Alpha-Lipoic Acid, But Not Dihydrolipoic Acid, Activates Nrf2 Pathway in TNF-α-Challenged HUVECs

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Endothelial dysfunction, the shift from a healthy endothelium to a generalized damaged phenotype, is an early event in many pathologies including atherosclerosis, hypertension, diabetes, and hyperlipidemia. Oxidative stress and inflammation are considered among the prominent pathways of vascular endothelial dysfunction. Leukocyte adhesion and migration to the subendothelium, in response to chemoattractants and other activating molecules, is mediated by adhesion molecules expressed on endothelial cells. It has been reported that induction of endothelial adhesion molecules by inflammatory cytokines, such as tumor necrosis factor α (TNF-α), depends on activation of the transcription factor nuclear factor kB (NF-kB). In fact, agents that block NF-kB signaling and, hence, adhesion molecule expression and leukocyte–endothelial interactions in vitro also exert marked effects on inflammatory responses in vivo. Alpha-Lipoic acid (ALA) is a sulfur-containing coenzyme required for the mitochondrial dehydrogenase reactions leading to ATP formation. LA is readily taken up by a variety of cells and tissues and is reduced in mitochondria to the potent antioxidant dihydrolipoic acid (DHLA). Due to their antioxidant properties ALA and DHLA have been recently proposed as pleiotropic compounds with potential therapeutic use in many inflammatory diseases. In this study, we investigated the in vitro effect of physiological concentrations of ALA and DHLA (250-500 nM) against endothelial dysfunction induced by TNF-alpha in human umbilical vessel endothelial cells (HUVECs). Our data show that ALA, but not DHLA, inhibits TNF-α-induced activation of NF-kB pathway, as observed by reduced p65 nuclear levels and cytoplasmic IkB-α phosphorylation. At the same extent, only ALA is able to reduce, in a dose-dependent way, the TNF-α-induced downstream expression of the endothelial adhesion molecules ICAM-1 and E-selectin. Part of these effects can be explained by the observed specific stimulatory activity on Nrf2 pathway. Nrf2 is the master of redox homeostasis, acting as regulator or inducer for the expression of several antioxidants and cytoprotective genes. In fact, ALA appeared able to activate a cellular adaptive response by inducing, in a dose-dependent way, Nrf2 nuclear translocation and its nuclear targets, such as HO-1 and NQO-1, at baseline and after TNF-α treatment. On the contrary, DHLA failed to induce Nrf2 nuclear translocation and its transcriptional activity. Furthermore, ALA, but not DHLA, is able to reduce TNF-α-induced caspase-3 activation (a marker of cellular apoptosis) in a dose dependent way. Interestingly these effects were observed both when HUVECs were treated with ALA and DHLA for 2 hrs before or after TNF-α-induced exposure.

This data support the hypothesis that ALA could give rise to a “hormetic” phenomenon where Nrf2 and NF-κB pathways are surely involved. Therefore, ALA could have a role in protection against vascular diseases. Interestingly, for the first time, we demonstrated that DHLA, the physiological reduced form of LA, fails to induce endothelial protection; however, further studies will be necessary in order to improve our understanding of the observed effects.