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*MEETING ABSTRACT*

## A1.13

### Role of aldehyde dehydrogenase 2-catalyzed nitric oxide formation in nitroglycerin-induced vasorelaxation

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**Background:** The antianginal drug nitroglycerin (GTN) causes vasodilation through activation of soluble guanylate cyclase (sGC) by release of nitric oxide (NO) or a related species, resulting in accumulation of 3′,5′-cyclic guanosine monophosphate (cGMP) in vascular smooth muscle. In 2002, Chen *et al.* showed that aldehyde dehydrogenase-2 (ALDH2) catalyzes bioconversion of GTN to 1,2-glyceryl dinitrate (1,2-GDN) and nitrite [1]. Nitrite was proposed to be reduced to NO by components of the mitochondrial respiratory chain. However, we found that a minor pathway of ALDH2-catalyzed GTN bioconversion, accounting for about 5% of total turnover, results in direct formation of NO. Site-directed mutagenesis revealed that two vicinal cysteine residues adjacent to the catalytically active C302 are essential for the major nitrite pathway but not involved in GTN reduction to NO. Mutation of C301 and C303 to serine led to > 95% loss of 1,2-GDN formation but enhanced sGC activation and NO formation. It was the aim of the present study to test whether the direct NO formation that was observed with purified C301S/C303S ALDH2 explains GTN bioactivation in vascular smooth muscle.

**Methods:** Wild-type ALDH2 and the C301S/C303S mutant were overexpressed in murine ALDH2-deficient aortic smooth muscle cells by recombinant adenoviral vectors. Protein expression of wild-type and mutated ALDH2 was analyzed by western blot. Activation of purified sGC by transfected aortic smooth muscle cells was studied with increasing concentrations of GTN. GTN denitration was assayed as 1,2-GDN formation by thin-layer radio-chromatography.

**Results:** Adenoviral overexpression led to virtually identical protein expression levels of wild-type and mutated ALDH2. In the presence of wild-type and C301S/C303S-transfected cells, GTN activated purified sGC with $EC_{50}$ values of 2.87 ± 0.68 and 0.11 ± 0.01 µM, respectively, demonstrating more than 10-fold higher GTN affinity of the mutant. Denitration activity of wild-type and C301S/C303S ALDH2 was 2.39 ± 0.33 and 0.62 ± 0.29 pmol/min/ng ALDH2 (determined in western blots using the purified protein as standard).

**Discussion:** Our results demonstrate that bioactivation of GTN in vascular smooth muscle involves direct reduction of GTN to NO. This reaction is catalyzed by ALDH2 in a minor pathway that is not linked to clearance-based GTN metabolism yielding 1,2-GDN and inorganic nitrite.

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**Reference**

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