**Nitrite-mediated Vasodilation Quantified from *In Vivo* Studies in Rat Mesentery**

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**Introduction:** Nitric oxide (NO) generated from nitrite through red blood cell nitrite reductase activity is proposed to play a major role in hypoxic vasodilation [1]. However, our mathematical model [2] predicts that much more NO can be derived from nitrite reductase activity in tissue. *In vitro* studies using heart and liver samples or isolated blood vessels [3-5] report greater nitrite reductase activity in tissue under acidic and hypoxic conditions that is associated with xanthine oxidase and/or aldehyde oxidase.

**Methods:** *In vivo* exteriorized rat mesentery experiments under isoflurane anesthesia were conducted to measure perivascular NO or PO2 with microelectrodes, arteriolar diameter (D) from video imaging, tissue perfusion by laser Doppler (LDF), and small artery blood flow using an ultrasonic flow probe (~270 micron diameter). All physiological signals were sampled at 10 Hz using a computer-controlled data acquisition system. Vasodilation in response to nitrite in the superfusion medium bathing the mesentery equilibrated with 5% O2 (normoxia) or zero O2 (hypoxia) at either normal or acidic pH was quantified. Experiments were also conducted following intraperitoneal (IP) injection of nitrite. Reponses to IP nitrite were repeated for the same arterioles after delivering allopurinol to inhibit xanthine oxidase in some animals or raloxifene to inhibit aldehyde oxidase in other animals.

**Results and Discussion:** The largest vascular responses were found with nitrite > 10 mM in the superfusion solution. Arteriolar blood flow, calculated from the Hagen-Poiseuille equation for laminar flow in a cylindrical vessel, doubled for some arterioles. However, no significant differences in average responses to superfused nitrite under hypoxic and acidic conditions (pH = 6.6-6.7) were found compared to hypoxic conditions with pH = 7.4. Attenuated responses were observed after inhibiting xanthine oxidase with allopurinol (average dose 4.2 mg/kg IP). Nitrite-mediated vasodilation was reduced or abolished after inhibiting xanthine oxidase (*p*<0.001). Increases in small artery blood flow and LDF after low O2 + nitrite were also reduced or eliminated after allopurinol. Similar results were observed for responses to nitrite delivered by IP injection (3 to 9 mg/kg), which were attenuated after allopurinol. Results to IP nitrite injection before and after inhibiting aldehyde oxidase with raloxifene were not as consistent, although a reduction in vasodilation was observed for some experiments.

**Conclusions:** Our results provide further evidence for a role of tissue nitrite reductases in nitrite-mediated hypoxic vasodilation. However, high nitrite concentrations (> 1 mM) were needed in the superfusion solution, much greater than used for intra-arterial infusions in humans (up to 7.8 M/min), which demonstrate greater vasodilation under hypoxia than normoxia [6].

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