AMPK mediates the anti-proliferative effects of Celecoxib in CML cell lines

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Beyond its use as anti-inflammatory drug, Celecoxib (CELE), the most characterized among the COX-2 inhibitors, is known to exert an anti-proliferative control in many models of solid tumours. However, its effects on 'liquid' tumours have not been fully elucidated. In order to fill this void in knowledge, we studied the impact of CELE on cell viability and its mechanism of action by using three human cell lines, K562, LAMA84 and JURL-MK1, established from patients affected by Chronic Myelogenous Leukemia (CML), at the 'blast crisis' stage of the disease.
MTT and 'clonogenic' assays on methyl-cellulose evidenced the ability of CELE to impair cell proliferation in a dose-dependent manner. These effects appeared irreversible considering that inhibition of cell growth caused by a single administration of the drug was more evident by increasing the length of exposure (as confirmed by MTTs in the 24-72 h time range and, even more evidently, by colony counting after 10 days, in methyl-cellulose, semi-solid milieu). In particular, the LAMA-84 cell line appeared as the most sensitive to this treatment with an EC50 of 23,8 μM, after 24h, and of 8,2 μM, after 6 days from treatment.
Flow cytometry of propidium iodide stained cells and morphological evaluation of nuclear DNA fragmentation (Hoechst-staining) demonstrated that treatments with CELE, at concentrations close to the EC50 at 24 h (25 μM), were mainly cytostatic (with a G1-block of the cell cycle and a paucity of cells with fragmented or subG1 content of DNA). Similarly to what observed with MTT, the LAMA-84 emerged as the most sensitive cell line to CELE, while JURL-MK1 cells were resistant.
Next, we assessed the ability of CELE to induce autophagy as a possible mechanism explaining the time-dependency of the anti-proliferative effects exerted onto CML cells. Indeed, by western blot and immunofluorescence, we evidenced that treatments with CELE induced the conjugation of phosphatidylethanolamine to LC3 protein (a typical marker of autophagy), although after at least 24 h from drug administration.
Under a mechanistic point of view, the cytostatic effect of CELE was not evoked by rofecoxib (ROFE), another COX-2 inhibitor, suggesting that its anti-leukaemic activity may rely on the modulation of a different molecular target. Thus, considering the centrality of the AMP-activated protein kinase (AMPK) in the events leading to autophagy, we assessed the activation of this protein following to treatments with CELE. Indeed, western blot analysis evidenced a clear phosphorylation of the threonine-172 epitope on the catalytic subunit of AMPK, already after 15 minutes from administration of 25 μM CELE to CML cell lines (more evidently for K562 and LAMA-84 cell cultures)
In agreement with these findings, lentivirally delivered shRNA, targeting AMPKa expression, abrogated the effects of CELE on the viability of LAMA-84 cells.
Also in line with literature, that describes AMPK as capable, under metabolic stress, to exerts an inhibitory control on the mTOR complex 1 (mTORC1), immunoblot analysis of protein extracts from LAMA-84 cell cultures exposed to CELE displayed a significant reduction in the activation of mTORC1, but not of mTORC2 (already evident after 8 h). These data were confirmed further by the reduced phosphorylation of down-stream targets (4E-binding protein and S6 kinase), as assessed by immunoblottings.
In conclusion, our work demonstrates the anti-leukaemic potential of CELE and suggests the AMPK-dependent inhibition of the mTOR pathway as a 'non COX-2' mechanism of action for this drug.