

**INHIBITION OF HYPOXIA-INDUCED TOLERANCE TO
SUBSEQUENT HYPEROXIC INJURY IN LUNG CELLS BY
PHOSPHATIDYL INOSITOL 3-KINASE INHIBITORS: LY294002
AND WORTMANNIN**

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Human lung microvascular endothelial cells (HLMVEC) and lung epithelial type II-like A549 cells preexposed to 0% O₂ for 24h demonstrated a marked tolerance to hyperoxia (95% O₂, 6-8 days) induced cell death. The cell death in preexposed HLMVEC vs. non-preexposed HLMVEC was 31±5% vs. 58±3%. Hyperoxic exposure of non-preexposed A549 cells resulted into 29±2% cell death whereas only 16.6±3% cell death was observed in the cells that were preexposed. Previously our studies have shown that rats acclimated to hypoxia survive better in subsequent hyperoxia. Hypoxic preconditioning has also been demonstrated to be beneficial for various organs, like heart, against oxidative stress. The molecular mechanism underlying the protection, however, is incompletely understood. Addition of the specific PI3-K inhibitors Wortmanin and LY294002 totally abolished the resistance towards cell death in hyperoxia, suggesting the involvement of PI3K in the protection. In order to directly demonstrate the role of PI3K in the hypoxic signalling, PI3K activity was immunoprecipitated from the cell extracts. Hypoxia induced a 1.5-2 fold increase in PI3K activity in both the cell lines. Role of PI3K was further confirmed by over-expression of P110 α (catalytic subunit of PI3K) in A549 cells. The overexpressors exhibited a clear survival advantage over the empty vector controls. These results indicate that hypoxia-induced activation of PI3-K is an important event in the acquisition of resistance against subsequent hyperoxic toxicity.

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