REDUCTIVE ACTIVATION OF RICIN AND RICIN A-CHAIN IMMUNOTOXINS BY PROTEIN DISULFIDE ISOMERASE AND THIOREDOXIN REDUCTASE SYSTEM.

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Intracellular activation of ricin and of the ricin A-chain (RTA) immunotoxins requires reduction of their intersubunit disulfide(s). This crucial event is likely to be catalyzed by disulfide oxidoreductases and precedes dislocation of the toxic subunit from the ER lumen to the cytosol. We investigated the role of protein disulfide isomerase (EC 5.3.4.1, PDI), thioredoxin (Trx) and thioredoxin reductase (EC 1.8.1.9, TrxR) in the reductive activation of ricin and of a ricin A-chain immunotoxin by combining enzymatic assays, SDS-PAGE separation and immunoblotting. We found that, whereas PDI, Trx and TrxR used separately were unable to directly reduce ricin and the immunotoxin, PDI and Trx in the presence of TrxR and NADPH could reduce both ricin and immunotoxin in vitro. TrXR dependent activation of disulfide reductase activity of PDI was confirmed by "in vitro" results of FRET analysis on double labeled fluorescent and single disulfide bonded substrate. The reductive activation of ricin was more efficient in the presence of GSH:GSSG ratio of 3:1. Pre-incubation with the gold(I) compound auranofin, which irreversibly inactivates TrxR, resulted in a dose-dependent inhibition of ricin and immunotoxin reduction. Similar results were obtained with microsomal membranes or crude cell extracts where pre-incubation with auranofin inhibited the reductive activation of ricin and immunotoxin. Colocalization of PDI and TrxR in the ER was obtained by indirect fluorescence confocal microscopy and reduced or absent ricin activation was observed in microsomes depleted of TrxR and in cell extracts depleted of both PDI and Trx. Pre-incubation of U-937, Molt-3, Jurkat and DU145 cells with auranofin significantly decreased ricin cytotoxicity with respect to mock-treated controls ($p<0.05$). Conversely, auranofin failed to protect cells from the toxicity of pre-reduced ricin which does not require intracellular reduction of disulfide between the two ricin subunits. We conclude that TrxR, by activating disulfide reductase activity of PDI, can ultimately lead to reduction/activation of ricin and immunotoxin in the cell.