**DIFFERENT EFFECT OF HYDROGEN SULFIDE DONORS AP39 AND AP123 IN ENDOTHELIAL CELLS GROWN IN HYPERGLYCAEMIC ENVIRONMENT**

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Diabetes represents a major disease associated to severe vascular complications, such as atherosclerosis and other inflammatory-based conditions, that converge in hypertension [1-4]. The mechanism underlying these events have been widely disclosed and involve, among other factors, endothelial nitric oxide synthase (eNOS). In particular, eNOS activity is suppressed in hyperglycaemic conditions, resulting in a reduced NO bioavailability. This change is coupled with reduced levels of eNOS and/or increased levels of caveolin-1 (Cav-1), a protein that negatively regulates eNOS function, and to reduced eNOS phosphorylation (p-eNOS) [5,6]. In addition, diabetic conditions are also linked to reduced circulating hydrogen sulfide (H2S) levels [7], further undermining vascular homeostasis with respect to endogenous vasorelaxant response [8] and suggesting that diabetes is a condition of "H2S deficiency" [9]. Here, we wanted to test whether the novel H2S donor molecules, AP123 and AP39 [10,11], could affect the changes to NO-signalling observed in diabetic conditions.

For this purpose, we used an established model of in vitro hyperglycaemia, where bovine aortic endothelial cells (BAEC) were grown in high glucose (HG, 50mM) environment for 3h [5,6]. AP123 and AP39 were added at same time as HG induction (time 0) or 1h later (time 1). After incubation, cells were challenged with calcium ionophore A23187 (1μM, 30min), to stimulate eNOS activation, and then collected to perform western blot analysis. Supernatants were used for fluorometric evaluation of nitrite/nitrate (NOx) levels.

Incubation of BAEC with AP39 or AP123 (3h, 0.1-1μM) restored NOx levels in a similar fashion and independently from administration time. However, the results obtained following concentration-response experiments at time 1, showed that AP39 and AP123 affetced NOx concentration in a different manner. AP39 induced an "on/off-like" effect, while AP123 modulated NOx levels in a concentration-dependent manner. Western blot analysis showed that AP39 was unable to restore physiological activation (peNOS) and expression of eNOS, altered by HG conditions. Conversely, reduction in eNOS activation showed as peNOS and expression of eNOS were positively modulated by AP123.

Overall, these data highlight that AP39 and AP123 modulate NOx levels in a different manner, being AP123 more efficient in restoring eNOS/NO signalling in hyperglycaemic conditions. Although preliminary, these studies suggest that H2S is a crucial vasculoprotective mediator in diabetic vasculature and that H2S-releasing molecules may be useful in counteracting the detrimental effects to the vasculature of "H2S deficiency" and high glucose environment, typical of diabetic state. In addition, it is feasible that different donors can possibly supply H2S through diverse mechanisms and therefore triggering different pathways.

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