Clinically-used nitrocatechols and EGCG synergistically inhibit lung cancer cells *in vitro*

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Polyphenols are likely to be responsible for the anticancer effects of green tea, with (-)-epigallocatechin-3-gallate (EGCG) the most well-studied. EGCG reduces lung cancer cell viability effectively *in vitro*, yet it is methylated by catechol-*O*-methyl transferase (COMT), limiting its effectiveness in inhibiting cancer cell growth. The nitrocatechol drugs entacapone and tolcapone are potent inhibitors of COMT and are used to mitigate effects of Parkinson's disease. Here, we investigated the synergistic effects of entacapone/tolcapone and EGCG in human (H1299) and murine (CL-13) cell lines. The IC50s of entacapone, tolcapone, and EGCG were 76.8, 29.3, and 174.9 μM, respectively in H1299 cells, and 50.7, 19.7, and 181.5 μM, respectively in CL-13 cells following treatment for 72 h. The IC50s were dramatically reduced when entacapone and tolcapone were used in combination with EGCG. The interactions were determined to be synergistic using isobolographic analysis. Inhibition of COMT-mediated methylation of EGCG by entacapone and tolcapone was also measured by LC-MS over 30 min and the IC50 values were 10 and 20 μM, respectively. The anticancer effects of the nitrocatechols and EGCG were also evaluated in pathways of apoptosis (TUNEL) and cell cycle (flow cytometry). In conclusion, the combination of EGCG and entacapone or tolcapone has greater lung cancer growth inhibitory activity than treatment with any of the single compounds. The mechanistic basis for this synergy is likely due in part to COMT inhibition, yet other mechanisms are also likely to play a role. *Funded by NIH grant AT004678*.