

Mechanisms mediating growth inhibitory effects of combined celecoxib and gamma tocotrienol treatment on neoplastic mouse mammary epithelial cells *in vitro*.

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The present study was aimed at characterizing the combined anticancer effects of celecoxib, a COX-2 inhibitor, and gamma tocotrienol on highly malignant neoplastic +SA mouse mammary epithelial cells. Treatment with 3-4 mM gamma tocotrienol or 7.5-10 mM celecoxib alone resulted in significant decrease in cell growth. IC₅₀ values were calculated as 3.3 mM for gamma tocotrienol and 8.6 mM for celecoxib. Combination treatment with a range of subeffective doses of celecoxib and gamma tocotrienol resulted in a significant decrease in neoplastic mammary epithelial cell growth in a dose-responsive manner. Combined treatment with 2.5 mM celecoxib and 0.25 mM gamma tocotrienol showed significantly greater growth inhibitory effects than either compound alone. Additional studies were conducted to characterize the mechanisms involved. Since prostaglandin E₂ (PGE₂) level is a direct indicator of COX-2 activity in cells, PGE₂ Enzyme Immuno Assay was carried out to assess the effect of individual and combined treatments on COX-2 activity. Treatment with celecoxib alone resulted in a dose-dependent decrease in cellular levels of PGE₂. Interestingly, a similar decrease in PGE₂ level was observed in gamma tocotrienol treated cells. Combined treatment with celecoxib and gamma tocotrienol showed an additive decrease in PGE₂ levels. This observation suggests that one of the mechanisms involved in enhanced growth inhibition is a combined suppressive effect on COX-2 activity. Additional studies were conducted to assess the signaling mechanisms involved in enhanced growth inhibition. +SA cells were treated either alone or in combination with a subeffective dose of celecoxib (2.5 mM) and gamma tocotrienol (0.25 mM). Western blot analysis was carried out to estimate the levels of COX-1, COX-2, Akt and pAkt, pNFkB proteins. No significant changes were observed in COX-1 levels at all the sub-effective and effective treatment doses. Dose-dependent decrease was observed in COX-2 levels in individual treatments of celecoxib and gamma tocotrienol. Combined treatment with subeffective doses showed additive decrease in COX-2 levels. These results were in accordance with the observations in PGE₂ EIA assay. Total Akt levels did not change at all the treatment doses. Treatment with effective doses of celecoxib and gamma tocotrienol alone showed decrease in pAkt and pNFkB levels. Combined treatment with subeffective doses resulted in decreased pAkt levels as compared to the treatments with same doses of either drug alone. These studies suggest that enhanced growth inhibitory effects of celecoxib and gamma tocotrienol are partly mediated by combined suppressive effects of these drugs on COX-2, Akt and NFkB activity. Further studies are required to understand the interrelationship of these pathways suppressing mammary epithelial cell growth.