Modulation of liver X receptor-beta (LXRβ) expression by natural and synthetic steroids in rat C6 glioma model

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Introduction: The highly lethal brain cancer glioblastoma (GBM) is remarkably dependent on cholesterol for survival, rendering this tumor sensitive to Liver X receptor (LXR) agonist-dependent cell death. EGFR mutations and PI3K hyperactivation are common in GBM, promoting tumor growth and survival, including through SREBP-1-dependent-lipogenesis.Targeting LDL-R (LDL-Receptor) with the Liver X Receptor (LXR) agonist caused IDOL (Inducible Degrader of LDL-R)-mediated LDL-R degradation and increased expression of the ABCA1 cholesterol efflux transporter, potently promoting tumor cell death in an *in vivo* GBM model. EGFR can promote tumor survival through PI3K-SREBP-1 dependent up-regulation of LDL-R, and suggest a role for LXR agonists in the treatment of GBM patients. In this context, we evaluated on C6 rat glioma cells the cytotoxic properties of natural and synthetic steroids as well as their ability to modify the expression of LXR.

Materials and Methods: 7β-hydroxycholesterol (7β-OHC) was used as positive control to induce cell death. We used 11 animal steroids (22R-hydroxycholesterol; cholesterol-α-epoxide; cholesterol-β-epoxide; dihydrocholesterol; 6β cholesterol; 6α-cholesterol; 7α-cholesterol; 7-ketocholesterol; 7β-hydroxycholesterol; 4α–hydroxycholesterol; 4β-hydroxycholesterol), 2 plant steroids (3β, 5α, 6α-trihydroxy-sitosterol and diosgenin) and 13 synthetic steroids (Litomet; Chenomet; Lith-11-oxo; 22R-Iso-hydroxycholesterol; Cholest-5-ene-3-beta, 22R, 26-triol; 5-Cholesten-3β, 7α-diol; 5,25R-Cholesten-3β, 26-diol, maleic anhydrides (1a; 1b; 1f) and maleimides (7a; 7b; 7f)) (***Samadi et al., Steroids. 2017; 125: 124-130***). C6 rat glioma cells were cultured without or with natural and synthetic steroids in a range of concentration from 5.5 to 180 µM for 24 h. Steroids-induced cell death was characterized by various criteria: loss of cell adhesion (phase contrast microscopy); membrane esterase activity after staining with fluorescein diacetate (FDA) (fluorimetry); succinate dehydrogenase activity after staining with MTT; morphological aspect of the nuclei after staining with Hoechst 33342 (fluorescence microscopy); lysosomal integrity after staining with acridine orange, plasma membrane permeability after staining with propidium iodide (PI), transmembrane mitochondrial potential (ΔΨm) after staining with $DiOC_6(3)$; and analysis of the repartition of the cells in the different phases of the cell cycle after staining with PI (flow cytometry). Apoptosis and autophagy were evaluated by western blotting with antibodies raised against cleaved caspase-3 and LC3I - LC3II, respectively. The LXR α and β mRNA levels were determined by real-time PCR.

Results: The cytotoxic molecules were selected based on the observations realized by phase contrast microscopy, and on the IC50 values obtained with the MTT and FDA tests. 13 steroids were found cytotoxic (5 animal steroids; 1 plant steroids; 7 synthetic steroids). The cytotoxic concentrations of the steroids selected were in the range of 35-70 µM with the MTT test and of 5.5-100 µM with the FDA test. These cytotoxic steroids induce a loss of ΔΨm (increased percentages of cells with depolarized mitochondria), an increase plasma membrane permeability (increase of PI positive cells), and a destabilization of lysosomes (increase or decrease of orange/red mean fluorescence intensity). The cell cycle was also strongly modified, mainly with 22R-hydroxycholesterol, and cholesterol-β-epoxide. Compared to the control, no nuclear modification characteristic of apoptosis was observed: the nuclei keep their round shape and show no chromatin marginalization, condensation and/or fragmentation. Only LXRβ mRNA was detected in C6 cells. This nuclear receptor was down regulated by some cytotoxic steroids which also induce an increase of the ratio [LC3II / LC3I] which is an autophagic criteria. With the different molecules studied no cleaved caspase-3 was identified.

Conclusions: The cytotoxic steroids able to induce cell death on C6 cells also down regulate LXRβ expression and activate autophagy. Some of these molecules, potentially active on the lipid metabolism via LXRβ, could constitute new drugs to for the treatment of glioblastoma.