ABSTRACT

Advanced glycation end products (AGEs) of proteins and lipids once formed in the body, persist in the circulation, thus interact with the cells all the time and cause micro and macro-vascular pathologies. Hemodynamic force is another important determinant of endothelial cell functions that plays an active role in many physiological and pathophysiological conditions. Focus of the present study was to investigate the interaction of disturbed flow simulated by orbital shear stress (OSS) and metabolic stress simulated by advanced glycation end products of human serum albumin (AGE-HSA) in modulating certain aspects of endothelial cell (EC) function pertaining to vascular health. ECs were isolated from human umbilical cord vein (HUVEC) and characterized by their typical cobblestone morphology and acetylated low-density lipoprotein 1,1'-dioctadecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate (DIL-Ac-LDL) uptake. AGE-HSA was synthesized in-vitro by incubating human serum albumin with three different concentrations of glucose, equivalent to normo-glycemic, moderately-hyperglycemic and poorly controlled hyperglycemic conditions, for 14 weeks. Extent of glycation was found to be a function of glucose concentration, confirmed by fluorescence spectroscopy and MALDI-TOF methods. AGE-HSA was found to modulate EC functions by altering NO synthase activity and concentration of receptors of AGEs (RAGE and galectin-3). Additionally it affects the expression of a characteristic endothelial derived molecule, ESM-1. It was also found to govern the expression of sICAM-1 and MMP-9. Generally higher level of OSS together with higher extent of glycation showed more prominent effect on the inflammatory mediators. Expression of RAGE on the other hand was up-regulated at lower OSS possibly due to the enhanced chance of AGE-RAGE interaction at low shear stress. This is consistent with the theory correlating low
oscillatory shear stress with increased atherogenic potential. Glucose even at a higher concentration did not display significant change in the expression of inflammatory proteins in present experimental set-up.

AGE-HSA and OSS both exerted a modulating role in the expression of ESM-1 at transcriptional and translational level in a dose dependent manner. AGE-induced expression of ESM-1 is regulated by the AGE receptors (RAGE and galectin-3), confirmed through gene silencing study. RAGE was found to be positively linked with ESM-1 expression whereas galectin-3 exhibits negative correlation. Mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinases (JNK) signaling pathways were not found to be involved in the regulation of ESM-1 expression whereas phosphatidylinositol 3,4,5 triphosphate kinase (PI3K) pathway regulated ESM-1 expression negatively.

S-allyl cysteine sulphoxide (SACSO) or alliin, the main sulphur containing bioactive constituent of garlic, has anti-diabetic property. We demonstrate that it has ameliorating effect on the AGE and OSS activated inflammatory condition in HUVECs. It affects two of the AGE receptors in different ways, down-regulating AGE induced RAGE expression while up-regulating AGE induced expression of galectin-3. Inhibition of RAGE expression by SACSO indicates the protective role of SACSO under metabolic stress condition which possibly derives from reduction in AGE-RAGE interaction and its downstream pro-inflammatory effects. On the other hand, up-regulation of galectin-3 expression by SACSO protects AGE induced tissue damage by binding it and thus reducing AGE availability for RAGE. Availability is also reduced by degradation of galectin bound AGEs through endocytosis. SACSO also inhibits AGE-HSA induced sICAM-1 and ESM-1 expression while increasing NO synthase activity, that supports its
anti-inflammatory and anti-diabetic role. Partial restoration of NO synthase activity indicates improvement in the vasoactive function of endothelium. The inhibitory effects of SACSO on the expression of pro-inflammatory molecules may be via the regulation of AGE-RAGE interaction. No obvious toxic effect was observed in HUVECs, however further studies are needed to confirm the safety and quality before using it as therapeutic agent.