CHLORIDE INTRACELLULAR CHANNEL 1INHIBITION DICTATES ANTITUMOR EFFECTS OF OLD AND NEW BIGUANIDE COMPOUNDS IN GLIOBLASTOMA STEM CELLS

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Introduction: Repositioning of "old" drugs to treat different diseases including cancer is an attractive therapeutic strategy. Metformin, the standard drug for type 2diabetes of the biguanide class, showed preclinical and clinical antitumor effects in various cancer types, but mechanism underpinning its activity are not yet well defined. Metformin preferentially exerts its antiproliferative activity on cancer stem cells (CSCs), the subset of tumor cells able to self-renew, survive therapies, expand and sustain the development and relapse of several tumor types, including glioblastoma (GBM). GBM is the most aggressive and common primary brain cancer in adults, characterized by invasive growth and resistance to current multimodal treatments. Chloride intracellular channel 1(CLIC1) represents a promising biomarker and therapeutic target in GBM. CLIC1is constitutively active into the plasma membrane of CSCs, controlling GBM cell proliferation and migration. Preclinical studies revealed that high concentrations of metformin (mM range), hardly manageable in a clinical setting, are required to exert antitumor effects. This scenario makes compelling the search for more potent compounds among known and novel biguanides. Therefore, we tested the activity of phenformin (a withdrawn antidiabetic drug), moroxydine (an antiviral agent), the antimalarial proguanil and its active form cycloguanil (with a cyclized biguanide moiety), and four new biguanide analogs (2linear and 2cyclized structures) in GBM CSCs, to functionally target CLIC1.

Materials and methods: Drug efficacy was analyzed in 7CSC cultures derived from post-surgical samples of human GBM. Normal human stem cells from umbilical cord (mesenchymal stem cells, MSCs) were used as reference. Cell proliferation and survival were analyzed by cell count and MTT assay; annexin V-FITC staining was used for apoptosis analysis; self-renewal ability was assessed by sphere-formation efficiency of serially passaged CSC cultured as neurospheres. Invasiveness was evaluated by 2D and 3D invasion assays. Electrophysiological measurements were used for CLIC1current detection.

Results: All the known biguanides significantly impaired CSC proliferation, at IC_{50} values in the range 0.05–0.5mM markedly lower than metformin (9.4mM), as well as self-renewal, migration and invasion. Drugs showed the same higher potency than metformin potency in the inhibition of CLIC1ion flux, except proguanil which was ineffective. Two new derivatives (1linear and 1cyclized) displayed a dose-dependent antiproliferative activity, high potency (IC_{50} in the low micromolar range) and efficacy (up to 80% inhibition at the highest concentrations), ability to impair spherogenesis and invasiveness. Conversely, the other new analogs did not significantly affect any of these CSC functions, and failed to interfere with CLIC1activity as confirmed by electrophysiology.
All efficacious biguanides specifically affect CSCs, since in normal MSCs no cytotoxicity was observed. MSC viability was reduced faintly by phenformin and markedly by proguanil.

Discussion and conclusions: Old biguanides, unrelated to the antidiabetic drugs (moroxydine and cycloguanil) and two new derivatives significantly reduce proliferation, self-renewal and invasiveness of CSCs, showing higher in vitro potency than metformin, selectively affecting CSCs while sparing normal stem cells. These effects are mediated by the direct interaction of drugs with the extracellular portion of the active CLIC1, impairing channel activity. Overall, these results suggest that antitumor activity against the GBM CSCs is a shared feature of biguanides acting through the common target CLIC1, although each drug studied had different potency, efficacy and selectivity as CLIC1inhibitor. The inhibition of CLIC1activity might represent a biguanide class-effect to impair GBM CSC aggressive phenotype, granting high antitumor efficacy and safe toxicological profile.