Title:

A combination of fenofibrate and mirabegron improves redox conditions in coronary artery endothelial cells under oxidative stress.

Aims:

To assess the effect of fenofibrate and mirabegron on s-glutathionylation of endothelial nitric oxide synthase, a redox-sensitive reaction that is shown to be caused by hyperglycaemia.

Methods:

Human coronary artery endothelial cells were seeded into a 96-well plate. Cells were cultured in 5mM glucose or 25mM glucose for 48 hours to mimic normal and diabetic conditions, respectively. 25mM mannitol was used as an osmotic control. 15 minutes after seeding, treatments were added to triplicate wells. These treatments were 50μM fenofibric acid, 1μM mirabegron, 10μM mirabegron and in combination. 500μM diamide was used as a treatment mimicking extreme oxidative stress conditions for 1 hour before fixing. Cells were stained using goat anti-human eNOS and mouse anti-glutathione with respective Alexa Fluor secondary antibodies. Immunofluorescence was imaged at 63X magnification using the Opera Phenix confocal microscope.

Results:

After 48 hours, high glucose shows increased s-glutathionylation compared to normal glucose. Mirabegron shows decreased s-glutathionylation back to normal levels in high glucose-treated cells. In the diamide-treated wells, all drugs decreased s-glutathionylation, foreshadowing redox-protective mechanisms. A combination of 50μM fenofibrate and 1μM Mirabegron shows the greatest reduction of s-glutathionylation in diamide treated cells. All samples displayed a weak positive Pearson’s correlation coefficient, suggesting that other proteins are potentially s-glutathionylated.

Conclusion:

Our findings suggest that mirabegron and fenofibrate may be drug candidates for vascular conditions underpinned by oxidative stress, like diabetic foot ulcers and coronary and peripheral arterial disease.