## Abstract

The changes in homeostasis $Ca^{2+}$ intracellular was one of the basic mechanisms of vascular endothelial dysfunction, which has an important role in the development of vascular complication of diabetes mellitus. The objective of this study was to explain the influence of $H_2O_2$, lipid peroxide and the degree of mitochondrial respiration on basal $Ca^{2+}$ intracellular concentration ([$Ca^{2+}$] in vascular endothelial cells exposed to high glucose concentration.

The study was done in cultured human umbilical vein endothelial cells (HUVECs) exposed to normal (5mM/L) and high d-glucose concentration (11, 22 and 33 mM/L) for 3,5,7 and 9 days. [$Ca^{2+}$]i was measured using the $Ca^{2+}$ probe Fura 2AM and observed by spectroflurometric and optical method. $H_2O_2$ was determined using spectrofluorometric, whilst lipid peroxide (MDA) was measured using spectrophotometric. The level of mitochondrial respiration was measured using spectrophotometric. In addition, we also examined duration of the decreasing phase of [$Ca^{2+}$] transient evoked by ATP with a supramaximal concentration, the cell density and the cellular structure.

The result showed that [$Ca^{2+}$]i, $H_2O_2$, and MDA in cells exposed to high glucose for 3,5,7 and 9 days, respectively were significantly increased, whereas the level of mitochondrial respiration was significantly decreased. The analytic results of simultaneous equivalent system showed that $H_2O_2$ had a significant positive role toward basal [$Ca^{2+}$]i during the exposure of high glucose for 3,5, and 7 days respectively. MDA had a significant positive role toward basal [$Ca^{2+}$]i during the exposure of high glucose for 3 days. The level of mitochondrial respiration had a significant negative role toward basal [$Ca^{2+}$]i during the exposure of high glucose for 5 days, but there was a significant positive role during 9 days exposed to high glucose. The exposure of cells to glucose 22 mM/L for 9 days resulted in the prolonged of decreasing phase of ATP-induced $Ca^{2+}$ signaling. The conditions was not showed in cells exposed to glucose 22 mM/L for 3,5, and 7 days. There was a decrease in the density of cultures exposed to glucose 33 mM/L for 7,14 and 21 days. The exposure of cells to glucose 22 and 33 mM/L for 9 days resulted in the changes in the cellular structure.

In conclusion, the increase of basal [$Ca^{2+}$]i in vascular endothelial cells exposed to high glucose for 3,5,7 and 9 days are caused by the roles of $H_2O_2$ and the level of mitochondrial respiration, while the role of lipid peroxide is seen in glucose exposure for 3 days merely. There is a strong suggestion that the high glucose exposure for 9 days alters the homeostasis of $Ca^{2+}$ intracellular, the production of $H_2O_2$ and the synthesis of ATP, which is followed by changing in cellular structure, thus it gives the disadvantages of cellular prognosis.


Key words: endothelial cells - glucose - [$Ca^{2+}$]i - $H_2O_2$ - lipid peroxide - ATP