ประสิทธิผลของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิตในการรักษาริ้วรอยบริเวณหางตา

The effectiveness of peripheral blood stem cell therapy on crow's feet wrinkles.

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นิสิตระดับปริญญาโท สาขาวิชาเวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ มหาวิทยาลัยแม่ฟ้าหลวง

Abstract

Peripheral blood stem cell therapy (PBSC) has long been known as an effective treatment in various medical conditions. The effectiveness of peripheral blood stem cell therapy on crow's feet wrinkles is an application that is being explored in this thesis. The study is an open labeled study aiming to evaluate the efficacy and safety of PBSC in the treatment of crow's feet wrinkles.

In a 7 wk, 25 healthy volunteers, 21 females and 4 males were recruited. All subjects gave their written consent prior to participate in the study. Rao-Goldman 5 point visual scoring scale and VisioScan® VC 98 were used for evaluating crow's feet wrinkles at wk0, wk2, wk4, wk6 and patient's satisfaction questionnaires were evaluated at the end of study. On day 5th, PBSCs were harvested from 20 ml of the blood samples and subsequently injected in the lateral periorbital areas. The peripheral blood stem cell mobilization was done by filgrastim (GCSF) administration for 4 consecutive days.

The improvement in crow's feet wrinkles were demonstrated at wk2 and more improvement were observed at wk4 and wk6. Statistical differences $p<0.001$(wk2,4,6) were shown by repeated measure ANOVA. The side effects found only in wk0 were mild and included mild pain, redness and skin irritation at the sites of injection. All of side effect was gone in a few hours.

This study clearly demonstrated the effectiveness of PBSC in the treatment of crow's feet wrinkles.

Keywords: Peripheral blood stem cell / Crow's feet wrinkles / filgrastim (GCSF)

บทคัดย่อ

เซลล์ต้นกำเนิดจากเม็ดโลหิต ได้รับการยอมรับมานานแล้วว่า มีประสิทธิผลในการรักษาสภาวะต่าง ๆทางการแพทย์ วิทยานิพนธ์ฉบับนี้ ศึกษาประสิทธิผลและความปลอดภัยของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ในการรักษาริ้วรอยบริเวณหางตา

ทำการศึกษาในอาสาสมัครที่มีสุขภาพแข็งแรง จำนวน 25 ราย เพศหญิง 21 ราย เพศชาย 4 ราย เป็นระยะเวลา 7 สัปดาห์ โดยเซ็นต์ใบให้ความยินยอม ก่อน เข้าร่วมในโครงการวิจัย ใช้สาร filgrastim(GCSF) เพื่อกระตุ้นให้ร่างกายสร้างเซลล์ต้นกำเนิดจากเม็ดโลหิตในปริมาณมากขึ้น เป็นระยะเวลา 4 วันติดต่อกัน จากนั้นในวันที่ 5 จึงเริ่มทำการรักษาริ้วรอยบริเวณหางตา โดยนำเลือดจากอาสาสมัครปริมาณ 20ซีซี คัดแยกเฉพาะส่วนที่เป็นเซลล์ต้นกำเนิดจากเม็ดโลหิต ฉีดกลับไปที่บริเวณหางตาทั้งสองข้าง (สัปดาห์ที่ 0) นัดติดตามประเมินผลการรักษา ความปลอดภัย และผลข้างเคียง ในอาทิตย์ที่ 2, 4 และ 6 โดยใช้เครื่องมือ Rao-Goldman 5 point visual scoring scale and VisioScan® VC 98 เมื่อครบการรักษา (สัปดาห์ที่ 6) อาสาสมัครจะประเมินความพึงพอใจจากการรักษา

จากการศึกษาพบว่าริ้วรอยบริเวณหางตาทั้งสองข้าง เริ่มมีการลดลงที่สัปดาห์ที่ 2 และลดลงมากขึ้นในสัปดาห์ที่ 4 และ 6 ตามลำดับ อย่างมีนัยสำคัญทางสถิติ p-value < 0.001 (สัปดาห์ที่ 2, 4 และ 6) โดย repeated measure ANOVA ผลข้างเคียง พบเฉพาะในสัปดาห์ที่ 0 หลังจากมีการฉีดเซลล์ต้นกำเนิดจากเม็ดโลหิตไปที่บริเวณหางตาทั้งสองข้าง โดยพบอาการเจ็บเล็กน้อย แดง และระคายเคืองของผิวหนังบริเวณหางตา ซึ่งอาการต่าง ๆหายไปในเวลา 1–2 ชั่งโมง

การศึกษาวิจัยในครั้งนี้ แสดงให้เห็นถึงประสิทธิผลในการรักษาริ้วรอยบริเวณหางตา โดยการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต

คำสำคัญ เซลล์ต้นกำเนิดจากเม็ดโลหิต / ริ้วรอยบริเวณหางตา / ฟิลกราสทิม

Introduction

Facial rejuvenation is the cosmetic procedure or medical procedure used to increase or restore the appearance of younger and healthier looking skin. To be more precise, it aims to restore skin appearance of youth by using a combination of brow lift, elimination of eye bags, elimination of aging spots and decreasing wrinkles. In this thesis, crow's feet wrinkles are selected as the main indicator of facial rejuvenation.

There are various treatments available including chemical peels, microdermabrasion, topical cream applications, botox, fillers injections of various substances and cosmetic surgery. These agents work to plum up wrinkles or immobilize muscles and prevent the appearance of wrinkles. There have been several reports on the adverse effects of most of these procedures (Draelos, 1997; Bailin, P.L. & Bailin, M.D., 1988). During the last decades cell-based therapies including platelet rich plasma (PRP), growth factors and cytokines have received much attention due to the fact that they are likely to provide longer lasting effects with minimal side effects (Moulin, 1995).

PRP has been widely used in facial rejuvenation and aesthetic medicine (Foster, 2009; Bolta, 2007). It is an extract of the patient's own blood that contains three to five times more platelets and numerous Platelet Derived Growth Factors (PDGF) (Bolta, 2007; Arora, Ren & Romanos, 2009).These growth factors stimulate proliferation of fibroblasts and keratinocytes, which produce collagen and keratin (Marx, Carlson & Eichstaedt, 1998; Kevy & Jacobson, 2001; Weibrich & Kleis, 2002; Don, 2007; Foster, 2009; Redaelli, 2010). PDGF have a

significant role in forming new blood vessels, promoting the cellular division of fibroblasts and the synthesis of collagen and extracellular matrix, including hyaluronic acid (Marx *et al*, 1998; Kevy & Jacobson, 2001; Weibrich & Kleis, 2002; Schmitz & Hollinger, 2001; Marx, 2001). Collagen production has been shown to decrease the wrinkles. Improvement of skin texture and tone can be noticeable within three weeks and continues to be improved for the next 8 months (Bailin, P.L. & Bailin, M.D., 1988). In this thesis, PBSC was selected as the innovative cell-based therapies to be evaluated for its efficacy and safety for the treatment of crow's feet wrinkles.

Peripheral blood stem cells (PBSCs) are hematopoietic stem cells that circulate in the blood. They have the plasticity potential to differentiate into osteoblasts, chondroblasts, adipocytes, skeletal muscles, skin cells, etc (Abkowitz, 2002; Zavan, 2010; Waese & Kandel, 2008**;** Oh & Choo, 2010). It is most likely that this type of stem cells can be effective in decreasing wrinkles as well. The PBSCs extraction is considered to be non-invasive. In addition, much higher number in the circulation can be achieved by administering certain cytokines such as granulocyte colony-stimulating factor (G-CSF) or when hematopoietic recovery occurs following chemotherapy (Kiatpongsan & Tannirandorn, 2006).

In this thesis, Filgrastim (Human granulocyte colony stimulating factor (Hu-G-CSF)) was used to stimulate bone marrow production. Filgrastim has been approval by U.S. Food and Drug Administration since 5/29/2002. Filgrastim is produced by recombinant DNA technology. There are many indication and usage include patients undergoing peripheral blood progenitor cell collection and therapy. Filgrastim is contraindicated in patients with known hypersensitivity to E coli-derived proteins, Filgrastim or any component of the product.

The peripheral blood stem cells extracted were injected to crow's feet wrinkles of the healthy volunteers of both sexes in a systemic manner. The efficacy and safety of PBSCs in treatment of crow's feet wrinkles were evaluated comparing before and after results of the same subjects.

Objective

To study the effectiveness of peripheral blood stem cell therapy on crow's feet wrinkles.

Methodology

The 25 subjects were recruitment from healthy volunteer groups of both sexes with ages ranging between 35-60 years old, using inclusion and exclusion criteria. Written consent was given before participating in the study.

Injected Filgrastim 10 µg/kg/day subcutaneous at the abdominal area for 4 consecutive days. On the 5^{th} day 20 ml of blood were drawn and divide equally into two tubes. The blood samples collected were centrifused at a speed of 1000 RPM, 5 min for separate blood components. The red blood cells were move to the bottom of the tube, the plasma fraction was

float as the top layer and the buffy coat which contains the majority of platelets was between the plasma and above the red blood cells. Aspirated plasma and buffy coat from two tubes. Mixed it then divide equally into two tubes, centrifused at a speed of 3000 RPM, 10 min. Throw away the upper plasma, used the white bottom residual (PBSC). Three points injection of 0.1 ml PBSC id at both eyes, the 1st 1.5 cm from the lateral cantus, the 2nd and 3rd were given 1cm above and below the first.

The skin wrinkles were assessed by Rao-Goldman 5-point visual scoring scale and VisioScan® VC98 before and after treatment (wk0, wk2, wk4, wk6). Side effects and complications were also assessed. Patient's satisfaction questionnaires were evaluated at the end of study.

Statistics used for data analysis

Wilcoxon signed ranks test used to compare before and after treatment data collect due to Rao Goldman 5 point visual scoring scale and for patient's satisfaction questionnaires. Repeated measure ANOVA used for VisioScan® VC98. Compare Laboratory test before and after injection of GCSF used paired t-test and Wilcoxon signed rank test.

Results

Visioscan® VC98 evaluation. Statistic analysis by repeated measure ANOVA show significant improvement both right and left crow's feet wrinkles in every follow up (wk2, wk4, wk6). P-value < 0.001.

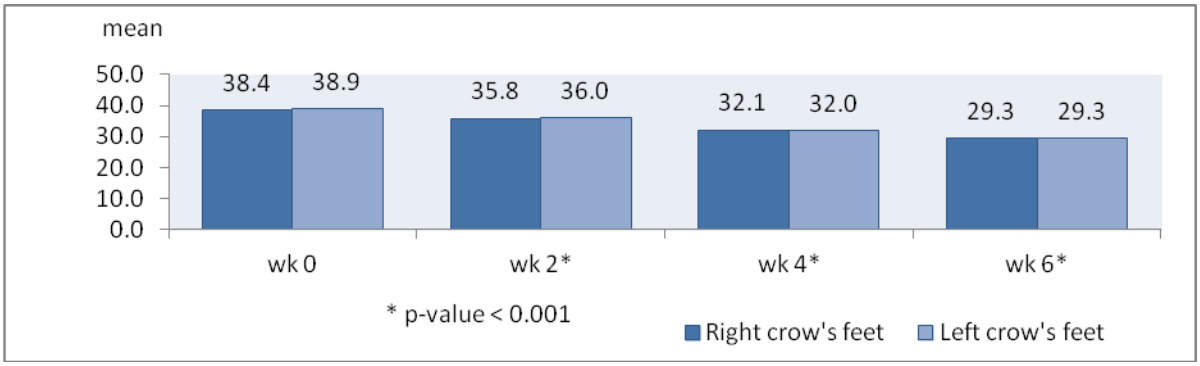

Fig.1 Visioscan® VC98 evaluation

Compared RAO Goldman 5 point visual scoring scale with wk0. Statistic analyses by wilcoxon signed ranks test data indicated significant decrease at wk 4 and wk6. Right crow's feet wrinkles in wk4 and wk6 means were 3.0 and 2.8, p-value=0.001 and <0.001 consecutively. Left crow's feet wrinkles in wk4 and wk6 means were 3.1 and 2.8, p-value =0.001 and <0.001 respectively.

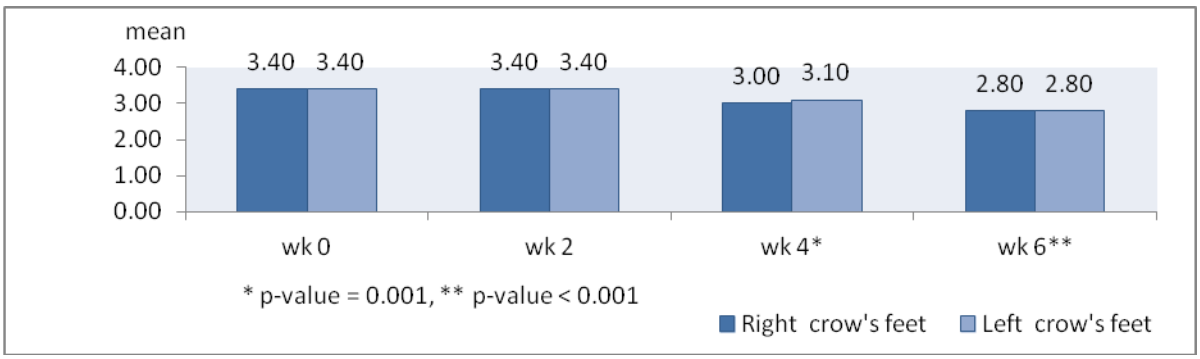

Fig.2 Compared RAO Goldman 5 point visual scoring scale with wk0

Side effect and complication. Statistic analysis by wilcoxon signed ranks test revealed that only 11 volunteers had side effects and complications in right crow's feet wrinkles and 10 volunteers had side effects and complications in left crow's feet wrinkles. All of the adverse events were found at wk0 (right at the end of the procedure). Side effects and complications included pin point bleeding found in 2 volunteers (8%) in right crow's feet wrinkles, redness was found in 5 volunteers (20%) and 6 volunteers (24%) in right and left crow's feet wrinkles. Three volunteers (12%) were noticed to have slight edema in both crow's feet wrinkles. Ecchymosis was found in 1 volunteer (4%) in both crow's feet wrinkles.

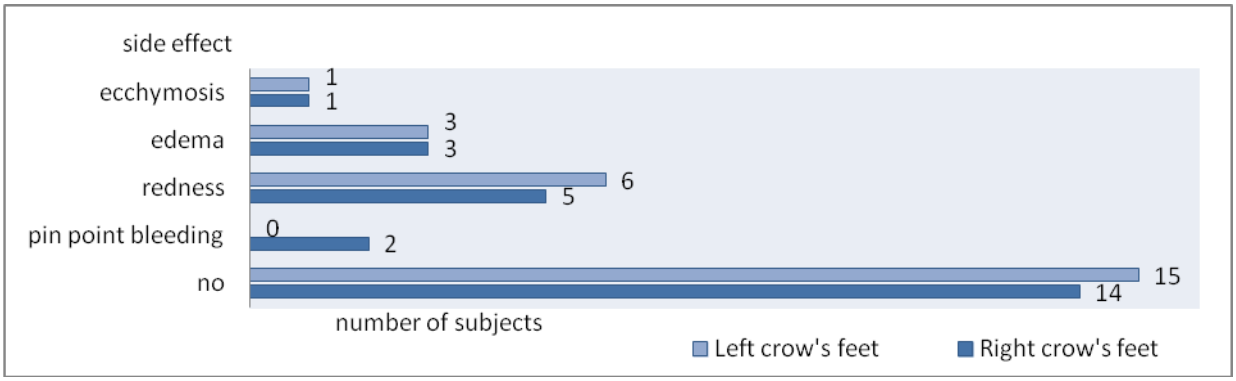

Fig.3 Side effect and complication

Laboratory test evaluation. There were significant increase in WBC p-value < 0.001 but significant decrease in platelet p-value = 0.031 as determined by paired t-test and Wilcoxon signed rank test.

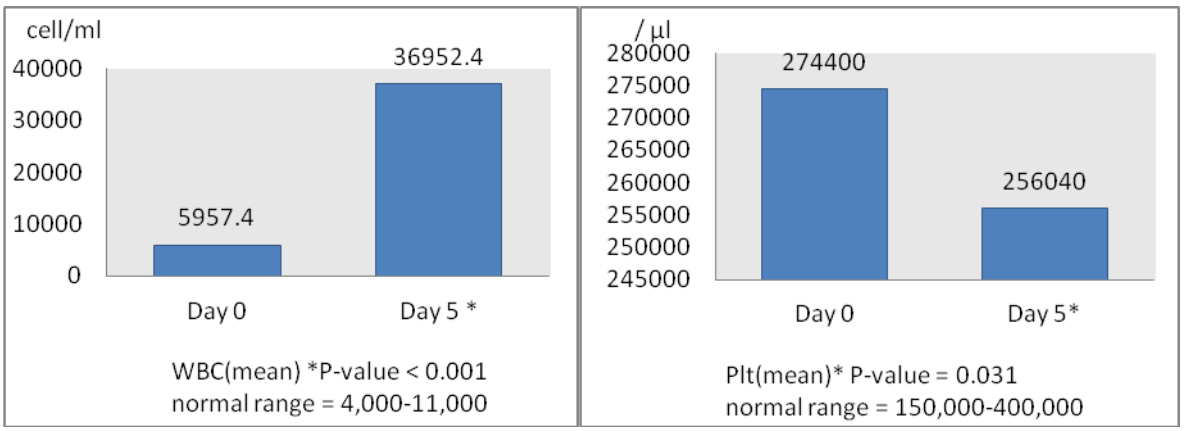

Fig.4 Laboratory test evaluation

Patient's satisfaction questionnaires. Statistic analysis by Wilcoxon signed ranks test of changes in right crow's feet wrinkles showed that there were 11 volunteers (44%), 11 volunteers (44%) and 3 volunteers (12%) who had very good, good and moderate satisfaction consecutively. In left crow's feet wrinkles, there were 10 volunteers (40%), 10 volunteers (40%), 4 volunteers (16%) and 1 volunteer (4%) who had very good, good, moderate and unchanged satisfaction consecutively.

Discussion

Fetal mesenchymal stem cells from amniotic fluid have similar characteristics and differentiation capacity to the pluripotent bone marrow derived mesenchymal stem cells (You et al., 2012). They had excellent wound healing effect in the skin fibroblast, excellent wound closure and skin regeneration effects in the wounded mouse skin, another benefit in clinical study show excellent effects of improving whitening, skin damages, skin regeneration, skin lifting and wrinkles (You et al., 2012). PBSCs have similar characteristics and differentiation capacity to the pluripotent bone marrow derived mesenchymal stem cells too, so they could improve crow's feet wrinkles.

Conclusion

The results of this study clearly indicated that PBSCs are effective in decreasing crow's feet wrinkles. All parameters measured revealed similar results. The anti wrinkle effects took place starting from 2 weeks with more improvement observed at 6 weeks. All subjects reported to have satisfaction with the treatments received.

No serious adverse effects were observed in all tested subjects. The redness and signs of skin irritation noted were judged to be due to injection reactions. Due to the autologous nature of the treatment, allergic reactions are unlikely to occur. The increase in white blood cell counts (WBCs) on day 5^{th} of the study was expected to be induced by filgastrim administration. In fact, the increase in granulocytes is correlated with stem cell mobilization.

The level of WBCs were known to come down to baseline levels within 4 day after stopping filgastrim administration. The decline in platelet counts observed in some patients are also associated with filgastrim injection. However, there should not be any clinical significance regarding the slight decline in platelets.

Suggestions

There should be further study to increase the number of subjects. In addition, long term follow up should also be performed in order to determine the long lasting effect of PBSC.

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