Microglia is an active player in how glibenclamide improves stroke outcome

Francisco J Ortega[1,2], Jukka Jolkkonen[2], Manuel J Rodríguez[1]

[1]*Unitat de Bioquímica i Biologia Molecular*, the *Facultat de Medicina*, the *Institut d'Investigacions Biomèdiques August Pi i Sunyer* (IDIBAPS), the *Universitat de Barcelona* and the *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas* (CIBERNED), Barcelona, Spain.

[2]Institute of Clinical Medicine – Neurology, University of Eastern Finland, Kuopio, Finland

Correspondence to: Dr. Francisco J Ortega
Unitat de Bioquímica i Biologia Molecular
Facultat de Medicina, UB
c/ Casanova 143
E-08036 Barcelona, SPAIN
Phone: +34 93 402 4525
FAX: +34 93 403 5882
e-mail: j.ortega@ub.edu

Running title: Microglia mediate glibenclamide-induced stroke recovery

Abstract

Glibenclamide has beneficial effects in animal models of cerebral ischemia. The cellular targets of glibenclamide are proposed to be neurons, endothelial cells, oligodendrocytes and astrocytes. However, independent studies have shown that the SUR1-formed ATP-dependent potassium (K_{ATP}) channels are expressed by microglia, which reinforces the idea that glibenclamide may also target microglia and modulate their inflammatory phenotype. This comment to 'Sulfonylurea receptor 1 in central nervous system injury: a focused review' provides new insights on the putative role of the microglial K_{ATP} channel in mediating, at least in part, the neuroprotective and neurorestorative effects of glibenclamide after stroke.

Keywords: Brain Ischemia, Chemokines, Glial Cells, Inflammation, Microglia, Trophic Factors

The recent review article 'Sulfonylurea receptor 1 in central nervous system injury: a focused review'[1] is an extensive summary of the current knowledge of the role of the sulfonylurea receptor 1 (SUR1) and SUR1-regulated $NC_{Ca\text{-}ATP}$ channels in acute brain injuries. The review also highlights the potential translational applicability of the use of glibenclamide in treating brain pathologies such as cerebral ischemia or traumatic brain injury. It is proposed that the cellular targets of glibenclamide are neurons, endothelial cells, oligodendrocytes and astrocytes, and that glibenclamide resolves the cytotoxic edema after cerebral ischemia by blockade of the astroglial $NC_{Ca\text{-}ATP}$ channel. However, independent studies have shown that the SUR1-formed ATP-dependent potassium (K_{ATP}) channels are expressed by microglia,[2-6] which reinforces the idea that glibenclamide may also target microglia and modulate their inflammatory phenotype in brain pathologies. More important, we recently showed that microglia after cerebral ischemia increase the expression of Kir6.2 and SUR1 components of the K_{ATP} channel in the lesion core[3] and also in the medial striatum of the ischemic hemisphere[4] (Figure 1A).

K_{ATP} channel's subunits possess an endoplasmic reticulum (ER)-retention motif, which prevents trafficking of mismatched subunits to the membrane. Our studies in rat primary microglial cultures suggested that microglial activation involves translocation of SUR1 from its internal reservoir toward the cell surface (Figure 1B). Furthermore, *in vitro* studies using BV2 microglia cells and primary microglial cultures have demonstrated that reactive microglia are sensitive to different K_{ATP} channel drugs regulating the phagocytic activity and the release of cytokines and chemokines.[2,3,5,6] Our findings of the glibenclamide-mediated enhancement of microglial *in vitro* phagocytosis was correlated with *in vivo* experiments, where increased clearance of cell debris and calcium was found in the infarcted hemisphere, and consequently provided an optimal neuroprotection in the surrounding tissue.[3] Although glibenclamide also blocks other ion channels that some belong to the ATP-binding cassette proteins (e.g., the Cystic fibrosis transmembrane conductance regulator) expressed by microglial cells, it is unlikely that glibenclamide will bind to these channels because they present much lower affinity to the drug than the used in our studies. Thus, our findings shed a new light on the putative role of the microglial K_{ATP} channel in mediating at least in part, the neuroprotective and neurorestorative effects of glibenclamide after stroke.

Simard and colleagues have described that the activation of $NC_{Ca\text{-}ATP}$ channels in astrocytes causes cell blebbing characteristic of cytotoxic edema. The glibenclamide-induced beneficial effect in MCAO animals was only linked with the blockade of these channels, whereas the involvement of K_{ATP} channels in the process has been excluded.[7,8] Interestingly,

Simard *et al*[7] only used inside-out patches of large neuron-like cells isolated from the core 2 h and 6 h after MCAO or isolated native reactive astrocytes type 1 (ref. 9). The K_{ATP} channel biophysical properties in other cell types isolated from the core or the peri-infarct area after brain ischemia, which could be also expressing SUR-1 regulated channels, were not assessed. Intriguingly, despite the massive neuronal loss observed, immunoblots revealed no concentration changes of Kir6.1 and Kir6.2 proteins in the ischemic core.[7,10] Our findings are consistent with these results and argue for a contribution of the microglial K_{ATP} channels to the neurorestorative effects of glibenclamide by reducing the severity of lesion. We observed that reactive microglia enhances SUR1, Kir6.1 and Kir6.2 protein expression, and amoeboid microglia express K_{ATP} channels in the necrotic core of the lesion.[3,4] Therefore this upregulation is certainly contributing to the enhancement of SUR1 found by Simard *et al*,[7] and helped to compensate for a putative decrease in Kir6.1 and Kir6.2 subunits due to the massive neuronal loss in the infarct zone.

On the other hand, we also observed that glibenclamide increased the number of migrating neuroblasts toward the ischemic core 72 h after reperfusion, thereby indicating that glibenclamide modifies the cell lineage choice or enhances progenitor cell proliferation and migration.[4] As adult neural precursor cells do not express the Kir6.1 neither the Kir6.2 subunits, is unlikely that they will express functional K_{ATP} channels or present sensitivity to the glibenclamide treatment. However, microglia which are the primary immune effector cells in the brain and as a component of the neurogenic niche, participate in promoting the proliferation, migration and differentiation of neural precursors cells by the release of a wide panel of bioactive molecules, including neurotransmitters, purines, cytokines and growth factors. Interestingly, reactive microglia in the medial striatum expressed the K_{ATP} channel components SUR1 and Kir6.2 at 72 hours after ischemia (Figure 1A), suggesting participation over longer time intervals in post-ischemic regeneration and neurogenesis. Complementary *in vitro* studies showed that the specific blockade of the microglial K_{ATP} channel cause the release of soluble factors that enhance the activation of neural precursors cell from the subventricular zone (unpublished data). Therefore, ongoing studies will help us to elucidate the complex interplay of ischemic and inflammatory signals responsible for the role of the microglial K_{ATP} channel in endowing microglia to a new distinct phenotype promoting brain repair after injury.

Taken together, the involvement of the K_{ATP} channel expressed by microglia can contribute to the beneficial effects of glibenclamide on stroke models. Our data on the glibenclamide-mediated control of the microglia activity through K_{ATP} channel blockade

argues for a multifunctional neuroprotective effect of SUR targeting after brain injuries. This is consistent with the idea of cross-talk between multiple cell types and death mechanisms after cerebral ischemia. By briefly summarizing the current state of knowledge in this area, this commentary hopefully provides a new and complementary insight on the neuroprotective and neurorestorative effects of glibenclamide after stroke.

Disclosures

MJR holds an EU patent (No. WO2006/000608). The other authors report no disclosures.

Ethics statement

All of the experiments reviewed were approved by the Ethics Committee of the Universitat de Barcelona, in accordance with the regulations from the Catalan government (Generalitat de Catalunya) or the Animal Ethics Committee (Hämeenlinna, Finland). Animals were handled following European legislation (86/609/EEC) and all efforts were made to minimize the number used and animal suffering, in accordance with the ARRIVE guidelines.

References

1. Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V. Sulfonylurea receptor 1 in central nervous system injury: a focused review. *J Cereb Blood Flow Metab* 2012; 32: 1699-1717.
2. Liu X, Wu J-Y, Zhou F, Sun X-L, Yao H-H, Yang Y *et al*. The regulation of rotenone-induced inflammatory factor production by ATP-sensitive potassium channel expressed in BV-2 cells. *Neurosci Lett* 2006; 394: 131-135.
3. Ortega FJ, Gimeno-Bayon J, Espinosa-Parrilla JF, Carrasco JL, Batlle M, Pugliese M *et al*. ATP-dependent potassium channel blockade strengthens microglial neuroprotection after hypoxia-ischemia in rats. *Exp Neurol* 2012; 235: 282-296.
4. Ortega FJ, Jolkkonen J, Mahy N, Rodriguez MJ. Glibenclamide enhances neurogenesis and improves long-term functional recovery after transient focal cerebral ischemia. *J Cereb Blood Flow Metab* 2013; 33:356-364.
5. Virgili N, Espinosa-Parrilla JF, Mancera P, Pastén-Zamorano A, Gimeno-Bayon J, Rodríguez MJ *et al*. Oral administration of the K_{ATP} channel opener diazoxide ameliorates disease progression in a murine model of multiple sclerosis. *J Neuroinflammation* 2011; 8:149.
6. Zhou F, Yao H-H, Wu J-Y, Ding J-H, Sun T, Hu G. Opening of microglial K(ATP) channels inhibits rotenone-induced neuroinflammation. *J Cell Mol Med* 2008; 12: 1559-1570.
7. Simard JM, Chen M, Tarasov KV, Bhatta S, Ivanova S, Melnitchenko L *et al*. Newly expressed SUR1-regulated $NC_{Ca\text{-}ATP}$ channel mediates cerebral edema after ischemic stroke. *Nat Med* 2006; 12: 433-440.
8. Simard JM, Yurovsky V, Tsymbalyuk N, Melnichenko L, Ivanova S, Gerzanich V. Protective effect of delayed treatment with low-dose glibenclamide in three models of ischemic stroke. *Stroke* 2009; 40: 604-609.
9. Chen M, Dong Y, Simard JM. Functional coupling between sulfonylurea receptor type 1 and a nonselective cation channel in reactive astrocytes from adult rat brain. *J Neurosci* 2003; 23: 8568-8577.
10. Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. Glibenclamide is superior to decompressive craniectomy in a rat model of malignant stroke. *Stroke* 2010; 41: 531-537.

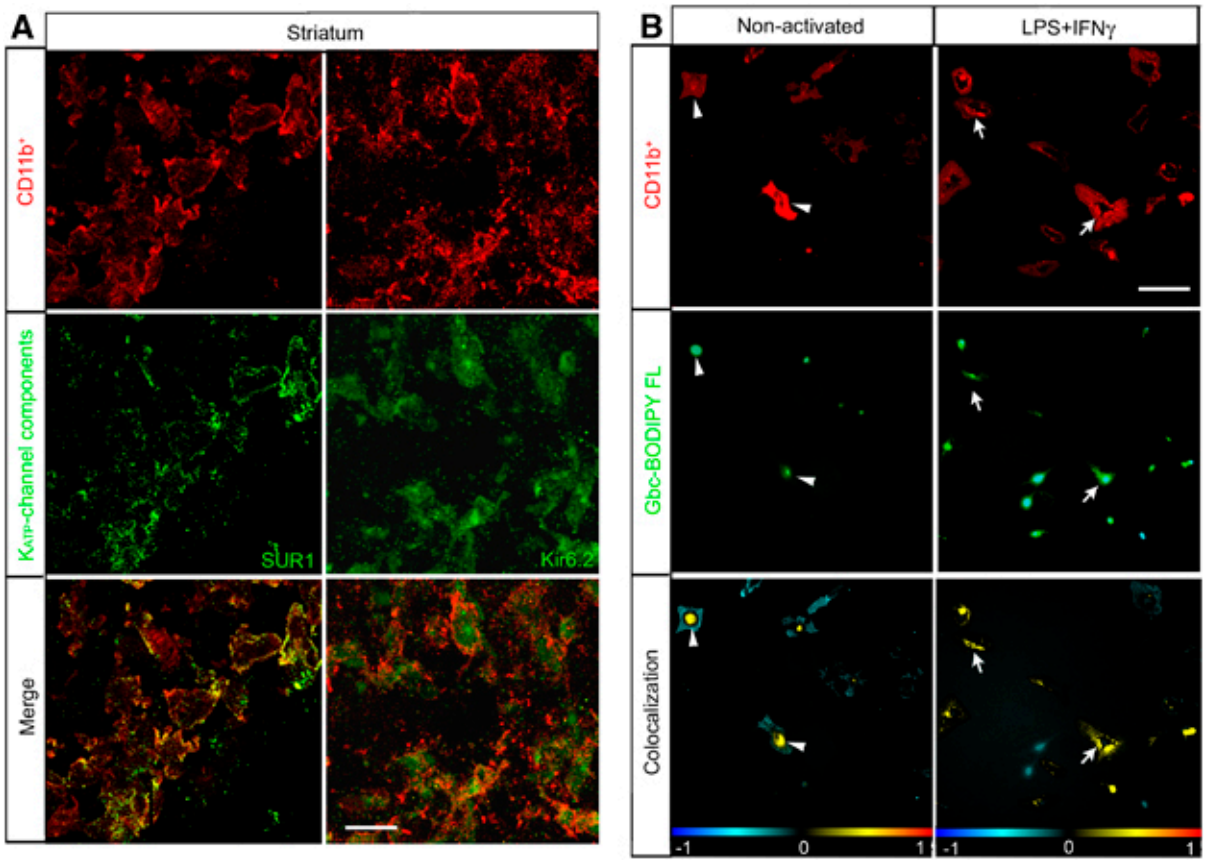

Figure 1- Reactive microglia express and translocate sulfonylurea receptor 1 (SUR1) to the cell surface. (**A**) Confocal photomicrographs of SUR1 and Kir6.2 (green) in reactive microglia (CD11b-positive; red) localized to the medial striatum in middle cerebral artery occlusion rats. Yellow in the merge image denotes colocalization, whereby reactive CD11b-postive cells expressed SUR1 or Kir6.2 72 hours after ischemia. (**B**) Localization of glibenclamide (Gbc) (Gbc BODIPY FL; green fluorescence) in rat microglial primary culture. Non-activated or cultures activated with lipopolysaccharide (LPS)+interferon gamma (IFNγ) for 48 hours are shown in the upper row. Microglial cells were labeled with an anti-CD11b (red) antibody and Hoechst (blue) to stain the nuclei. Lower row shows respective colocalization of the red and green channels, where the yellow denotes the presence of the Gbc binding in microglial cells. Arrowhead denotes perinuclear colocalization and arrows show surface labeling. The data shown are representative of four experiments each. Scale bar in (**A**) is 15 μm and in (**B**) is 20 μm. From Ortega *et al.*[4]