IN VITRO EFFECTS OF BRIVARACETAM AND LACOSAMIDE ON PROLIFERATION AND MIGRATORY POTENTIAL OF HUMAN GLIOBLASTOMA CELLS

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BACKGROUND - Epilepsy is a frequent complication in patients with glioma, however the use of antiepileptic drugs is not always sufficient for seizure control. The MRPs (multidrug resistance proteins) and P-gp, (P-glicoprotein) were found to be overexpressed in brain tissue of patients with drug-resistant epilepsy, suggesting their involvement in the clearance of antiepileptic drugs.
In addition to the anticonvulsant mechanism, for some antiepileptic drug a cytotoxic effect has been previously suggested (Eyal S et al., Epilepsia, 2004).
AIM – Aim of the work was to evaluate possible *in vitro* cytotoxic effects of two new-generation antiepileptic drug on human glioma cell lines. Expression of MRPs was also evaluated on the same cells before and after *in vitro* exposure to sub-cytotoxic drug concentration.

METHODS

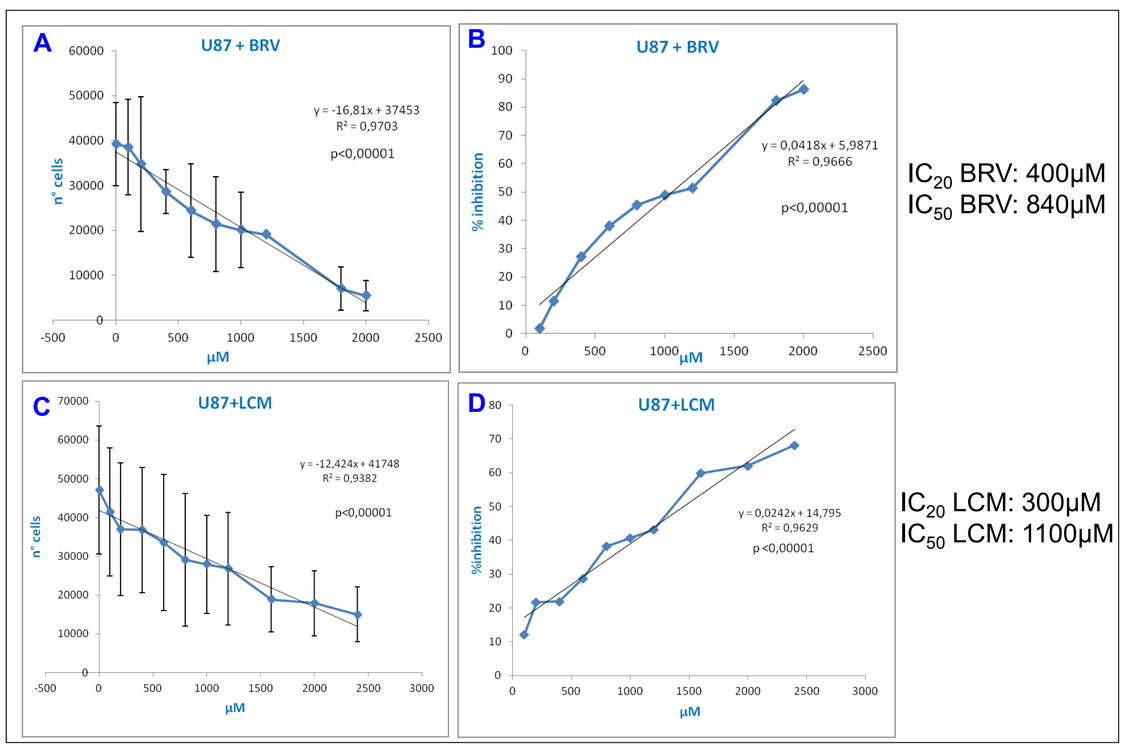
CYTOTOXICITY ASSAY
We studied the *in vitro* effects of two new-generation antiepileptic drugs, brivaracetam (BRV) and lacosamide (LCM), by cell proliferation assay (MTS), on two human glioblastoma cell lines (A172 and U87MG). Inhibitory concentrations (IC) were calculated from the regression line that correlates percentage of growth inhibition and drug concentrations calculated as:

100-(100*average n. cells [0-2500µM] /n. cell basal level)

APOPTOSIS
Evaluation of apoptotic cells was performed in untreated and treated cells (IC_{20} BRV or IC_{20} LCM for 24-48-72h) using Annexin V binding assay - Annexin V-FITC Apoptosis detection kit- by flow citometry (FacsVantage SE, Becton Dickinson, CA, USA) . Data are expressed as percentage of apoptotic cells.
MICRORNA EXPRESSION PROFILE
Total RNA from U87 cells, treated with BRV or LCM (IC_{20}) fo 24, 48 and 72h, was purified, labelled and hybridized on "Human miRNA Microarray V19" slides (Agilent), that contain probes for 2000 human microRNA. Scanning and image analysis were performed using the scanner "Agilent DNA Microarray Scanner (P/NG2565BA)". "Feature Extraction Software" (versione 10.5) was used to extract data.
MIGRATION CELL STUDY
Migratory ability of U87 cells transfected with mimic control or mimic miR-107 was evaluated by plating cells in serum-free medium on transwell with 8µm porous membrane (BD falcon) using medium additioned with 10% serum as chemoattractant. After 16h membranes were cut from the insert and migrated cells were visualized by DAPI staining and counted.

RESULTS

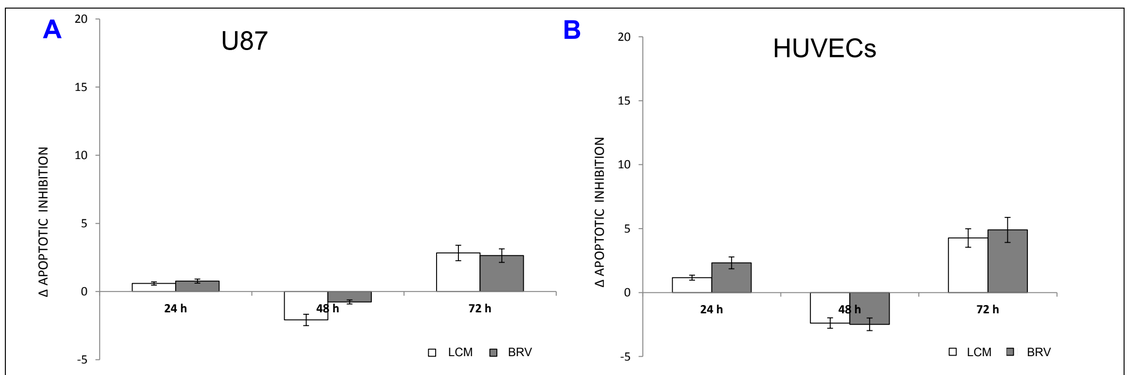
CYTOTOXICITY ASSAY AND APOPTOSIS
Results show a cytotoxic effect of brivaracetam (Pearson correlation index p <0.00001, fig.1A-B) and lacosamide (Pearson correlation index p <0.00001, fig.1C-D) for U87 at 72h of treatment. No cytotoxicity was detected in A172 cell line (data not shown).
Antiproliferative effect of BRV and LCM on U87 cell line does not seem to correlate with an apoptotic mechanism (fig. 2A). Human umbilical vein endothelial cells (HUVECs) did not show an increase in apoptosis after *in vitro* exposure to the two drugs (Fig. 2B)



IC_{20} BRV: 400µM
IC_{50} BRV: 840µM

IC_{20} LCM: 300µM
IC_{50} LCM: 1100µM

Fig. 1



Graphs show Δ apoptotic inhibition (%apoptotic cells IC_{20}BRV or IC_{20}LCM - % apoptotic ctrl cells) on U87 cell line and on HUVECs, after IC_{20} BRV and LCM treatment at 24-48-72h.

Fig. 2

MICRORNA EXPRESSION PROFILE
The analysis of microRNA expression profiles on U87 cells enable us to identify microRNA modulated by BRV (fig. 3A) and LCM (fig. 3B). In detail, we identify 37 microRNA modulated by BRV and 30 miR by LCM. We validate our results with RealTime PCR on miR-107 and miR-195, that were induced by both treatments (fig. 3C). In literature there are several evidences that demonstrate an anticancer role of both microRNAs that act by inhibiting both the proliferation that the migration of cancer cells (fig. 3D).

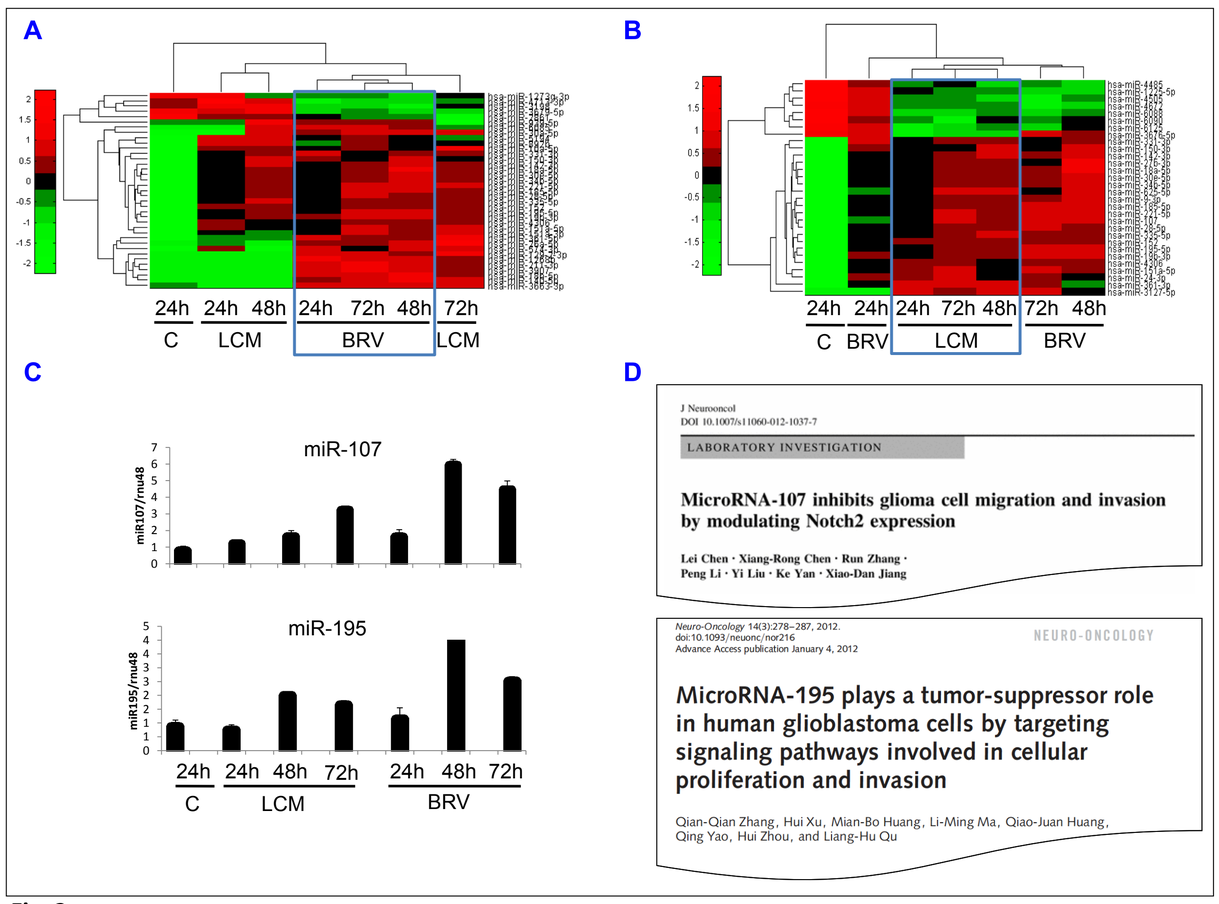


Fig. 3

CELL MIGRATION STUDY
U87 cells migration assay following induction of mir-107 expression shows microRNA inhibitory effect on migration ability in glioblastoma cells (fig. 4A). This effect is also reflected in a decreased expression of the main proteins involved in migration such as EGFR, N-cadherin and cyclin-D1 (Fig. 4B).

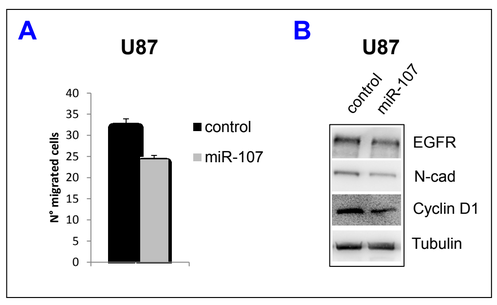


Fig. 4

CONCLUSIONS

- Brivaracetam and lacosamide showed a cytotoxic effect on glioblastoma cell lines U87 *in vitro*.
- The cytotoxic effect of BRV and LCM does not seem to be related to induction of apoptosis.
- U87 cells treatement with BRV and LCM cause modulation of different microRNA like miR-107 and miR-195, which may partly explain the effect exerted by the two drugs. Moreover our data suggest a possible involvement of miR-107 in inhibition of cell migration.

REFERENCES

•Baltes S et al. Differences in the transport of the antiepileptic drugs phenytoin, levetiracetam and carbamazepine by humanand mouse P-glycoprotein. Neuropharmacology. 2007
•Beyreuther BK et al. Lacosamide: a review of preclinical properties. CNS Drug Rev. 2007
•Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: a summary of the Ninth Eilat Conference (EILAT IX). Epilepsy Res. 2009
•Bialer M et al. Progress report on new antiepileptic drugs: a summary of the Eleventh Eilat Conference (EILAT XI).Epilepsy Res. 2013
•Bobustuc GC et al.Levetiracetam enhances p53-mediated MGMT inhibition and sensitizes glioblastoma cells to temozolomide. Neuro Oncol. 2010
•Calatozzolo C et al. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST-pi in human glioma. J Neurooncol. 2005
•Chen L et al. MicroRNA-107 inhibits U87 glioma stem cells growth and invasion. Cell Mol Neurobiol. 2013
•Chufan EE et al. Multiple transport-active binding sites are available for a single substrate on human P-glycoprotein(ABCB1). PLoS One. 2013
•Ciusani E et al.Valproic acid increases the in vitro effects of nitrosureas on human glioma cell lines.Oncol Res. 2007
•Kwan P et al. Drug-resistant epilepsy. N Engl J Med. 2011
•Kwiecińska P et al. Effects of valproic acid and levetiracetam on viability and cell cycle regulatory genes expression in the OVCAR-3 cell line. Pharmacol Rep. 2012
•Li XN et al. Valproic acid induces growth arrest, apoptosis, and senescence in medulloblastomas by increasing histone hyperacetylation and regulating expression of p21Cip1, CDK4, and CMYC. Mol Cancer Ther 2005
•Luna Tortós C et al. The Antiepileptic Drug Topiramate is a Substrate for Human P-glycoprotein but Not Multidrug Resistance Proteins. Pharm Res 2009
•Neuman MG, Nanau RM, Shekh-Ahmad T, Yagen B, Bialer M. Valproic acid derivatives signal for apoptosis and repair in vitro. Clin. Biochem. 2013
•Perucca E. What is the promise of new antiepileptic drugs in status epilepticus? Focus on brivaracetam, carisbamate, lacosamide, NS-1209, and topiramate. Epilepsia. 2009 Porojan D,
•Prakash O, Lukiw WJ, Peruzzi F, Reiss K, Musto AE. Gliomas and seizures. Med Hypotheses. 2012
•Stander M et al. Anticonvulsant drugs fail to modulate chemotherapyinduced cytotoxicity and growth inhibition of human malignant glioma cells. J Neurooncol 1998
•Zhang C et al. Potential role for human P-glycoprotein in the transport of lacosamide. Epilepsia. 2013
•Takai N et al., Human ovarian carcinoma cells: histone deacetylase inhibitors exhibit antiproliferative activity and potently induce apoptosis. Cancer. 2004 Dec 15;101(12):2760-70.
•Zhang QQ et al. MicroRNA-195 plays a tumor-suppressor role in human glioblastoma cells by targeting signaling pathways involved in cellular proliferation and invasion. Neuro Oncol. 2012 .
•Zhou Y et al. Histone deacetylase inhibitor, valproic acid, radiosensitizes the C6 glioma cell line in vitro. Oncol Lett. 2014 Jan;7(1):203-208. Epub 2013 Nov 7.
•Eyal S, Yagen B, Sobol E, Altschuler Y, Shmuel M, Bialer M. The activity of antiepileptic drugs as histone deacetylase inhibitors. Epilepsia 2004 Jul;45(7):737–744.