Pengaruh Asidifikasi pH Ekstraseluler PBMCs terhadap Stres Oksidatif dan Viabilitas Sel serta Kaitannya dengan Ekspresi mRNA CA9 dan HIF-1alfa = Effect of Extracellular pH Acidification of PBMCs on Oxidative Stress and Cell Viability: The Relationship With Expressions of CA9 and HIF-1alpha mRNA

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Abstrak

Latar Belakang: pH ekstraseluler (pHe) perlu dipertahankan dalam sel normal untuk menjalankan fungsi sel dengan baik. Adanya perubahan di lingkungan seluler meliputi asidifikasi akan berdampak pada fisiologi sel dan menginduksi kematian sel. Namun, studi terntang pengaruh asidifikasi pHe terhadap stres oksidatif dan regulasinya masih terbatas. Tujuan penelitian ini adalah menganalisis pengaruh asidifikasi pHe PBMCs terhadap stres oksidatif dan viabilitas serta kaitannya dengan ekspresi mRNA CA9 dan HIF-1alfa.
Metode: PBMC dikultur dengan berbagai pH medium selama 24, 48, dan 72 jam. pH medium kultur diatur menjadi pH 7,4, 7,2, 7,0, dan 6,6 menggunakan 0,01 HCl. Viabilitas sel dihitung menggunakan Trypan blue. Kadar ROS diukur menggunakan DHE dan DCFH-DA probes. Ekspresi mRNA HIF-1alfa, CA9, dan MnSOD dianalisis menggunakan qRT-PCR. Aktivitas spesifik MnSOD dianalisis menggunakan RanSOD Kit dan aktivitas CAT juga dianalisis. Konsentrasi MDA diukur menggunaan metode Wills.
Hasil: pHe meningkat secara bertahap pada waktu inkubasi 24, 48, dan 72 jam. Kadar ROS dan ekspresi mRNA HIF-1alfa, CA9, dan MnSOD meningkat, sementara aktivitas MnSOD menurun dan CAT meningkat. Konsentrasi MDA meningkat dan berdampak pada penurunan viabilitas sel.
Kesimpulan: Asidifikasi pHe PBMCs berdampak pada peningkatan stres oksidatif dan penurunan viabilitas sel. Selain itu, respons pada mRNA CA9 dan HIF-1alfa masih cukup baik.
.....Background: The extracellular pH (pHe) needs to be maintained in normal cells to carry out cell functions properly. Changes in the cellular environment including acidification affect to cell physiology and induce cell death. However, studies about effect of pHe acidification on oxidative stress and its regulation are still limited. This study aimed to analyze the effect of pHe acidification of PBMCs on oxidative stress and cell viability with expressions of CA9 and HIF-1alpha mRNA.

Methods: PBMCs were cultured with various pH medium for 24-, 48-, and 72-h. The pH of culture medium was adjusted to pH 7.4, 7.2, 7.0, and 6.6 by using 0.01 M HCl. Cell viability was calculated using trypan blue. ROS levels was measured using DHE and DCFH-DA probes. HIF-1alpha, CA9, dan MnSOD mRNA expressions were analyzed using qRT-PCR. MnSOD spesific activity was analyzed using RanSOD Kit and CAT acitivity was analyzed. MDA concentration was measured by Wilss method.

Results: pHe increased gradually at 24-, 48-, and 72-h incubation. ROS levels and HIF-1alpha, CA9, MnSOD mRNA expressions were increased, while the MnSOD spesific activity decreased and CAT activity increased. MDA concentration incease and had an impact on decreasing cell viability.

Conclusions: pHe acidification increased oxidative stress levels and decreased cell viability. In additon,

PBMCs had a response to of CA9 and HIF-1alpha mRNA.