IV Abstract

Glioma treatment is a hurdle in medicine because of the deep penetration of glioma cells in the brain parenchyma. Recent studies highlight the role of microglia in the glioma cellular micro-milieu and, consequently, signify microglial cells as potential players in the treatment strategies. The role of microglial cells can also be extended to other brain pathologies such as neuromyelitis optica, a supposedly autoimmune disease of the central nervous system. Astrocytes, the most frequent glial cells in the brain, have come into focus, especially because of their prominent role in the pathology of neuromyelitis optica and their interactions with glioma cells. Microglial cells are native immune cells in the brain that interact with neurons, astrocytes, glioma, and other immune cells via direct or indirect pathways. Because of the abundance of glial cells in the brain and their role in different brain pathologies, especially in glioma and neuroautoimmune diseases, this study addressed the molecular-cellular level of the interactions between astrocytes, microglia, and glioma cells. These interactions were examined under anti-inflammatory and anticonvulsant drugs as well as under female sex steroid hormone. Anti-inflammatory agents and anticonvulsants are frequently used in a multitude of brain illnesses. In addition, the female sex steroid hormone was selected, since the predominance of either gender has been frequently noticed in brain illnesses such as glioma and neuroautoimmune diseases. For example, unlike neuromyelitis optica, glioma is less frequent in females than in men.

The expression of two proteins, connexin 43 (Cx43) and aquaporin 4 (AQP4), along with other biological parameters were investigated in vitro. Cx43 is the most abundant gap junction in the brain that is mainly expressed in the astrocytes. Gap junctions can facilitate the rapid transfer of certain molecules and ions in a cell syncytium. In addition, Cx43 has a multitude of actions in the cell proliferation, growth, adhesion, and migration. AQP4, on the other hand, is a selective bidirectional water channel that is widely expressed in the feet processes of astrocytes and regulates water homeostasis in the brain. Moreover, AQP4 has been implicated in the cell adhesion and migration.

Microglia are abundant in several brain pathologies. Therefore, a model of high or low microglia content, M30 or M5, was used to investigate the role of microglia in the regulation of AQP4 and Cx43 expression channels under medical treatments. Two rat glioma cell lines, F98 and C6, were selected because they are from different glioma categories and they are known to express different native levels of Cx43 expression. It was demonstrated that the number of microglia as well as the type of glioma cells exert differential responses in Cx43 and AQP4 expression. 17β-estradiol, a
female sex steroid hormone, induced opposite responses of Cx43 expression in glioma cells relative to the estradiol receptor expression. This finding advances our understanding for the etiology of glioma as well as for the gender differences and gender-based therapies in glioma. Estradiol increased AQP4 expression in the astrocytes of M30 cultures that implies more interacting binding sites in the astrocytes and possible worsening of NMO in pregnancy during which, a high level of estradiol is produced. In addition, levetiracetam and valproic acid, the commonly used anticonvulsants in glioma treatment, differentially regulated Cx43 expression and cell migration in F98 and C6 cell lines. This result shows that anticonvulsants have the potential to affect glioma progression with mechanisms beyond neuronal inhibition. Furthermore, levetiracetam and valproic acid significantly increased amoeboid type of microglia in the astrocyte-microglia-glioma co-cultures. The increased level of interleukin-6 along with the activation of microglia indicate that these anticonvulsants can promote glioma progression. Moreover, levetiracetam, but not valproic acid, increased the AQP4 expression in M30 and M30+C6 cultures which adds to our current understanding of the mechanism of action of levetiracetam in the brain. Microglia increased the glioma cell proliferation which is in line with the previous findings of this kind. Dexamethasone increased AQP4 expression in M30 cultures, but decreased this level in M5 cultures, indicating a differential role for dexamethasone in the physiologic and pathologic brain condition. Dexamethasone did not significantly alter AQP4 expression, whereas prednisolone differentially modified AQP4 expression in astrocyte-microglia-glioma co-cultures. This shows that prednisolone more likely interferes in glioma-induced brain edema treatment by modulating AQP4 expression. In addition, glioma cells which do not express AQP4 in monocultures, weakly expressed this protein in the astrocyte-microglia-glioma co-cultures. These findings suggest that glioma cells probably require a challenging environment to express AQP4. Lastly, the expression of AQP4 and Cx43 by microglia in astrocyte-microglia-glioma cultures provide new evidence for possible direct glioma-microglia interactions.