

## Prevention of oxidant-induced cell death by intralysosomal iron binding

**Lennart Persson**

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Fakultetsopponent: Professor Rolf Hulcrantz, Karolinska sjukhuset, Stockholm

### **Abstract**

The lung is particularly prone to oxidative stress by its exposure to ambient oxygen and inhaled environmental oxidants. Abnormal assimilation and accumulation of iron are found in many lung disorders, which in redox-active form will exacerbate oxidative tissue damage. It may be that the most important cellular pool of reactive iron exists within lysosomes. As a result, these organelles are very vulnerable to oxidative stress and may burst due to peroxidative membrane destabilization. Support for the importance of intralysosomal iron in cellular oxidant damage includes the observation that the iron chelator, desferrioxamine, which almost exclusively localizes within the lysosomal compartment, will protect cells against oxidative challenge. Iron chelators targeted to the lysosomes may therefore be a particularly efficient therapeutic strategy for cells under conditions of substantial oxidative stress.

The present study, employing cultures of human respiratory epithelial cells and murine macrophage-like cells, explores the protective effects by iron binding agents upon H<sub>2</sub>O<sub>2</sub> and gamma radiation induced lysosomal damage and cell death. Using these *in vitro* models, the present study shows: (1) that chelation of intralysosomal iron efficiently prevents lysosomal rupture and ensuing cell death induced by either H<sub>2</sub>O<sub>2</sub> or gamma radiation; (2) that cell permeable lysosomotropic iron-chelators are much more efficient than those being internalized by endocytosis; (3) that intralysosomal iron is the most important cellular pool of redox-active iron for chelation therapy; (4) that iron-catalyzed peroxidative lysosomal destabilization is a decisive and early event in the apoptotic machinery.

Although apoferritin and desferrioxamine suppress the reactivity of lysosomal iron, their efficacy is considerably restrained by their uptake by fluid-phase endocytosis. Apoferritin is digested intralysosomally which further decreases its iron sequestering potential, while desferrioxamine by its intralysosomal retention may disturb normal cellular functions and cause iron-starvation. Amongst cell permeable iron binding agents, we tested  $\alpha$ -lipoic acid,  $\alpha$ -lipoamide, and a synthetic amine derivative of  $\alpha$ -lipoamide,  $\alpha$ -lipoic acid-plus (5-[1,2] dithiolan-3-yl-pentanoic acid (2-dimethylamino-ethyl) amide). The large difference in the protective potential of these cell permeant iron-chelators derives from their being localized in different cellular compartments, which lends further support that lysosomes contain the most important pool of chelatable redox-active iron. Indeed,  $\alpha$ -lipoic acid-plus by its lysosomotropism was by all means the most efficient iron chelator. On a molar basis  $\alpha$ -lipoic acid-plus was 4,000 to 5,000 times more effective than desferrioxamine to prevent lysosomal rupture and cell death induced by H<sub>2</sub>O<sub>2</sub> or gamma radiation.

We conclude that iron chelating therapy targeted to the lysosomes is an efficient strategy to protect oxidatively stressed cells *in vitro*. A corresponding efficacy of such treatment *in vivo*, and in iron dependent pulmonary disorders in particular, needs to be explored.