IN VITRO EFFECTS OF BERBERINE ON PEDIATRIC LOW GRADE ASTROCYTOMA CELLS: A PILOT STUDY TOWARD PRECLINICAL EVALUATIONS

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Introduction: Pilocytic astrocytoma (PA) is a pediatric low-grade glioma and the most common pediatric brain tumor corresponding to WHO grade I. PA can arise anywhere in the CNS, but it is most commonly localized in the cerebellum followed by the optic pathway/hypothalamic region. Current standard of care therapy includes maximal safe resection where possible and chemotherapy and/or irradiation. PA can be aggravated by recurrence or progressive disease, which occurs in up to 80% of patients, depending on location and extent of initial resection. Therefore, the development of new therapies is urgently needed in order to specifically target the disease and improve the clinical course of patients suffering from PA. Berberine (2,3-methylenedioxy-9,10-dimenthoxyprotoberberine chloride; BBR) is an isoquinoline alkaloid isolated usually from Huang Lian (Rhizoma coptidis). BBR has pharmacological properties and its anticancer activity has been detected in various cancers, including neuroblastoma. Its ability to suppress cell migration, invasion and metastasis in many tumor types has been studied as well. In our study we have investigated the effect of BBR on PA cell lines (i.e. Res186and Res259), investigating the apoptotic effect and evaluating if BBR could represent a candidate drug for the treatment of this pediatric tumor.

Materials and methods: Cell Culture and treatments: Human astrocytoma Res186, Res259cells were cultured and maintained in DMEM medium (Euroclone, Milano, Italy), supplemented with 10% FBS, 100u/mL penicillin, 0.1mg/mL streptomycin, and 1% L-glutamine, and kept at 37°C in a 5% CO_2. BBR dilutions were obtained from a 10^{-2}M BBR chloride (Sigma) stock solution prepared in DMSO (Sigma). Viability and apoptosis cytofluorimetric analysis after BBR administration: Cells viability was quantified using the Muse Count and viability Kit (Merck). Live, dead, early-, or late-apoptotic cells were assayed using the Muse Annexin V & Dead Cell Assay (Merck) according to manufacturer's instruction. Immunoblotting analysis: Cells were lysated in ice-cold RIPA buffer (150mM NaCl, 50mM Tris-HCl pH8.0, 0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% sodium dodecylsulphate. Proteins were denatured and separated on 12% SDS-PAGE according to the protein size. After electrophoresis, proteins were transferred onto nitro-cellulose membrane Hybond-C Extra (GE Healthcare), using the Trans-Blot Turbo (Biorad). Membranes were blocked 1h with 5% (w/v) non-fat milk in TBS containing 0.1% Tween-20 and incubated over-night at 4°C with primary antibodies.

Results: Effects of BBR on cell viability: Res186and Res259cells were cultured with a range of BBR doses (0, 10, 20, 40, 80, 160 mM) for 24, 48and 72h. A significant reduction in cell viability was observed in a dose- and time-dependent manner after 24h of BBR treatment in both cell lines. The viability of Res186and Res259further decreases after 48and 72h of BBR treatment. Effects of BBR on cell apoptosis: Res186and Res259cells were cultured as before. A significant increase of total apoptosis, in correspondence to the highest BBR concentartions, (43-75%) has been observed 24h p.t. in both cell lines. The apoptotic index of Res186and Res259further decreased after 48and 27h of BBR treatment.

Discussion: In the present study, we proved that BBR significantly reduced the viability of Res186and Res259cells and this observation suggested that BBR treatments may be effective adjuvant anticancer options against PA. Future studies will be performed to clarify the mechanism regulating the reduction of cell viability and, at the same time, chemical changes will be made on BBR molecules to improve the effect on pediatric astrocytomas.