The Effects of Acute or Chronic Hydrogen Sulfide on Human Pulmonary Fibroblasts and HEK-293 Cells.

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Hydrogen sulfide (H$_2$S) is a gasotransmitter that modulates various biological functions through complex mechanisms which may involve Transient Receptor Potential (TRP) channels such as TRPA1. TRPA1 is postulated to play a prominent role in pulmonary inflammation and airway hypersensitivity (1). H$_2$S also modulates cell growth and survival, with high concentrations leading to reactive airway disease, acute respiratory failure and pulmonary fibrosis (2). The latter is a condition characterized by fibroblast accumulation, excess collagen deposition, and matrix remodelling that leads to progressive decline in lung function. N-Acetylcysteine (NAC) is a thiol precursor of L-Cysteine and Glutathione, which elicits antioxidant effects and has been implicated in preventing the progression of pulmonary fibrosis (3). The aim of the study was to examine the effects of Sodium Hydrosulfide (NaHS) an H$_2$S donor on cell growth.

Untransfected human pulmonary fibroblasts, and HEK-293 cells transfected with either TRPA1 (TRPA1-HEK) or an empty vector (EV-HEK), were seeded onto twelve or 24-well plates. Cells were treated with either a 30-minute acute application of NaHS (dissolved in water at concentrations of 1µM – 10mM) or chronic exposure to NaHS at these concentrations. Alternatively cells were treated for 1.5 hours with 100nM – 10mM NAC. Cells were then allowed to grow for 5 days in DMEM with 10% FCS, subsequently fixed in situ, stained and analysed as per the Sulforhodamine B colorimetric assay (4).

TRPA1-HEK or EV-HEK cells exhibited a significant reduction in growth on day 5 when chronically treated with 10mM NaHS (P<0.0001 n= 3 separate experiments each with up to 3 repeats; one-way ANOVA with Dunnett’s test). The NaHS IC$_{50}$ for TRPA1-HEK cells was 4.1mM, but 3.7mM in EV-HEK. Similarly acute treatment with 10mM NaHS significantly reduced growth on day 5 of TRPA1-HEK cells (P<0.0001; n= 3, statistical analysis as previously) and pulmonary fibroblasts (≥3 mM, P<0.001; n= 5). A 1.5 hour exposure to 1mM NAC also induced a significant reduction in growth of pulmonary fibroblasts at day 5 (P=0.01; n=3, one-way ANOVA with Dunnett’s test). In contrast, there was no significant effect on growth of 100nM to 10mM NAC on TRPA1-HEK cells (P=0.72; n= 3).

These results indicate that chronic treatment with high concentrations of NaHS attenuates cell growth in HEK-293 cells transfected with or without TRPA1. Interestingly, this effect on growth is also observed following a brief 30min exposure to NaHS in both TRPA1-HEK and cultured primary human pulmonary fibroblasts. The fibroblasts appeared more sensitive to the effects of NaHS; this seems to contradict the in vivo pro-fibrotic effects of H$_2$S (5). The pulmonary fibroblasts also exhibited growth inhibition by 1mM NAC; this is consistent with studies on hepatic fibroblasts, and provides in vitro evidence for the potential anti-fibrotic actions of NAC in the lung (6). The effect of acute NaHS treatment on cell growth is a novel
finding that requires further investigation to elucidate the mechanism of action. This could potentially provide a better understanding of the effects of H₂S in modulating the progression from inflammation to fibrosis.

(1) Bautista DM et al. (2013). *Annu Rev Physiol.* **75**:181-200


