ATP cytotoxicity on GL261 mouse glioma cell line and primary cultures of glioblastoma: evidences of $P2X_7$ purinoceptor involvement in glioma cell death

Alessandra S.K. Tamajusuku[1], Romela Paulus[1], Adriana Ritter[2], Bernardo Garicochea[2], Robson Coutinho-Silva[3], Ana M.O. Battastini[4], Márcia R. Wink[5] and Guido Lenz[1].

[1]Biophysics Department, UFRGS, [2]Dep. of Internal Medicine, PUC-RS, [3]Biophysics Institute, UFRJ; [4]Biochemistry Department, UFRGS, [5]Biochemistry Department, UFCSPA - Brazil.

Background: Gliomas are the most common and aggressive CNS tumor and their therapy resistance are well described. Previously we showed that interference with the purinergic signaling could reduce glioma growth. The objective of this study was investigating high ATP doses in glioma survival. **Methods:** Citotoxicity was measured by LDH release, propidium iodide incorporation and MTT assay. RT-PCR, qRT-PCR and Western Blot protocols were performed to analyze $P2X_7$ mRNA and protein presence. **Results:** Here we show that ATP is cytotoxic in the mouse glioma cell line GL261 and a primary culture of a glioblastoma (JJS). ATP 5mM induced PI incorporation and reduced cellular viability in GL261 and JJS cells. Experiments performed with the GL261 showed that ATP was toxic only at concentrations above 2mM, as indicated by increased LDH release, whereas other purinergic receptors agonists such as adenosine, ADP and UTP were not toxic at 5mM. BzATP, also at mM concentrations, stimulated cell death. The P2 receptors antagonists PPADS and oATP significantly reversed ATP-induced toxicity. $P2X_7$ expression, at mRNA and protein levels, in GL261, was confirmed. Besides, GL261 subpopulation more sensitive to ATP presents 1.7 times more P2X7 mRNA. **Conclusion:** These results showed that GL261 and JJS cells are sensible to ATP and suggest a substantial involvement of $P2X_7$ receptor in ATP-induced death, which were corroborated by the expression of the mRNA and protein of this receptor in GL261. Others P2Xs receptors can not be excluded, but their contribution in ATP citotoxicity seems to be of lower importance.

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