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The Tyrosine Kinases Inhibitors (TKI) Staurosporine/ Midostaurin Act as Cation Channel Inhibitors with Antiproliferative Effects in DIPG36 and DIPG50 Cells

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Ion channels and transporters are up-regulated in 33% and down-regulated in 48% of cases in paediatric tumour brain. No data are available on ion channels in Diffuse Intrinsic Pontine Glioma (DIPG) which is a rare, paediatric high-grade glioma of pons. Tyrosine kinases have been proposed as molecular targets. Staurosporine (STS) is a multitarget TKI with ion channel modulatory actions. Investigations are carried out on DIPG patient-derived H3.1K27M (DIPG36) and H3.3K27M (DIPG50) cells using staurosporine (STS) and its structural analogue midostaurin (MIDO). We investigated their antiproliferative effects using crystal violet staining and the 96 multi-well CCK-8 assay. On DIPG36, staurosporine 2.14 μM reduced cell survival by −68.94±0.82% and −100±0.40% after 48h and 72h of incubation time, respectively, while midostaurin 2.14 μM was more potent reducing cell survival by −100±18% at all incubation time. On DIPG50 cells, staurosporine 2.14 μM reduced cell survivals by −47±15.14% after 48h and −94±12.6% after 72h of incubation time, and midostaurin 2.14 μM reduced it by −63.85±14.56% after 48h and −97±12.9% after 72h of incubation time. These data were confirmed using clonogenic assay on DIPG36 cells, in which midostaurin 2.14 μM reduced the number of colonies from N = 345 to N = 0 after 72h of incubation time. We failed to collect data on clonogenic assay on the DIPG50 cells because they were not adherent. On concentration-response relationship investigations, both staurosporine and midostaurin (0.1μM-100μM), reduced cell proliferation in the sub-micromolar concentrations. The IC50 of STS in 96-wells CCK-8 assay after 48h of incubation time were: $5x10^{-10}$ M in DIPG50 and $8.9x10^{-8}$ in DIPG36 cells. Midostaurin showed an IC50 of 10^{-7} M and $4.739x10^{-7}$M respectively, after 48h and 72h of incubation times. The KATP channel blocker repaglinide (0.1μM-200μM) reduced cell proliferation with an IC50 of $80x10^{-6}$M on DIPG36 and $20x10^{-4}$M on DIPG50 cells, respectively, after 48h of incubation time. It also caused a partial reduction of the colonies formation while MIDO fully reduced it. Patch-clamp investigations of the whole-cell inward and outward cation currents showed that, the currents of DIPG36 cells (N cells = 20) were acutely inhibited by −74%±56 (+20mV Vm), −50%±43 (+40mV Vm) and −46%±30 (+60mV Vm) by STS 2.14μM vs controls. The STS-responsive currents were fully inhibited by the not selective K^+ ion channel blockers TEA-BaCl2 (5 mM) and were sensitive to the KATP channel blocker glibenclamide (100 mM). STS reduced the capsazepine-sensitive current recorded at +80mV Vm in the same cells. No data were collected with MIDO in this cell type because of seal instability. On DIPG50 cells (N cells = 3), midostaurin 2.14 mM at t = 0 failed to inhibit the control currents at negative potentials while after 20 min of incubation time reduced currents by −22% (−60mV Vm) and −28% (−80mV Vm) vs control. At positive potentials, at t = 0, MIDO reduced the control currents by −64% (+60mV Vm) with an increasing inhibitory effect of −97% after 20 min (+60mV Vm). These findings suggest that the unselective TKI STS and MIDO showed antiproliferative effects in either DIPG36 and DIPG50 cells and their action can be mediated by inhibition of cation channels.