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Meeting abstract

Cellular antioxidant activity of bilirubin in the human endothelial cell line EA.hy 926 is mediated by bilitranslocase

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Background

Oxidative stress plays an important role in the pathogenesis of cardiovascular degenerative diseases. Bilirubin is known to be a potent endogenous antioxidant, both in *in vitro* and *in vivo*. The latter can be ascribed to an efficient amplification cycle whereby bilirubin, acting as antioxidant, is itself oxidized to biliverdin and then recycled back to bilirubin by NADPH-dependent biliverdin reductase. Bilitranslocase, a bilirubin-specific membrane carrier that mediates cellular uptake of bilirubin, has recently been found in the vascular endothelium. However, the levels of albumin-free bilirubin in plasma and tissues are only 10 to 50 nM. Therefore, the objective of this study was to elucidate the antioxidant activity of low concentrations of bilirubin and the involvement of bilitranslocase-mediated plasma membrane transport in human endothelial cells.

Methods

In this study we used the cellular antioxidant activity (CAA) assay developed by Wolfe and Liu [1]. Briefly, the assay is designed to trigger an acute oxidative stress into cells (by adding the radical initiator ABAP to the cell medium) and to fluorimetrically follow the subsequent increase of an intracellular radical-sensitive fluorescent dye. Substances with antioxidative properties that have free oxygen radical scavenging properties have been found to decrease the formation of fluorescence. By using the CAA assay, we have quantitatively evaluated the antioxidant activity of bilirubin in the endothelial cell line EA.hy 926. In our experiments, the cells were pre-incubated with anti-bilitranslocase antibodies (studied group) or bovine IgG (control group) before starting the CAA assay with bilirubin (0.5-100 nM).

Results

The intra-cellular antioxidant activity of bilirubin was concentration-dependent with an apparent saturation obtained at higher concentrations. The half-maximal effect was obtained at concentrations as low as 5 nM. The pre-incubation of the cells with anti-bilitranslocase antibodies reduced the antioxidant activity by about 50%.

Conclusion

Bilirubin has a potent intracellular antioxidant activity if applied to cells at concentrations close to its albumin-free plasma levels. The observed cellular antioxidant activity depends on bilitranslocase-mediated plasma membrane transport and therefore confirms the functional role of bilitranslocase as a membrane transporter in the endothelium.

References

1. Wolfe KL, Liu RH: **Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements.** *J Agric Food Chem* 2007, **55:**8896-8907.