THE MECHANISM OF THE REGULATION OF PAI-1 GENE EXPRESSION BY REACTIVE OXYGEN SPECIES

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The signal transduction pathway involved in the induction of PAI-1 expression is not yet well characterized. The inhibition of TNF-α-induced expression of PAI-1 by antioxidant N-acetyl-L-cysteine (NAC) indicated redox-sensitive mechanisms acting in the signaling pathway. TNF-α-induced reactive oxygen species (ROS) production in the endothelial cells was attenuated by NAC, so we investigated the possible involvement of ROS in the activation of PAI-1 by the cytokine. We found redox-regulated effects of TNF-α on the expression of PAI-1 via the activation of NF-κB in endothelial cells. In this study, we focused on the mechanisms of the regulation of PAI-1 expression by reactive oxygen species in human endothelial cells. Upregulation of PAI-1 expression by the activation of the cells with TNF-α (50 ng/ml) or H₂O₂ (10-200 µM), observed by means of antigen level measurement, was reversed in the presence of NAC (20 mM). The stimulatory effect of ROS was detected at the level of the PAI-1 promoter in endothelial cells transfected with plasmid p800 LUC containing the PAI-1 promoter fragment (+71 to -800). PAI-1 promoter activity increased in the presence of ROS, and was suppressed by up to 75% in the presence of antioxidants. The investigation of oxidant-mediated upregulation of PAI-1 by the activation of the MAPK cascade showed that MAPK activity increased in response to TNF-α or H₂O₂, and was blunted when the cells were first treated with an antioxidant, N-acetyl-L-cysteine. These results suggest that reactive oxygen species are involved in a pathway leading to the activation of PAI-1 expression by the activation of the MAPK cascade. Our data suggest that PAI-1 protein expression is under the control of NF-κB, and in addition, the inhibition of TNF-α-induced PAI-1 activation was achieved when NF-κB activity was inhibited with the antioxidant N-acetyl-L-cysteine. We observed the inhibition of the stimulatory effect of TNF-α on the expression of PAI-1 in the presence of the relatively specific IκBα 26S proteasome inhibitor MG132. Antisense oligonucleotides for NF-κB inhibit the synthesis of PAI-1 mRNA. Our finding suggests that TNF-α- and H₂O₂-induced over-expression of PAI-1 is transcriptionally regulated by NF-κB. This study showed that reactive oxygen species are important in the cytokine-induced activation of PAI-1 expression, and may act as a signal transduction messenger for NF-κB.