Optimal temperature for normal human skin fibroblast proliferation and glucose uptake, an in vitro study

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ABSTRACT

Devi Artami Susetiati, Laily Noor Qomariah, Sunardi Radiono, Yohanes Widodo Wirahadidjojo - Optimal temperature for normal human skin fibroblast proliferation and glucose uptake, in vitro study

Background: Wrinkles is caused by a decrease in collagen synthesis and an increase in collagen degradation. Collagen synthesis depends on fibroblast proliferation. Collagen synthesis needs glucose, so that collagen synthesis may be expressed by the increase in glucose uptake. Skin rejuvenation with heating may increase the collagen synthesis. The effect of short-term heating and optimal temperature on fibroblast proliferation and glucose uptake has not yet been defined.

Objectives: This study was aimed to determine the optimal temperature of short-term heating for normal human skin fibroblasts proliferation and glucose uptake.

Methods: A simple experimental study was conducted on 3rd passage subculture of normal human skin fibroblasts culture, isolated from 2 patients. Normal human skin fibroblasts with complete DMEM were classified into 5 groups, and then heated for a minute with thermocycler-machines at 38°C, 46°C, 56°C, 66°C, and 72°C. Those cells were incubated for 7 days in complete DMEM and subsequently fibroblasts proliferation was measured by MTT-assay. Glucose uptake was measured by glucometer Mediasafe-Terumo. The differences in glucose uptake and fibroblasts proliferation were analyzed with one-way ANOVA.

Result: Optimal temperature for fibroblasts proliferation was 46°C, and 66°C for collagen synthesis.

Conclusion: Skin rejuvenation based on heating could be performed at two different temperatures, each cycle consisted of 65°C on first heating, and seven days later, at 46°C on second heating.

Key words: fibroblast proliferation - glucose uptake - skin rejuvenation

ABSTRAK

Devi Artami Susetiati, Laily Noor Qomariah, Sunardi Radiono, Yohanes Widodo Wirahadidjojo - Suhu optimal pada proliferasi dan konsumsi glukosa fibroblas kulit normal, studi in vitro


Tujuan: Penelitian ini ditujukan untuk menentukan suhu optimal pemanasan jangka pendek untuk proliferasi dan konsumsi glukosa fibroblas kulit normal manusia.


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INTRODUCTION

Wrinkle skin exposed to the sunlight shows drastic decrease of collagen fibres due to the decrease in procollagen type I and III synthesis and the increase in collagen degradation. The decrease in collagen synthesis occurs due to fibroblast aging and the decrease in mechanical stimulation of fibroblast proliferation. The decrease in mechanical stimulation is caused by a decrease in the interaction between the fibroblasts and loose fibres.

One of the treatment of skin wrinkles due to solar exposure is the utilization of selective photothermolysis energy which improves the appearance of skin wrinkles clinically. This selective photothermolysis effect using temperature between 45°C-50°C can induce the expression of heat shock protein 70 (HSP 70) and HSP 47. The expression of HSP 70 and HSP 47 can improve tissue repair and wound healing process that will increase collagen synthesis.

In normal human skin, collagen synthesis depends on the level of fibroblast glucose, so that a decrease in glucose level will cause a low collagen level. Glucose-rich medium can stimulate the collagen synthesis. The increase in collagen synthesis can be seen in skin wrinkles therapy using heating (photothermolysis). Increased collagen synthesis will be followed by the increase in consumption of glucose, as the addition of insulin in glucose-rich medium will stimulate fibroblast to synthesize collagen, thus the increased glucose consumption can be used to describe the increase in collagen synthesis.

Study on the effect of heating and optimal temperature on normal human skin fibroblast proliferation and glucose consumption has not been reported previously.

METHODS

It was an in vitro study with simple experimental design. Research subjects were normal skin fibroblasts from two volunteers passage 3 subculture. The inclusion criteria included all normal human skin, and the exclusion criteria included human skin that received topical therapy in the previous one month that may affected skin fibroblasts. The study was carried out in the Department of Dermatovenereology Faculty of Medicine Gadjah Mada University.

Fibroblast culture in complete DMEM (Dulbecco’s Modified Eagle’s Medium) was divided into 5 groups, then was heated with thermocycler machine at 36°C, 46°C, 56°C, 66°C, and 72°C for 1 minute. Before the heating process started, glucose level was measured with glucometer Medisafe-Terumo, and then the heating was conducted in quadruple. After heating, sample was cultured for the next 7 days in complete DMEM. Second glucose measurement was conducted after heating and 7 day incubation. Fibroblast proliferation was measured using MTT-assay with 570 nm wavelength. Glucose consumption was the glucose level after the heating minus glucose level before the heating.

Statistical analysis was conducted with SPSS program. The difference of fibroblast proliferation average and glucose consumption was analyzed with one-way variant analysis, followed by posthoc tests using Tukey HSD. Significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Temperature that caused a significant increase in fibroblast proliferation was found 46°C ($p = 0.004$). At other temperatures, the increase in fibroblast proliferation was not significant, as seen in FIGURE 1.
The effect of heating temperature on the average percentage of glucose consumption can be seen in FIGURE 2. Increased glucose consumption was significant at 56°C (p = 0.016), 66°C (p = 0.005), and 72°C (p = 0.029).

FIGURE 1. The effect of temperature on fibroblast proliferation

The average fibroblast proliferation measured using MTT-assay increased significantly at 46°C temperature (p = 0.004), as shown in the FIGURE 1 that describes the effect of temperature on average fibroblast proliferation. Heating at another temperature did not produce any significant increase.

Fibroblast proliferation increased at 46°C temperature, caused by HSP 47 and HSP 70, which played role in the increase of fibroblast proliferation and collagen synthesis. They were released at temperature around 42°C-45°C. These HSP would induce the extracellular signal-regulated kinase (ERK) pathway that would mediate intracellular signal transduction in fibroblast proliferation. ERK induced p43 cytokine which stimulated dermal fibroblast proliferation and played a role in wound healing. The number of fibroblast at around 56°C was not significantly different from that of control, because cells began to lose their viability or die at 53.2°C.

FIGURE 2 illustrates the effect of temperature on average glucose consumption. The effect was significant at 56°C (p = 0.016), 66°C (p = 0.005), and 72°C (p = 0.029). This result is comparable with the result of a study by Reeves, who found that cells which were given hyperthermia stress could increase their glucose consumption ability. At 66°C glucose consumption started to decrease, this was possibly because the higher the temperature was the more cells would lose their viability. Glucose-rich medium could increase the fibroblast proliferation. When the cells were actively proliferating, the glucose consumption was increased and it started to decline when the cells stopped to proliferate.

Skin rejuvenation using radiofrequency at 45°C-65°C would increase the synthesis of collagen. The condition would be followed by the increase in glucose consumption, so that the increased glucose consumption could be used to describe the increase in collagen synthesis.

Based on these findings, the heating for skin rejuvenation should be conducted in two different temperature cycles. The first heating was to increase collagen synthesis at 66°C, and the second heating was to increase the number of skin fibroblast cells at 46°C temperature. The interval between two heating should range about seven days, because according to Lawrence et al., the increase in mRNA expression of collagen type I reached its maximum point on the seventh day post-heating.

CONCLUSION AND SUGGESTION

The heating of normal human skin fibroblast for 1 minute could increase its proliferation. The optimal temperature for fibroblast proliferation was 46°C, while the optimal temperature for glucose consumption was 66°C.

It is suggested that heating-based skin rejuvenation is conducted at two different tempe-
REFERENCES


