

PRO-OXIDANT AND ANTITUMOR EFFECTS OF CURCUMIN AND N-ETHYLMALEIMIDE IN THE HA22T/VGH MODEL OF OVERT HEPATOCELLULAR CARCINOMA

Carina Valeria, Labbozzetta M., Poma P., Alaimo A., Maurici A., Inguglia L., Notarbartolo M., D'Alessandro N.

Dipartimento di Scienze Farmacologiche, Università di Palermo

The dietary polyphenol curcumin (CUR) is endowed with interesting antitumor activities, both in the chemoprevention setting and in the treatment of overt tumors. A matter still debated is that of the possible relationship of such activities to the complex potential, either anti- or pro-oxidant, properties of the agent. We have compared the antitumor effects of CUR with those of the cysteine alkylating agent N-ethylmaleimide (NEM) in the HA22T/VGH hepatocellular carcinoma cell line. Both the compounds, in 1-30 μ M amounts, exhibited dose-dependent growth inhibitory (evaluated by MTS assays) and cell death (evaluated by flow cytometry analysis of PI stained DNA) effects in the cells. In addition, CUR, but not NEM, potentiated the antitumor effects of cisplatin; the combination of CUR and NEM produced substantially additive effects. The antitumor effects of CUR were clearly antagonized by pre- or post-treatments with 1 or 2 mM N-acetylcysteine (NAC) and increased by pre-treatment with the glutathione depleting agent buthionine sulfoximine at 100 μ M; for NEM, they were prevented by pre-exposure to NAC, but only slightly reversed by post-treatment with the same agent.

Further, the influences on two critical redox-sensitive targets, such as the nuclear levels of activated NF- κ B (p65 subunit) and the catalytic activity of telomerase were examined in the cells by TransAMTM and TRAP assays, respectively. In a representative experiment, after 4 h of exposure, CUR 25 μ M remarkably up-regulated (by 355% of the control) nuclear NF- κ B, an effect that was partially blunted (to increases of 197% or 238%) by pre-treatment with NAC or the combination with NEM 25 μ M, respectively. NEM alone caused a modest increase (of 156%) in the nuclear activated transcription factor, that was marginally reduced (to 131% of the control) by NAC. For telomerase, exposures to CUR or NEM for 8 h caused minor inhibitions (22 and 17%, respectively) of the catalytic activity of the enzyme, which were not modified by NAC pre-exposure. The effects of the combination of CUR and NEM on telomerase activity were sub-additive (inhibition of 27%). On the basis of these findings showing the different behaviour of CUR and NEM, we suggest that modifications of cellular thiols, possibly determined mainly by oxidation rather than alkylation, are involved in the antitumor activity of CUR in the HA22T/VGH model of overt hepatocellular carcinoma. They appear to be also required for the synergistic effects exerted by CUR in combination with cisplatin, which were antagonized by NAC pre-administration.