Peran sitoglobin pada hipoksia jaringan fibrosis dengan keloid sebagai model = The Role of cytoglobin in fibrosis hypoxia with keloid as a model

Endah Wulandari, author

Abstrak

Latar belakang: Sitoglobin (Cygb) adalah protein pengangkut O2 yang diekspresikan oleh fibroblas dan fibroblast like cells aktif. Keperluan O2 dan energi meningkat pada fibrosis akibat proliferasi fibroblas dan sintesis kolagen. Pada fibrosis terjadi hipoksia yang ditandai oleh stabilisasi hypoxia inducible factor-1α (HIF-1α), yang kemudian membentuk HIF-1 yang merupakan faktor transkripsi untuk ekspresi protein adaptasi (termasuk Cygb). Diduga Cygb berperan dalam suplai O2 pada fibrosis. Tujuan penelitian ini adalah untuk memperoleh informasi mengenai peran Cygb pada hipoksia jaringan fibrosis dengan keloid sebagai model.

Metode: Penelitian bersifat observasional deskriptif. Sampel keloid diperoleh melalui biopsi, sedangkan kontrol preputium diperoleh melalui sirkumsisi, masing-masing 10 sampel jaringan. Pengukuran ekspresi mRNA Cygb, HIF-1α, kolagen I dan III dilakukan dengan real time RT-PCR; kadar protein Cygb dan HIF-1α dengan ELISA; dan ekspresi protein Cygb, HIF-1α, FGF, kolagen I dan III di lapisan dermis dengan imunohistokimia (IHK). Pengukuran kadar MDA dan GSH (tingkat stres oksidatif) serta kadar hidroksiprolin (untuk pematangan kolagen) dengan spektrofotometri, sedangkan pengukuran kepadatan kolagen dengan pewarnaan Van Gieson. Data dianalisis secara statistik menggunakan uji-t.

Hasil: Pada keloid dibandingkan preputium, ekspresi mRNA Cygb meningkat 8,7 kali, protein Cygb meningkat bermakna (1,196 Vs 0,779 ng/mg protein dan 95% Vs 63% ; p <0,05). Ekspresi mRNA HIF-1α meningkat 5,1 kali, protein HIF-1α meningkat bermakna (0,201 Vs 0,122 ng/mg protein dan 80% Vs 38%; p <0,05). Terdapat korelasi kuat antara ekspresi protein HIF-1α dan mRNA Cygb (Pearson; R = 0,649; p <0,01). Ekspresi protein FGF keloid meningkat bermakna (78% Vs 41%; p <0,05). Demikian pula ekspresi mRNA prokolagen I dan III keloid meningkat bermakna (35 kali dan 27,1 kali), serta ekspresi protein kolagen I dan III (61% Vs 37% dan 39% Vs. 16%; p <0,05). Juga terdapat korelasi kuat antara protein HIF-1α dengan FGF, prokolagen I dan III (Pearson; R= 0,878; R=0,960; dan R=0884; p<0,01). Kadar hiroksiprolin lebih tinggi pada keloid (0,297 Vs 276 ng/mg protein; p >0,05) dan pematangan kolagen lebih tinggi bermakna (1,2 kali; p <0,05). Cygb berkorelasi kuat dengan pematangan kolagen (kadar hidroksiprolin) (Pearson; R = 0,790; p <0,001).

Kesimpulan: Cygb berperan pada hipoksia jaringan fibrosis yang ditandai dengan peningkatan ekspresinya. Peran Cygb terkait dengan ekspresi HIF-1α yang berkorelasi dengan peningkatan FGF, pro/kolagen I dan III yang merupakan faktor penting pada fibrosis. Cygb juga berperan pada pematangan kolagen.
<hr>
Background: Cytoglobin (Cygb) is an O2 carrier protein expressed by fibroblasts and active fibroblast like cells. O2 and energy demand increased in fibrosis due to proliferation of fibroblasts and synthesis of

collagen. In fibrosis hypoxia occurred which is characterized by stabilization of hypoxia inducible factor-1α (HIF-1α), which later forming the HIF-1, a transcription factor for the expression of adaptation protein (including Cygb). Cygb alleged role in the supply of O2 in fibrosis. The purpose of this study was to obtain information about Cygb role in fibrosis hypoxia with keloid tissue as a model.

Methods: This was an observational descriptive study. Keloid samples were obtained from biopsy, while the preputium as control were obtained from circumcision, 10 tissue samples each. Measurement of Cygb, HIF-1α, collagen I and III mRNA expression were carried out by real time RT?PCR. Cygb and HIF-1α protein level were measured by ELISA; while Cygb, HIF-1α, FGF, and collagen I and III protein expressions in the dermis layer by immunohistochemistry (IHC). Measurement of MDA and GSH levels (oxidative stress) and hydroxyprolin concentration (marker of mature collagen) by spectrophotometry, while the collagen density measurement with van Gieson staining. Data were analyzed statistically using t-test.

Results: In keloid compared preputium, Cygb mRNA expression increased 8.7 times compared to preputium, Cygb protein increased significantly (1.196 Vs 0.779 ng/mg protein and 95% Vs 63%, $p < 0.05$). HIF-1α mRNA expression increased by 5.1 times in keloid tissue, and protein HIF-1α increased significantly (0.201 Vs 0.122 ng/mg protein and 80% Vs 38%, $p < 0.05$). There is a strong correlation between the expression of HIF-1α protein and Cygb mRNA (Pearson; $R = 0.649$, $p < 0.01$). Keloid FGF protein expression increased significantly (78% Vs 41%; $p < 0.05$). Similarly, mRNA expression of procollagen I and III keloid increased significantly (35 times and 27.1 times), and protein expression of collagen I and III (61% Vs 37% and 39% Vs 16%, $p < 0.05$). There is also a strong correlation between HIF-1α protein with FGF, procollagen I and III (Pearson, $R = 0.878$, $R = 0.960$; and $R = 0.884$, $p < 0.01$). Hydroxyprolin concentration were higher in keloid (0.297 Vs 0.276 ng/mg protein; $p > 0.05$) and collagen maturation was significantly higher (1.2 times, $p < 0.05$). Cygb is correlated with maturation of collagen (hydroxyproline levels) (Pearson, $R = 0.790$, $p < 0.001$).

Conclusion: Cygb play role in fibrosis hypoxia which is characterized by its increased expression. Cygb role is associated with the expression of HIF-1α which are correlated with increased FGF, pro/collagen I and III, which are important factor in fibrosis. Cygb also play a role in the maturation of collagen.