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The effect of metformin on proliferation and glucose uptake in keloid fibroblast culture

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ABSTRACT

Nur Dwita Larasati, Sunardi Radiono, Yohanes Widodo Wirohadidjojo - ***The effect of metformin on proliferation and glucose uptake in keloid fibroblast culture***

Background: Metformin as an antihyperglycemic agent has a potential effect in increasing type I collagen synthesis and decreasing MMP, so that it has a potential to be an antiaging agent. One of aging failure processes is the development of keloids. Keloids are formed due to hyperproliferation of fibroblasts, an increase of collagen synthesis, particularly type I and III, and a decrease in MMP-1 and MMP-2. Fibroblast proliferation process and collagen synthesis need glucose uptake. The study on metformin ability to aggravate or stimulate the formation of keloid has never been conducted before.
Objective: The aim of this study was to know the difference of proliferation and glucose uptake between keloid fibroblasts given metformin and without metformin.
Method: A simple experiment was conducted using 3rd passage keloid fibroblasts culture. Keloid fibroblasts were divided into 2 groups, the first group was treated with metformin in the dose of 100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL, and control. Keloid fibroblasts proliferation in the first group was measured using spectrophotometer with MTT assay, and glucose uptake of keloid fibroblast in the other group was measured using glucometer. The difference in proliferation and glucose uptake of keloid fibroblast was analyzed using one-way anova.
Result: The result of this study showed that the average keloid fibroblast proliferation in the metformin treatment groups was not increased compared to that in control group. Meanwhile, the average keloid fibroblast glucose consumption in metformin treatment group significantly increased, at the dose of 300 lg/mL ($p = 0.044$) and 400 μg/mL ($p = 0.008$).
Conclusion: Metformin could not increase keloid fibroblasts proliferation, but it could increase glucose uptake of keloid fibroblasts.

Keywords: keloid - metformin - fibroblast proliferation - glucose uptake

ABSTRAK

Nur Dwita Larasati, Sunardi Radiono, Yohanes Widodo Wirohadidjojo - ***Pengaruh metformin terhadap proliferasi dan konsumsi glukosa pada biakan fibroblas keloid***

Latar Belakang: Metformin sebagai antidiabetes mellitus terbukti meningkatkan sintesis kolagen tipe I dan menurunkan MMP, sehingga memiliki potensi sebagai antipenuaan. Salah satu bentuk gagal menua adalah munculnya keloid. Keloid terjadi akibat hiperproliferasi fibroblas, peningkatan sintesis kolagen, terutama tipe I dan III, serta penurunan MMP-1 dan MMP-2. Dalam proses proliferasi fibroblas dan sintesis kolagen, diperlukan konsumsi glukosa. Kemungkinan pemakaian metformin dapat memperberat keloid atau memacu timbulnya keloid belum pernah dibuktikan.
Tujuan: Penelitian ini bertujuan untuk mengetahui perbedaan dalam proliferasi dan konsumsi glukosa fibroblas keloid yang mendapat metformin dibanding yang tidak mendapat metformin.
Metode: Rancangan penelitian adalah eksperimental sederhana, mengggunakan kultur fibrobas keloid *passage* 3. Fibroblas keloid dibagi menjadi dua kelompok, masing-masing kelompok diberi perlakuan dengan metformin 100 μg/ml, 200 μg/ml, 300 μg/ml, 400 μg/ml serta kontrol. Pada kelompok pertama dilakukan pengukuran proliferasi fibroblas keloid dengan spektrofotometer menggunakan MTT-*assay*, dan pada kelompok kedua dilakukan pengukuran

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konsumsi glukosa fibroblas keloid menggunakan glukometer. Perbandingan proliferasi dan konsumsi glukosa fibroblas keloid masing-masing diuji dengan analisis varian satu jalan.
Hasil: Hasil penelitian ini menunjukkan bahwa rerata proliferasi fibroblas keloid yang diperlakukan dengan metformin tidak meningkat dibanding kelompok kontrol. Sedangkan, rerata konsumsi glukosa kelompok perlakuan dengan metformin meningkat bermakna pada dosis 300 lg/mL ($p = 0.044$) and 400 lg/mL ($p = 0.008$) dibanding kontrol.
Simpulan: metformin tidak meningkatkan proliferasi fibroblas keloid tetapi metformin terbukti meningkatkan konsumsi glukosa fibroblas keloid.

INTRODUCTION

Metformin is a biguanide derivate (dimethyl-biguanide), which has anti-hyperglycemic effect, used as therapy for type II diabetes.[1] Metformin lowers the blood glucose level through two main mechanisms that will reduce liver glucose production[2] and increase musular glucose consumption[3] through stimulation of adenosine monophosphate-activated protein kinase (AMPK).[4] In addition to working on the glucose metabolism, metformin also affects the collagen synthesis and cell proliferation. Metformin was proven to increase type I collagen synthesis, increase cell proliferation[5] and decrease matrix metalloproteinase-2 (MMP-2) in normal fibroblast culture.[6]

The aging process of the skin is marked by the occurrence of wrinkles caused by the decrease in collagen synthesis, especially type I and III collagen due to the increase of collagen degradation by MMP.[7] Although the aging process goes on, many people still have a desire to look young and healthy. Nowadays, experts are working to develop skin antiaging drugs. One of the drugs that have antiaging effect is metformin.[8]

Keloid is a form of skin aging failure. This is related with the increased collagen synthesis in keloid, especially type I and III collagen, and the decrease in normal aging process.[9]

Keloid is a benign fibrous tissue tumor, caused by hyperproliferation of fibroblast and accumulation of extracellular matrix component, especially type I and III collagen[10] without being balanced by MMP collagen degradation.[11] Keloid fibroblast is characterized to secrete many growth factors, especially transforming growth factor-β1 (TGF-β1)[12], decrease cellular apoptosis[13], and increase several cytokines.[14]

Fibroblast activity is marked with cellular proliferation and collagen synthesis. Cell proliferation can be measured using MTT, which measures the reduction of yellow-colored salt tetrazolium (MTT) into blue-colored formazan. The formed formazan can be determined optically and its optical density is read using spectrophotometer.[15]

Collagen synthesis needs glucose.[16] It has been proven by Trevisan *et al.* that higher glucose consumption increased collagen synthesis[17], while lower glucose consumption decreased collagen synthesis.[18] Thus, the increased consumption of glucose can be used to describe the increase in collagen synthesis.

Glucose consumption is the glucose uptake that occurred in tissue, determined by measuring the glucose level before the consumption minus glucose level after the consumption. It can be measured using glucometer.[19]

Based on the nature of metformin on collagen synthesis, antiaging drugs, including metformin itself, can increase the risk or aggravate keloid formation. So far, the effect of metformin on keloid is unknown.

METHOD

This research was an *in vitro* study, using simple parallel multigroup research design, with metformin treatment on the fibroblast keloid culture.

Fibroblast culture was taken from keloid tissue that has been sliced into small pieces, and then primary culture technique was conducted in a sterile petri disk, contained complete Dulbecco minimal essential medium (DMEM) consisted of DMEM, 10% fetal bovine serum (FBS), amphotericin-B 250 lg/mL and penicillin-streptomycin 0.2% from Gibco. The culture was incubated in 5% CO_2 at 37 °C. Keloid fibroblast culture that has grown was trypsinized with 0.25% trypsin etilendiaminetetra-acetic acid (EDTA) (Gibco) and was subcultured until passage 3 was obtained and confluent fibroblast keloid cells reached 60-70%. Keloid fibroblast cells

were divided into two groups, and put into two 96 multiwell plates with an amount of $1x10^5$ cells in each well. The keloid fibroblast proliferation in the first group was measured with spectrophotometer using MTT-assay, and glucose consumption in the second group was measured using glucometer.

Keloid fibroblast proliferation measurement was conducted by addition of the tetrazolium bromide (MTT). After keloid fibroblasts were put into the 96 multiwell plate, the cells were treated with metformin in the dose of 100 ìg/mL, 200 ìg/mL, 300 ìg/mlL 400 ig/mL and control, then incubated in the incubator at 37°C with 5% CO_2 for 18 hours. To each well 50 ìg/mL MTT was added, and then the wells were wrapped with aluminum foil and incubated again for 4-8 hours. After that, all MTT and the medium were sucked out from the well, and 200 iL DMSO and 25 iL glycin buffer were added into each well. The optical density was immediately measured using spectrophotometer at 620nm to determine the number of cells that were still viable.

The measurement of keloid fibroblast glucose consumption was conducted by measuring the glucose level inside the complete DMEM medium that had been filled with keloid fibroblast cells using glucometer before treatment (glucose level before treatment). Later, metformin treatment was given in the dose of 100 ìg/mL, 200 ìg/mL, 300 ìg/mL, 400 ìg/mL, and incubated at 37°C with 5% CO_2 for 18 hours. After incubated, glucose level of each well was measured again using glucometer (glucose level after treatment). The glucose consumption was calculated by reducing the glucose level before treatment with glucose level after treatment.

Comparison of the average of keloid fibroblast proliferation and the average of glucose consumption between metformin treatment groups and the control groups was analyzed with one-way analysis of variance.

RESULTS AND DISCUSSION

The average of proliferation and glucose consumption between keloid fibroblast with metformin treatment groups and control groups were presented in FIGURE 1 and 2.

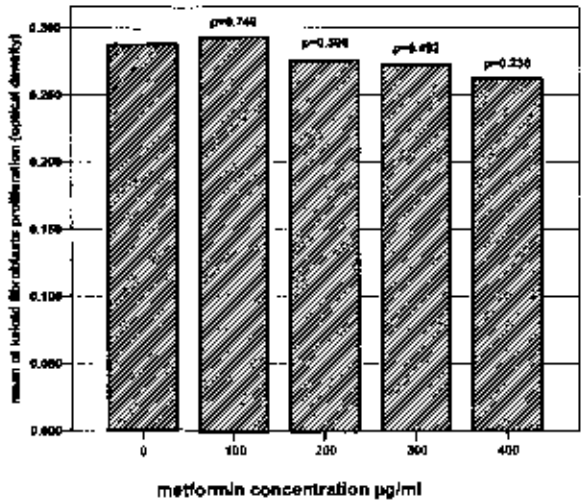

FIGURE 1. Average of keloid fibroblast proliferation in metformin treatment groups and control group

FIGURE 1 shows that the average keloid fibroblast proliferation in the metformin treatment groups did not increase compared to that in the control group. This result was contradicted with the study of Cortizo *et al.*[5], which proved that metformin enhanced the normal fibroblast cell proliferation rate at 300-3000 ìg/mL. The absence of the increase in keloid fibroblast proliferation in this research was possibly due to the effect of TGF-β−1 on keloid fibroblast proliferation was greater than of metformin, so that proliferation effect due to metformin treatment was not visible.

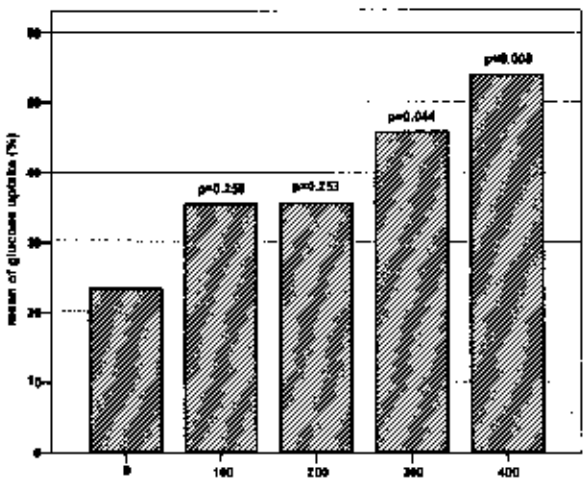

FIGURE 2. Average of keloid fibroblast glucose consumption in metformin treatment groups and control group

FIGURE 2 shows that the average of keloid fibroblast glucose consumption in metformin treatment group significantly increased, at the dose of 300 ìg/mL (p = 0.044) and 400 ìg/mL (p = 0.008). This result is comparable with the previous studies suggesting that metformin increased glucose consumption, either *in vivo* or *in vitro*.[20] Purello *et al*. demonstrated that metformin was able to increase glucose consumption in the lymphocytes at 33 mumol/L concentration.[21] Meanwhile, a research by Bertrand *et al*. proved that 10 mM metformin treatment on cardiomyocyte culture would increase its glucose consumption by 8-10 times.[22]

The high glucose consumption increases extracellular matrix protein synthesis, that is, type I and III collagen[23], and decreases MMP activity that play role in collagen degradation.[24]

Keloid fibroblast glucose consumption increased along with the increased metformin dose that in turn would increase the collagen synthesis.[17]

CONCLUSION

Metformin did not increase the proliferation of keloid fibroblast, but it increased the keloid fibroblast glucose consumption. Thus, metformin had a direct effect on keloid fibroblast glucose consumption and might be on collagen synthesis.

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