Nitric Oxide Decreases the Expression and Activity of the Ubiquitin-Conjugating Enzyme UbcH10

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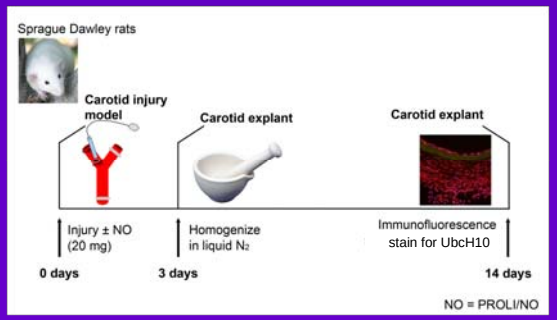
Background:

- Nitric oxide (NO) has been shown to limit the formation of neointimal hyperplasia in animal models of arterial injury.
- NO is also known to arrest vascular smooth muscle cells (VSMC) in the G_0/G_1 phase of the cell cycle.
- The ubiquitin-proteasome pathway is responsible for the degradation of proteins that regulate cell cycle progression.
- Ubiquitination proceeds via formation of thioester bonds and NO can act to disrupt those bonds.
- Since UbcH10 is responsible for the degradation of cyclins A and B, we hypothesize that NO prevents VSMC proliferation, and hence neointimal hyperplasia, by decreasing the expression and/or activity of UbcH10.

Methods:

- VSMC were exposed to the NO donor diethylenetriamine NONOate (DETA/NO, 0 – 1 mM) or transfected with a plasmid bearing UbcH10.
- Proliferation was assessed by tritiated thymidine incorporation or MTT assay.
- UbcH10 activity was assayed using recombinant UbcH10, ubiquitin, and dipropylenetriamine NONOate (DPTA/NO).
- The carotid artery balloon injury model was performed in 11-week male Sprague Dawley rats. Treatment groups included injury or injury + proline NONOate (PROLI/NO, 20 mg). Controls were uninjured arteries.
- UbcH10 expression was assessed by:
  - Western blot analysis on homogenized arteries 3 days after injury (n = 3/group)
  - Immunofluorescent staining of carotid artery sections 14 days after injury (n = 3/group).



Results:

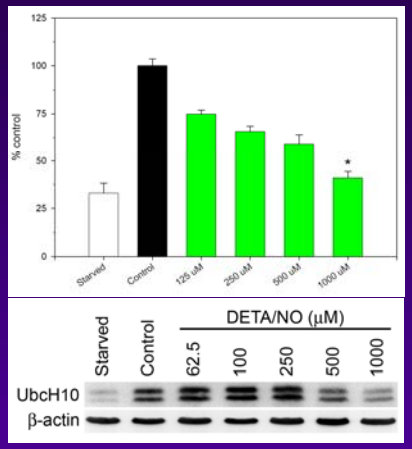


Figure 1. VSMC treated with the NO donor DETA/NO showed decreased expression of UbcH10. This was correlated with a decrease in VSMC proliferation. t = 24 hrs. *P<0.05.

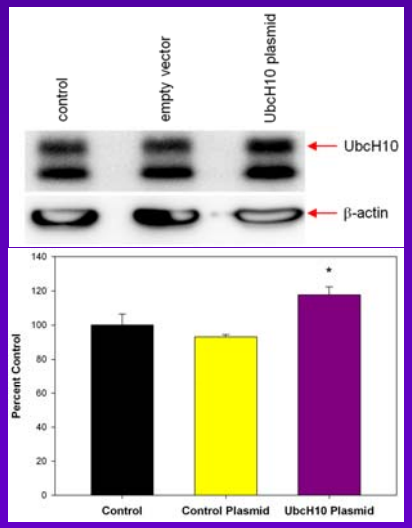


Figure 2. Tritiated thymidine incorporation assay showed increased proliferation in VSMC where UbcH10 had been overexpressed. *P=0.004, t = 48 hrs.

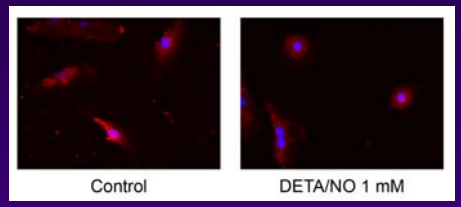


Figure 3. A distinct cytoplasmic staining pattern (red staining) was observed when VSMC were probed for UbcH10 using immunofluorescence (IF). NO treatment decreased this UbcH10 expression. Blue is DAPI staining. t = 24 hrs.

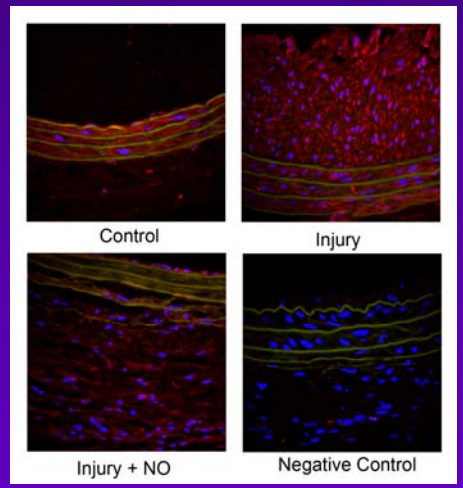


Figure 4. IF staining of balloon-injured rat carotid artery sections showed a marked increase in UbcH10 expression (red staining) after injury. This expression was reduced by treatment with the NO donor proline NONOate (PROLI/NO). Blue is DAPI staining, green is auto-fluorescence.

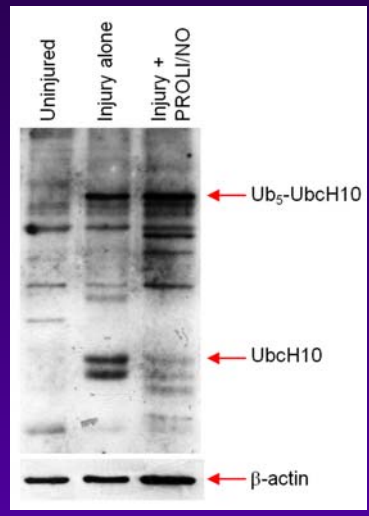


Figure 5. Western blot analysis of carotid artery lysates showed an increase in UbcH10 expression after balloon injury. This was reversed by administration of NO. Interestingly, NO induced an increase in ubiquitinated UbcH10 (Ub-UbcH10). t=3 days.

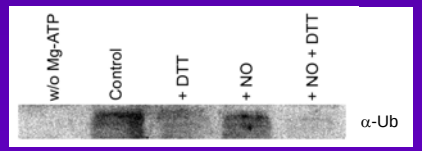


Figure 6. Cell-free activity assay showed that addition of DPTA/NO (0.5 mM) reduced formation of the thioester bond between ubiquitin and UbcH10. Addition of 5 mM dithiothreitol (DTT) also reduced thioester bond formation. t = 4 hrs.

Conclusion:

- Central to our hypothesis, we report that NO decreases the activity and expression of UbcH10 *in vitro*, and decreases the expression of UbcH10 following arterial injury *in vivo*. These changes in UbcH10 expression and activity correlate with VSMC proliferation and neointimal hyperplasia. Therefore, UbcH10 may be a promising therapeutic target for inhibiting neointimal hyperplasia.