THE INFLUENCE OF ZINC ON H-KININOGEN INTERACTION WITH CELL SURFACE


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The endocytosis of plasma kallikrein/kinin system proteins mediated by proteoglycans is established by our group. The H-kininogen (HK) binding to cells and extracellular matrix depends on zinc, which modulates the structure and function of numerous proteins. The aim of the present work is to study the influence of zinc on HK interaction with cell surface mediated by proteoglycans. The cell lines used were CHO-K1 (wild type) and CHO-745 (defective in glycosaminoglycans biosynthesis). The kininogenase activities present in cell lysate fractions were determined by immunoblotting. The cell death was analyzed by flow cytometry after annexin V and 7AAD staining. The HK-Alexa-488 endocytosis was determined in real time using an inverted confocal laser-scanning microscope. At pH 5.5, either CHO-K1 or CHO-745 lysate fractions cleaved HK (115 kDa) totally in small fragments (78, 62, 48 kDa) and at 200 µM zinc some HK remained uncleaved, 15% and 26% respectively. At pH 7.4, HK was cleaved partially by CHO-K1 lysate (72%) and CHO-745 lysate cleaved HK totally; at 200 µM zinc some HK remained uncleaved, 36% and 19% respectively. The CHO-K1 viability, after 24 h of treatment with HK and determined by the MTT assay, decreased significantly either with or without zinc (p<0.001). Nevertheless, the trypan blue assay showed a significant increase in cell death only without zinc (p<0.05). The flow cytometry indicated that cell death was characteristic of necrosis. The CHO-745 viability determined by the MTT assay also reduced significantly, either with or without zinc (p<0.01) and zinc also decreased cell viability of CHO-745 significantly either with (p<0.01) or without (p<0.05) HK. The trypan blue assays on CHO-745 showed no effects caused by zinc or HK. In the absence of zinc HK-Alexa-488 was not internalized by CHO-K1. Our data suggest that H-kininogen effects on cells may be influenced by zinc in pathophysiological processes.

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Key Words: kininogen, zinc, proteoglycans.