

INHIBITION OF VASCULAR SMOOTH MUSCLE CELL PROLIFERATION BY S-NITROSOGLUTATHIONE IS REVERSED BY THE ADDITION OF ASCORBIC ACID

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Introduction: Endogenous S-nitrosothiols release nitric oxide (NO) following a catalytic reaction with ascorbic acid (AA). NO has been shown to inhibit vascular smooth muscle cell (VSMC) proliferation. The purpose of this study was to evaluate the ability of AA to potentiate NO release through reaction with the S-nitrosothiol S-nitrosoglutathione (GSNO). We hypothesized that AA would potentiate the antiproliferative effects of GSNO due to increased NO production.

Methods: VSMC harvested from male Sprague Dawley rats were treated with increasing concentrations of AA, GSNO, or a combination of the two. NO release and proliferation were measured after 24 hours. NO release was quantified using the Greiss reaction. Proliferation was measured using tritiated-thymidine incorporation.

Results: In VSMC exposed to AA alone, NO did not increase above control levels. In VSMC exposed to GSNO alone, NO increased in a dose-dependent manner (0.125-0.5mM, $p < 0.05$). In VSMC exposed to a combination of AA and GSNO, NO release increased in a synergistic manner and was 3-fold greater than GSNO alone ($p < 0.05$) for all combinations tested. Proliferation of VSMC was inhibited by GSNO in a dose-dependent manner (90% reduction versus control at 0.5mM, $p < 0.05$). Interestingly, dose-dependent inhibition of VSMC was also observed after exposure to AA alone (40% reduction at 2.0 mM, $p < 0.05$), suggesting an NO-independent antiproliferative effect. However, the inhibitory effect of GSNO on proliferation was reversed when combined with AA (5% reduction versus control, $p = NS$).

Conclusions: VSMC proliferation is inhibited after exposure to either the NO donor GSNO or its catalyst for NO production, AA. Although NO production from cells exposed to GSNO was significantly augmented by the addition of AA, the addition of AA reversed the inhibitory effect of GSNO on VSMC proliferation. These observations suggest that the mechanisms by which GSNO and AA inhibit proliferation are distinctly different and require further study.