Perubahan ekspresi protein hif 1 ca ix dan glutaminase pada ginjal tikus rattus norvegicus sebagai adaptasi terhadap induksi hipoksia normobarik sistemik kronik = Regulation of ca ix and glutaminase gene expression by hypoxia inducible factor 1 as response of rats rattus norvegicus kidney tissue on chronic systemic normobaric hypoxia

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Abstrak

ABSTRACT
Kondisi hipoksia menyebabkan stabilisasi HIF-1, yang mengatur ekspresi beberapa gen seperti Carbonic Anhydrase 9 (CA9). CA9 merupakan enzim yang memediasi homeostasis pH melalui reaksi reversibel CO2 dan H2O menjadi HCO3 - and H+.

Aktivitas enzim CA pada sel tubulus ginjal yang meningkat seiring dengan kondisi hipoksia menyebabkan peningkatan ion H+ dalam urin. Pada kondisi tersebut, sel tubulus ginjal akan mensekresikan NH3 (amonia) ke dalam cairan tubulus sebagai penyangga sehingga sekresi H+ dari sel tubulus dapat terus berlangsung. NH3 akan bereaksi dengan H+ membentuk NH4 + (amonium). NH3 dihasilkan dari deaminasi glutamin oleh enzim glutaminase yang disintesis di dalam sel tubulus. 25 tikus jantan Sprague Dawley (Rattus norvegicus L.) dibagi menjadi 5 kelompok. Sebanyak 20 tikus diinduksi dengan hipoksia (O2 10%) sebagai pemicu stabilisasi HIF-1 dan diobservasi selama 1, 3, 5, dan 7 hari pasca induksi. Kelompok kontrol tidak mendapat perlakuan induksi hipoksia. Semua tikus kemudian didekapitasi. Dari sampel ginjal, dilakukan pemeriksaan ekspresi mRNA HIF-1, CA9, dan Gls1 (dengan real time RT-PCR), protein HIF-1 (dengan ELISA) serta aktivitas enzim CA total dan glutaminase. Ekspresi tertinggi mRNA HIF-1, dan Gls1 dicapai pada hari ke-5 sedangkan ekspresi tertinggi mRNA CA9, dicapai pada 7 hari pasca induksi hipoksia. Konsentrasi protein HIF-1 sendiri tidak berbeda bermakna untuk semua kelompok. Aktivitas tertinggi enzim CA dan glutaminase dicapai pada kelompok 5 hari. Peningkatan mRNA dan aktivitas CA9 dan Gls1 pada kondisi hipoksia menunjukkan peran penting keduanya dalam menjaga homeostasis pH pada ginjal. Peningkatan mRNA CA9 dan Gls1 juga seiring dengan peningkatan mRNA HIF-1 yang menunjukkan bahwa ada korelasi positif antara HIF-1 dengan kedua gen tersebut.
<hr><i>ABSTRACT
Hypoxia can stabilize HIF-1, a protein that regulates many of genes involved in angiogenesis, erythropoiesis, glycolysis, iron metabolism, and cell survival. One of these genes is Carbonic Anhydrase IX (CA IX). CA IX is an enzyme which maintains pH homeostasis by converting CO2 and H2O into HCO3 - and H+ ions.

The activity of Carbonic Anhydrase in renal tubulus cell can cause an increase of H+ ion in urine. H+ ion must be buffered to prevent its gradient increase that can obstruct H+ secretion. In kidney, NH3 and H+ play an important role to form NH4 +, so secretion of H+ will be continued for pH homeostasis. Glutaminase function in conversion of glutamine into glutamate and NH3 was observed in this study. The samples were obtained from kidney tissues of rat exposed to chronic systemic hypoxia (O2 10% : N2 90%) for 1, 3, 5 and

7 days. Expression of HIF-1, CA9, and Gls1 mRNA were examined by real time RT-PCR. HIF-1 protein was measured using Cusabio® ELISA, as with the specific activity of CA and glutaminase were measured by spectrophotometer. The maximum levels of HIF-1 and Gls1 mRNA, were achieved in 5 days after hypoxia induction, meanwhile CA9 mRNA expression was found to be the highest at 7 days after induction. HIF-1 protein did not differ significantly among the groups. The maximum CA and glutaminase specific activity was measured at 5 days group. The increase of mRNA and specific activity of both CA and Gls1 in hypoxia shows that both of these protein have an important role for encountering the changing of pH in kidney, especially in the first 5 days. The significant increase of CA9 and Gls1 mRNA is also in line with the increase of HIF-1 mRNA. It can be concluded that expression of CA9 and Gls1 gene is regulated by HIF-1, although the HIF-1 protein have no difference among the groups.</i>