AUTOPHAGY INHIBITION IMPROVES THE EFFICACY OF CURCUMIN/TEMOZOLOMIDE COMBINATION THERAPY IN GLIOBLASTOMAS.

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Abstract

Introduction and objectives: Glioblastoma is a devastating primary brain tumor resistant to conventional therapies. In this study, we tested the efficacy of combining temozolomide - the first-choice glioblastoma chemotherapeutic - with curcumin, a phytochemical inhibitor of cancer cells overexpressed pathways such as NFKB, STAT3 and PI3K/Akt, in cell culture and pre-clinical models of the disease. We investigated the potential synergy between curcumin and temozolomide as well as the molecular mechanisms of cell resistance to these drugs. Materials and methods: MTT and LDH cytotoxicity assays were used to evaluate the synergy between curcumin and temozolomide, and annexin V/PI and acridine orange flow cytometry to determine apoptosis and autophagy, respectively, in C6, U251MG and U87MG glioma cells. Activation of caspase-3 as well as STAT3, NFKb and MAPKs was carried out by immunobloting. The brain implanted C6 glioma in Wistar rats was used as an animal model. Results and conclusions: The data showed that synergy between curcumin and temozolomide was not achieved due to redundant mechanisms that lead to activating protective autophagy both in vitro and in vivo. Autophagy preceded apoptosis, and blocking this response with autophagy inhibitors (3-methyl-adenine, ATG7 siRNA and chloroquine) rendered cells susceptible to temozolomide and curcumin alone or combinations by increasing apoptosis. While curcumin inhibited STAT3, NFKb and PI3K/Akt to affect survival, temozolomide-induced autophagy relied on the DNA damage response and repair components ATM and MSH6, as well as p38 and JNK1/2. However, the most interesting observation was that both temozolomide and curcumin required ERK1/2 to induce autophagy. Blocking this ERK1/2-mediated temozolomide and curcumin induced autophagy with resveratrol, a blood-brain barrier permeable drug, improved temozolomide and curcumin efficacy in brain-implanted tumors. Overall, the data presented demonstrate that autophagy impairs the efficacy of temozolomide/curcumin, and inhibiting this phenomenon could provide novel opportunities to improve brain tumor treatment.

Acknowledgements: We acknowledge the Brazilian funding agencies CAPES, FAPERGS and CNPq. A. Zanotto-Filho was recipient of DOCFIX (Edital CAPES/FAPERGS n° 09/2012) and CNPq (Projeto Universal 485758/2013-0).

Key Words: autophagy, curcumin; temozolomide.