity after administration of CSE for 4 days,

4. The increase in leucine concentration is strongly correlated and contributes greatly to blood vessel formation (angiogenesis) after 8 days of CSE administration.

- In this study, it was found that CSE administration could increase serum amino acid concentrations, increase arginine and leucine concentrations, it can be said that:

Amino acids, especially leucine and arginine in CSE can stimulate protein synthesis through a signaling mechanism that does not depend on the presence of insulin so that it has the potential to accelerate the wound healing process in diabetic rats, by increasing serum amino acid concentrations, especially Arginine. and Leucine.

Conclusion

- Giving CSE to diabetic rats with wounds was found to increase the concentration of amino acids, arginine, leucine and albumin in serum and form blood vessels at the wound site (angiogenesis) significantly and had a strong correlation, after administration of ECS for 4 days or 8 days..

Thank you

Arigato Gozaimashu