Topically applied S-nitrosoglutathione-containing hydrogel for transdermal nitric oxide release

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Statement of Purpose: Nitric Oxide (NO) plays an important role in the control of blood pressure and regulation of vasomotor tone. S-nitrosothiols, like S-nitrosoglutathione (GSNO), are potent endogenous vasodilators. GSNO stores and transports NO within the body and can also be used as an exogenous source of NO. Endothelial dysfunction is characterized by impaired NO production leading to vasoconstriction and complications in the micro and macro circulation, which are characteristic of diabetic conditions. The aims of this work were to characterize the local vasodilator effect of a GSNO-containing hydrogel topically applied on healthy human skin and on the skin of diabetic animals.

Methods:. GSNO was synthesized by reacting glutathione (Sigma, St. Louis, MO, USA) with equimolar sodium nitrite ($NaNO_2$) (Aldrich, St. Louis, MO, USA) in aqueous solution. GSNO was incorporated in Pluronic F-127 hydrogels ((poly(ethylene oxide)$_{99}$-poly(propylene oxide)$_{65}$-poly(ethylene oxide)$_{99}$, MW ~ 12,0000, ICI corporation, USA), under an ice bath. At physiological temperature, GSNO-containing Pluronic F-127 undergoes a gelation process leading to a hydrogel. **Human volunteers**: 20 µL of GSNO containing-hydrogel was applied on the forearm skin of eight subjects. Local blood flow was measured by laser Doppler for three hours following application of the hydrogel. Laser Doppler probes were secured to the overlying skin to measure cutaneous blood flow and dermal microdialysis catheters were simultaneously used to measure NO concentration within the dermis. Dialysate samples were analyzed for nitrite concentration by reduction of nitrite to NO, which was measured by chemiluminescence. **Animal model**: GSNO-containing hydrogel was applied to the foot sole skin of streptozotocin (STZ)-induced diabetic Wistar rats and non-diabetic rats (as controls). Local blood flow in the treated skin area was measured by Laser Doppler flowmetry.

Results/Discussion:. **Human volunteers**: Fig. 1 shows the correlation between dermal blood flow values after application of Pluronic F-127 containing GSNO (0.3mol per gram of hydrogel) on the skin of healthy volunteers, with nitrite concentration measured in the dialysate. The results show that the maximum blood flow was achieved within 30 min and returned to the basal level after three hours. The blood flow values correlate directly with dermal nitrite levels (the oxidation product of NO) measured in the dialysate. Fig. 1 shows that topical application of GSNO-containing Pluronic F-127 produces a consistent, sustained, and biologically effective release of NO on human skin *in vivo*.

Animal model: Fig. 2 shows the changes in blood flow values due to the topical application of GSNO-containing Pluronic F-127 (0.23 mol per gram of hydrogel): in (A) control and (B) diabetic animals. The changes in blood flow values were equal for either control or diabetic animals. In both cases, GSNO application increased cutaneous blood flow within 30 minutes. These results show that transdermally applied GSNO can reverse the abnormal constrictor tone in diseased vessels in this animal model of diabetes, in which the endogenous NO synthesis is impaired.

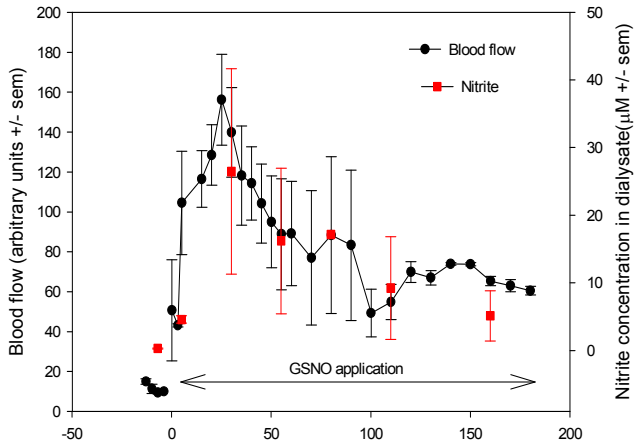

Fig 1. Correlation between blood flow and nitrite concentration measured in the dialysate.

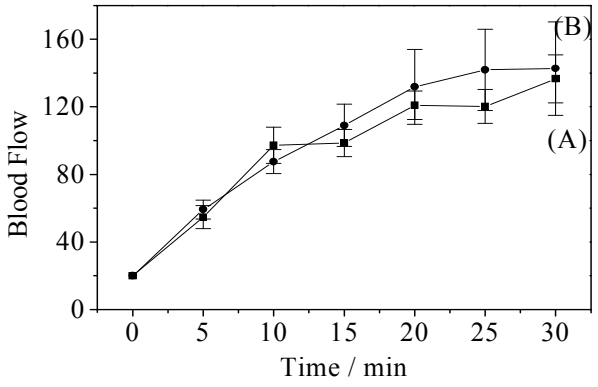

Fig 2. Blood flow after topical application of hydrogel containing GSNO in foot sole skin: (A) control animals, (B) diabetic animals.

Conclusions: Hydrogels containing GSNO expressively increase the local blood flow in healthy human skin and in both normal and diabetic animals. This effect offers perspectives for promoting vasodilation through local NO release in the treatment of microcirculatory disorders.

References: Seabra AB, Fiztpatrck A, Paul J, Weller R, de Oliveira MG., Brit J Dermatol (2004) 151:977.