RINGKASAN

Pengaruh Pemberian Metformin Terhadap Deposisi, Degradasi dan Kontraktilitas Kolagen Pada Model Fibrosis Kapsul Anterior Secara In Vitro (Studi Eksperimental)

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Katarak merupakan penyebab utama kebutaan dan gangguan penglihatan di dunia terutama di negara-negara berkembang. Kebutaan akibat katarak mencapai 32.4 juta orang dan 191 juta lainnya mengalami gangguan penglihatan di tahun 2010. *Posterior Capsule Opacification* (PCO) adalah kekeruhan kapsul posterior lensa yang terbentuk dari sisa sel epitel lensa setelah operasi katarak. Kejadian PCO berkisar 8.7% sampai 33.4% pada rentang waktu 16 sampai 22 bulan. Adanya Lensa Intra Okuler (LIO) di *capsular bag* dapat menginduksi jalur inflamasi seperti *Tumor Necrosis* Factor (TNF) α dan Interleukin (IL) 6 serta reaksi oksidatif dari faktor-faktor radikal bebas dan *Transforming Growth Factor* (TGF) β yang akan menstimulasi kolagen dan miofibroblas sehingga terbentuk fibrosis pada kapsul lensa posterior.

Metformin masih sangat jarang digunakan dalam bidang mata. Salah satu studi yang dilakukan oleh Kalariya *et al* secara *in vivo* menunjukkan adanya peningkatan *Adenosin Monophosphate-activated Protein Kinase* (AMPK) dan penurunan sitokin-sitokin pro inflamasi pada tikus yang dibuat uveitis dan diberikan metformin secara intraperitoneal dibandingkan kelompok kontrol.

Pada penelitian ini dilakukan studi *in vitro* pada kultur sel epitelial lensa atau *Lens Epithelial Cells* (LEC) dengan metode teknik *scratching* sebagai suatu model fibrosis kapsul lensa anterior. Penelitian ini bertujuan untuk mengetahui pengaruh metformin terhadap jumlah kolagen terdeposisi, jumlah kolagen terdegradasi, dan kontraktilitas kolagen sel epitel lensa. Sel kultur dibagi dalam 4 kelompok yaitu kelompok kontrol sel kultur LEC yang dibiakkan tanpa perlakuan

dan kelompok sel kultur LEC yang dibiakkan dengan pemberian metformin dengan dosis yang berbeda. Metformin diberikan dalam dosis 0,1 mM, 0,5 mM, dan 1 mM pada kelompok perlakuan sel kultur LEC.

Hasil penelitian menunjukkan bahwa metformin mempengaruhi deposisi kolagen secara signifikan dari konsentrasi 0,1 mM (17.91±6.15 ug/ml, $p<0.05$) dibandingkan dengan kontrol FBS 10%. Sedangkan untuk mempengaruhi degradasi kolagen dibutuhkan konsentrasi metformin minimal sebesar 0,5 mM (6.5900E2± 1.1599E2ug/ml, $p<0.05$) dibandingkan dengan kontrol FBS 10%. Pada variabel kontraktilitas sel, metformin sudah menunjukkan efek secara signifikan mulai konsentrasi 0,1 mM(16.38±3.88 um, $p<0.05$).

Dari data-data tersebut, dapat disimpulkan bahwa metformin secara umum dapat menghambat fibrosis pada biakan sel kapsul anterior lensa melalui penurunan kadar deposisi, peningkatan kadar degradasi serta penurunan kontraksi kolagen. Peningkatan konsentrasi metformin tidak memiliki efek peningkatan dalam kemampuan menurunkan kontraktilitas kolagen pada biakan sel kapsul anterior lensa. Sebaliknya, peningkatan dosis metformin sebanding dengan penurunan kolagen terdeposisi dan peningkatan kolagen terdegradasi.

SUMMARY

THE EFFECT OF METFORMIN ON COLLAGEN DEPOSITION, DEGRADATION AND CELL CONTRACTILITY IN ANTERIOR LENS CAPSULE FIBROTIC MODEL IN VITRO (LABORATORY EXPERIMENTAL STUDY)

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Cataracts are the main cause of blindness and vision impairment in the world, especially in developing countries. Blindness due to cataracts reached 32.4 million people and 191 million others experienced visual impairment in 2010. Opacification of the lens capsule after cataract surgery is a common case and it can be caused by some factors. Posterior Capsule Opacification (PCO) is a opacification of the posterior lens capsule that formed due to residual lens epithelial cell (LEC) after cataract surgery.

The incidence of PCO ranged from 8.7% to 33.4% in about 16 to 22 months. The presence of Intra Ocular Lenses (LIO) in capsular bags can induce inflammatory pathways such as Tumor Necrosis Factor (TNF) α and Interleukin (IL) 6 as well as oxidative reactions of free radical factors and Transforming Growth Factor (TGF) β which will stimulate collagen and α myofibroblasts so that fibrosis can be formed in the posterior lens capsule.

Metformin is rarely used in ophtalmology. One of in vivo study by Kalariya *et al* showed an increase of Monophosphate-activated Protein Kinase (AMPK) and a decrease of proinflammatory cytokines in mice which were made having uveitis and they were given metformin intraperitoneally compared to the control group.

In this study, the lens epithelial cell culture (LEC) was used in vitro by scratching technique method as a model of anterior lens capsule fibrosis. The aim of this study was to determine the effect of metformin on the amount of deposited collagen, the amount of collagen degraded, and the contractility of the lens epithelial cell collagen. The culture cells were divided into 4 groups: control group of LEC culture cells bred without treatment and groups of LEC culture cells cultured by administering metformin at different doses. Metformin was given in doses of 0.1 mM, 0.5 mM, and 1 mM in the LEC culture cell treatment group.

The results showed that metformin significantly affected collagen deposition starting at a concentration of 0.1 mM (17.91 ± 6.15 ug / ml, p <0.05) compared with a 10% FBS control. Whereas to influence collagen degradation, a minimum metformin concentration of 0.5 mM (6.5900E2 ± 1.1599E2ug / ml, p <0.05) was required compared with a 10% FBS control. For the cell contractility variable, metformin had shown a significant effect starting at a concentration of 0.1 mM (16.38 ± 3.88 um, p <0.05).

From those datas, it can be concluded that metformin generally can inhibit fibrosis in the anterior lens capsule cell culture by decreasing levels of deposition, increasing levels of degradation and decreasing collagen contraction. Increasing concentration of metformin does not increase the ability to decrease contractility of collagen in the anterior lens capsule cell culture. Conversely, increasing the dose of metformin is proportional to the decrease in deposited collagen and an increase in degraded collagen.

THE EFFECT OF METFORMIN ON CELL CONTRACTILITY, COLLAGEN DEPOSITION, AND DEGRADATION IN ANTERIOR LENS CAPSULE FIBROTIC MODEL IN VITRO (LABORATORY EXPERIMENTAL STUDY)

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Background : Posterior Capsule Opacification (PCO) is a frequent complication of cataract surgery. PCO is a fibrosis condition that is triggered by an inflammatory response due to lens capsule injury due to cataract surgery and is combined with an inflammatory reaction to the implanted intraocular lens.

Methods: HLEC were isolated from anterior lens capsule of cataract patient. HLEC were divided into 4 groups consist of FBS 10% control group, metformin 0,1 mM, 0,5 mM and 1 mM treated group. This study investigated the effect of metformin on cell contractility, collagen deposition and degradation in HLEC. The differences of cell contractility, collagen deposition and degradation among groups were analyzed using Anova or Kruskal Wallis test then followed by Posthoc test with significant level of $p<0.05$.

Results: Metformin 0.1mM (17.92 ± 6.16 ug/mL), metformin 0.5mM (12.92 ± 4.31 ug/mL) and metformin 1mM (11.25 ± 5.30 ug/mL) significantly reduced collagen synthesis in HLEC compared with the 10% FBS control (31.46 ± 7.52 ug/mL, p=0.002). Metformin 0.1 mM, 0.5 mM and 1 mM significantly increased collagen degradation (4.77 ± 9.27 ug/mL, 6.59 ± 1.16 ug/ml, 6.35 ± 1.90 ug/ml, respectively) compared with the 10% FBS control (2.21 ± 2.78 µg/mL, p = 0.002). Metformin 0.1mM (16.39 ± 3.89%), metformin 0.5mM (13.89 ± 2.59%) and metformin 1mM (11.93 ± 2.44%) significantly reduced collagen contractility in HLEC compared with the 10% FBS control (44.25 ± 4.95%, p=0.000). Increasing the dose of metformin did not provide a significant difference on collagen contractility ($p>0.05$). Increasing the concentration of metformin gave a significant difference on collagen deposition, at concentrations of 0.1 mM and 1 mM. ($p<0.05$), and also on collagen degradation, at levels of 0.1 mM and 0.05 mM ($p<0.05$).

Conclusion: Metformin has antifibrotic effect in HLEC through extracellular matrix remodeling.

Keywords: metformin, lens epithelial cell, fibrosis