1.1. Short summary

Diabetic complications are the leading cause of morbidity and mortality in diabetic patients. Oxidative stress and inflammation contributes to the loss of endothelial function and dysfunction of the vascular endothelium plays a major role in diabetic macrovascular and microvascular complications. We found that hydrogen sulfide can reduce the hyperglycemia-induced mitochondrial reactive oxygen species (ROS) production in endothelial cells and can prevent diabetic vascular dysfunction in aortic rings. Hydrogen sulfide is immediately released from its commonly used inorganic salts and its half-life is short *in vivo,* thus its protective effect is difficult to test against long-term diabetic complications and long-term clinical use of inorganic hydrogen sulfide donors is not plausible.

We generated novel slow-release hydrogen sulfide donors that release hydrogen sulfide at a constant rate over multiple days. Mitochondrial targeting moiety was incorporated to provide specific delivery of hydrogen sulfide since mitochondrial superoxide generation is a key pathogenic step in hyperglycemia-induced endothelial dysfunction. We found that the novel mitochondrial slow-release hydrogen sulfide donor compounds were effective against glucose-induced mitochondrial reactive oxygen species production at >1000-fold lower concentration than sodium sulfide. The novel hydrogen sulfide donor drugs normalize the mitochondrial membrane potential, improve the metabolic dysfunction and exert positive effect on cellular bioenergetics in hyperglycemic endothelial cells. We also confirmed that the mitochondrial sulfide donors increase the respiratory complex II/III activity in isolated mitochondria. The mitochondria-specific hydrogen sulfide release, the activity increase of respiratory complexes II and III, the normalization of the mitochondrial membrane potential and the increased ATP production suggest that positive effect of hydrogen sulfide supplementation in associated with direct electron supplementation to the respiratory chain via hydrogen sulfide metabolism in hyperglycemic endothelial cells. The beneficial effects of mitochondrial hydrogen sulfide donors in diabetes models also initiated formal toxicology studies that confirmed the regulated release of hydrogen sulfide and tolerability of the drugs.

The mechanism of action of hydrogen sulfide against mitochondrial ROS generation suggests that slow-release hydrogen sulfide donors may be applicable against diabetic complications in humans. These studies greatly contribute to clinical translation and the novel promising drugs may represent valuable treatment options in human diabetic complications in the future.