Dipping Our Toes In The Hot Springs: Protocol Development For Capturing The Endogenous Gasotransmitter Hydrogen Sulfide In Human Plasma

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BACKGROUND

Gasotransmitters are endogenously made, biologically active gases with unique physiologic properties. While nitric oxide is well known to the medical community the gases carbon monoxide and hydrogen sulfide have only more recently been appreciated as agents purposefully synthesized by an organism for physiologic use. In addition to participation in the hypoxic respiratory reflex of the carotid body it is thought that these gases may also play a role in more localized vasodilatory hypoxic tissue responses. While there is increasingly robust biochemical work in the field of endogenous hydrogen sulfide analysis little has been published addressing translational concerns for bringing these techniques into the human subject and hospital environment. This pilot project describes a method for hydrogen sulfide gas capture in human plasma utilizing the fluorescent trapping agent dansyl azide.

MATERIALS & METHODS

Under an IRB approved pilot project ten healthy male volunteers spontaneously ventilated room air, hypoxic (15% oxygen, 85% nitrogen), and hyperoxic (100%) gas mixtures via a non-rebreather system. Venous whole blood samples were collected at both internal jugular and antecubital sites following seven minutes of exposure to the tested oxygen environments. Resultant plasma aliquots were treated with dansyl azide and submitted to fluorescence reading (excitation 340nm, emission 517nm).

RESULTS

Fluorescent intensity of plasma samples significantly increased upon hypoxic exposure from baseline room air values; this was followed by a rapid return to near baseline values upon subsequent application of hyperoxic gas (Figure 1). Compiled mean data with matched t-Test from volunteer plasma samples demonstrated statistically significant findings ($p<0.05$) in measurement of increased fluorescent intensity between those samples collected under mildly hypoxic conditions compared to normoxic and hyperoxic samples submitted to the same laboratory criteria (Table 1).

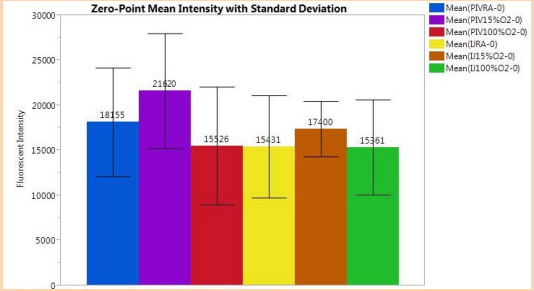

Figure 1: Compiled fluorescent intensity means from data of all subjects meeting inclusion criteria. (PIV: antecubital collection site, IJ: internal jugular collection site, RA: room air, 15%: 15%O_2/85%N_2, 100%: 100%O_2)

	p-values from Zero-Point Means Data		
	RA-PIV&IJ	15%O_2-PIV&IJ	100%O_2-PIV&IJ
RA-PIV&IJ		0.007	0.013
15%O_2-PIV&IJ	0.005		0.001
100%O_2-PIV&IJ	0.036	0.002	
	p-values from Internal Standard Means Data		

**p<0.05 shaded in purple*

Table 1: Combined t-Test by oxygen exposure

CONCLUSION

In order to study the role of hydrogen sulfide as a hypoxic responder in humans a reliable, robust, and safe protocol amenable to standard hospital laboratory procedures is needed. Through modification to methodologies described in the biochemistry literature this pilot project demonstrates the feasibility of utilizing a fluorescent hydrogen sulfide gas trapping agent for assessment of hypoxic response in humans within the confines of a typical clinical collection and analysis environment.

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