

## CALIXARENE GLYCOCONJUGATES INHIBIT C6 GLIOMA CELL PROLIFERATION AND MIGRATION: INTERACTION WITH GROWTH FACTORS

Viola Santa<sup>1</sup>, Consoli Grazia M.L.<sup>2</sup>, Merlo Sara<sup>1</sup>, Geraci Corrada<sup>2</sup>, Sortino Maria Angela<sup>1</sup>

<sup>1</sup>Department of Experimental and Clinical Pharmacology, University of Catania and <sup>2</sup>Institute of Biomolecular Chemistry, CNR, Valverde, Catania

Glioblastomas are among the most aggressive and invasive malignant tumours. A big effort is then made to identify novel therapeutic strategies to treat these tumours. We have tested the effect on the proliferation of C6 rat glioma cells of calixarene glycoconjugates exposing multiple copies of thioureyll *n*-acetyl-D-glucosamine (GlcNAc), C4- and C8-GlcNAc, based on the hypothesis that the interaction of GlcNAc with cell surface receptors could affect cell proliferation and migration. Treatment of C6 cells for 48 h with C4- and C8-GlcNAc (1-10  $\mu$ M), concentration-dependently, inhibited cell growth measured by the MTT assay (about 60-70% of control at maximal concentration). This effect was not accompanied by apoptotic cell death as shown by the lack of nuclear fragmentation following Hoechst staining. To our surprise, this effect was not due to interaction with the carbohydrate moiety as GlcNAc (1-10  $\mu$ M) *per se* was devoid of any effect whereas the calixarene scaffold bearing only the thioureyll group caused a marked inhibition of cell proliferation (about 50% at maximal concentration). Analysis of cell cycle after labelling with propidium iodide (50  $\mu$ g/ml) showed that, in the presence of C4- and C8-GlcNAc, C6 cells enter the S phase, but do not proceed to the G2/M phases of the cycle. Growth factors have been considered as potential targets of calixarene glycoconjugates. Accordingly, preincubation of 10  $\mu$ M C8-GlcNAc with epidermal growth factor (EGF) for 30 min prior to treatment of C6 cells, markedly reduced the inhibition of cell growth induced by C8-GlcNAc alone (65 vs. 81% of cell viability for C8-GlcNAc and C8-GlcNAc + EGF, respectively). In addition, assessment of ERK phosphorylation by a 10 min treatment with 20 ng/ml EGF was markedly reduced in C6 glioma cells pretreated with 10  $\mu$ M C8-GlcNAc. This effect appeared to be specific for the growth factor as the increase of ERK phosphorylation induced by exposure of C6 cells to 10 nM 17 $\beta$ -estradiol was not modified by C8-GlcNAc. C6 cell migration was evaluated using a monolayer scratch-wound model where confluent C6 cells were scratched with a pipette tip. Scratch-wound closure was then monitored by phase microscopy. Exposure of C6 cells to 10  $\mu$ M C8-GlcNAc markedly inhibited migration of cells into the gap, as by repeated observations at 8, 12 and 20 h after scratch-wound. Calixarene glycoconjugates, with mechanisms not completely identified as yet, seem to control glioma cell proliferation and migration without affecting cell viability, suggesting new potential strategies for the treatment of these tumours.