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Effect of Secretable SOD Delivered by Genetically Modified Cells on Xanthine/xanthine Oxidase and Paraquat - Induced Cytotoxicity in Vitro.

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We designed a new eukaryotic expression vector for secretable superoxide dismutase (SOD), which expresses human SOD cDNA by fusing it to 1 connecting amino acid and the signal peptide DNA sequence of the human interleukin-2 (IL-2) gene (IL-SOD(2) cDNA). The ILSOD(2) cDNA constructed by PCR‐based gene expression was ligated into the multicloning site of the pRc/CMV plasmid (pRc/CMV-ILSOD(2)). Rat lung epithelial-like cells (L2 cells) and rat skin fibroblasts (FR cells) were transfected with pRc/CMV-ILSOD (2) by lipofection. The extracellular SOD activities of IS(2)-L2 cells (L2 cells transfected with pRc/CMV-ILSOD(2)) and IS(2)-FR cells (FR cells transfected with pRc/CMV-ILSOD(2)) were 2-3 times higher than those of host cells.

Initially, we investigated the protective effect of extracellular SOD secreted from these transformed cells (IS(2)-L2 and IS(2)‐FR cells) on extracellular superoxide anion (xanthine/xanthine oxidase; X/XO treatment) -induced cytotoxicity in normal cells. The sensitivities of these transformed cells to X/XO-induced cytotoxicity was decreased significantly as compared with that of host cells. Although, the conditioned medium from IS(2)-L2 and IS(2)-FR cells protected against X/XO-induced cytotoxicity, the conditioned medium from host cells (L2 and FR cells) showed no significant effects on X/XO-induced cytotoxicity. Furthermore, The conditioned medium from transformed cells was more effective than that of host cells against lipid peroxidation by normal cells under conditions of oxidative stress.

Second, we generated superoxide anions in the intracellular space by paraquat treatment. The transformed cells were more sensitive to paraquat-induced cytotoxicity than host cells. Following addition of catalase, the sensitivity of these genetically modified cells to paraquat became equivalent to that of host cells. These results indicated a protective effect of transfection with secretable SOD genes against extracellular superoxide anion-induced cytotoxicity although no such protective effect was observed against the intracellular cytotoxicity generated by paraquat treatment.