Pola ekspresi neuroglobin dan sitoglobin jaringan otak tikus serta hubungannya dengan apoptosis sebagai respons adaptasi molekuler pada kondisi hipoksia sistemik kronik = The Expression pattern of neuroglobin and cytoglobin in the rat brain tissues and its correlation with the apoptosis as molecular adaptive response to the chronic systemic hypoxic

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Abstrak

Hipoksia berperan penting pada patofisiologi berbagai penyakit utama penyebab kematian seperti, penyakit jantung iskemia, strok, kanker, penyakit paru kronik, dan gagal jantung kongestif. Kedua protein golongan globin di otak, yaitu neuroglobin (Ngb) dan sitoglobin (Cygb) diduga berperan dalam suplai oksigen ke mitokondria dan melindungi jaringan otak dari kerusakan akibat hipoksia (neuroprotektan). Perubahan ekspresi protein merupakan salah satu bentuk adaptasi biokimia yang penting terhadap perubahan homeostasis. Oleh karena itu timbul pertanyaan bagaimana pola ekspresi Ngb dan Cygb serta peran neuroprotektan kedua protein tersebut di otak pada keadaan hipoksia sistemik kronik (HSK).
Penelitian bertujuan manganalisis perbedaan pola ekspresi Ngb dan Cygb serta kaitannya dengan apoptosis pada HSK. Parameter yang diukur adalah Ngb, Cygb, sitokrom c, MDA, GSH dan HIF-l. Rancangan penelitian yang digunakan adalah studi eksperimental in vivo model HSK pada tikus. Tikus sebagai hewan coba dibagi secara acak dalam 6 kelompok perlakuan, yaitu kelompok I adalah kelompok kontrol atau tanpa perlakuan hipoksia, sedangkan kelompok II, III, IV, V, dan VI mendapat perlakuan hipoksia dengan lama waktu hipoksia selama 1, 3, 5, 7, dan 14 hari.
Parameter yang diperiksa meliputi ekspresi Ngb dan Cygb dengan teknik real time-RT PCR, ELISA dan imunofluoresen FITC, stres oksidatif, HIF-1 sebagai penanda hipoksia, dan sitokrom c sebagai penanda apoptosis. Hasil yang diperoleh HSK meningkatkan ekspresi mRNA Ngb pada hipoksia 3, 5, dan 7 hari, namun ekspresi proteinnya menurun pada hipoksia 1, 3, 5, 7, dan 14 hari dibanding dengan kontrol. Berbeda dengan ekspresi mRNA Cygb yang menurun selama hipoksia 1, 3, 5, 7, dan 14 hari, namun protein Cygb meningkat pada hipoksia 1, 3, 5, 7, dan 14 hari dibandingkan dengan kontrol.
Korelasi Ngb dengan sitokrom c lemah tidak signifikan, sedangkan Cygb sangat lemah dan tidak signifikan. HSK menginduksi ekspresi HIF-l yang meningkat tertinggi pada hipoksia 7 hari, dan menyebabkan stres oksidatif yang ditandai dengan meningkatnya MDA pada hipoksia 1, 3 dan 5 hari, serta menurunnya GSH pada hipoksia 1, 3, dan 5 hari. Penelitian ini membuktikan bahwa terdapat perbedaan pola ekspresi Ngb dan Cygb pada HSK. Ekspresi Ngb sebagai respons adaptasi terjadi lebih awal dan lebih dipengaruhi oleh lama waktu hipoksia dibandingkan dengan ekspresi Cygb. Meskipun lemah, Ngb cenderung mempunyai peran menghambat apoptosis dibandingkan dengan protein Cygb.
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Hypoxia has an important role in the pathophysiology of high mortality diseases, such as ischemic cardiovascular disease, stroke, cancer, chronic lung disease, and congestive heart failure. The proteins belonged to globin protein group, included neuroglobin (Ngb) and cytoglobin (Cygb), have been presumed to play a role in regulating the oxygen supply into the mitochondria and protecting the brain tissues from

damage due to hypoxia (neuroprotectant). An alteration in protein expression due to a homeostatic shift is an important adaptation process in biochemistry. Therefore, the expression pattern of Ngb and Cygb as well as their protein roles in brain during a chronic systemic hypoxia condition (CSH) remain unclear.
This study aim to analyse the differences of the Ngb and Cygb expression patterns, and correlation of both protein to apoptosis in chronic systemic hypoxic condition. Ngb, Cygb, Cytochrome c, MDA, GSH, and HIF-1 . were examined. An in vivo experimental model of CSH was carried out using rat. The experimental rats were randomly divided into 6 treatment groups, i.e. group I was a control group or without hypoxic condition, groups II, III, IV, V, and VI were treated by hypoxic condition for 1, 3, 5, 7, and 14 days, respectively.
The Ngb and Cygb expressions were analysed using real time-RTPCR, ELISA, immunofluorescence with FITC, and the measurement of stress oxidative biomarkers, included HIF-1 as a biomarker of hypoxic condition and cytochrome c as a biomarker of apoptosis. The CSH was increased the mRNA expression of Ngb at 3, 5, and 7 days hypoxic groups, while the protein expression was decreased at 1, 3, 5, 7, and 14 days hypoxic groups compared to control group. The mRNA expression of Cygb was decreased at 1, 3, 5, 7, and 14 days hypoxic groups, whereas the Cygb protein expression was increased at 1, 3, 5, 7, and 14 days hypoxic groups compared to control group.
The correlation between Ngb with cytochrome c was weakly statistically insignificant, and Cygb with cytochrome c was statistically insignificant. The CSH induced the HIFl, which was shown by a high increase at 7 days hypoxic group, as well as stress oxidative which was represented by MDA at 1, 3, and 5 days hypoxic groups, and decreased GSH at 1, 3, and 5 days hypoxic groups. There are differences in expression pattern of Ngb and Cygb in CSH. The expression of Ngb, as an adaptive response, occurs earlier and is more influenced by the duration of hypoxic condition compared to Cygb. Although the correlation is weak, the Ngb seems more likely to inhibit apoptosis compared to Cygb protein.