



Diazoxide blocks or reduces microgliosis when applied prior or subsequent to motor neuron injury in mice

Bernat Nogradi^{a,b}, Valeria Meszlenyi^{a,b}, Roland Patai^a, Tamas F. Polgar^a, Krisztina Spisak^a, Rebeka Kristof^a, Laszlo Siklos^{a,*}

^a Institute of Biophysics, Biological Research Centre, Szeged, Temesvári krt. 62, 6726 Szeged, Hungary

^b Foundation for the Future of Biomedical Sciences in Szeged, Szeged Scientists Academy, Pálffy u. 52/d, 6725 Szeged, Hungary

HIGHLIGHTS

- Diazoxide blocks axotomy induced microgliosis when applied prior to the injury.
- Diazoxide reduces microgliosis when applied after the axotomy.
- Oculomotor nucleus exhibits reduced microgliosis compared to other motor nuclei.
- Diazoxide restores altered mitochondrial morphology in motoneurons after axotomy.

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ABSTRACT

Diazoxide (DZX), an anti-hypertonic and anti-hypoglycemic drug, was shown to have anti-inflammatory effects in several injured cell types outside the central nervous system. In the brain, the neuroprotective potential of DZX is well described, however, its anticipated anti-inflammatory effect after acute injury has not been systematically analyzed. To disclose the anti-inflammatory effect of DZX in the central nervous system, an injury was induced in the hypoglossal and facial nuclei and in the oculomotor nucleus by unilateral axonal transection and unilateral target deprivation (enucleation), respectively. On the fourth day after surgery, microglial analysis was performed on tissue in which microglia were DAB-labeled and motoneurons were labeled with immunofluorescence. DZX treatment was given either prophylactically, starting 7 days prior to the injury and continuing until the animals were sacrificed, or postoperatively only, with daily intraperitoneal injections (1.25 mg/kg; in 10 mg/ml dimethyl sulfoxide in distilled water). Prophylactically + postoperatively applied DZX completely eliminated the microglial reaction in each motor nuclei. If DZX was applied only postoperatively, some microglial activation could be detected, but its magnitude was still significantly smaller than the non-DZX-treated controls. The effect of DZX could also be demonstrated through an extended period, as tested in the hypoglossal nucleus on day 7 after the operation. Neuronal counts, determined at day 4 after the operation in the hypoglossal nucleus, demonstrated no loss of motor neurons, however, an increased Feret's diameter of mitochondria could be measured, suggesting increased oxidative stress in the injured cells. The increase of mitochondrial Feret's diameter could also be prevented with DZX treatment.

1. Introduction

Diazoxide (7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-

dioxide; DZX), which was originally introduced to lower blood pressure and treat hypoglycemia, is nowadays considered as a drug with multiple targets (Coetzee, 2013). Among other effects, it has been shown to

Abbreviations: A β_{1-42} , amyloid- β ; a.u., arbitrary units; K_{ATP}, adenosine-5'-triphosphate dependent potassium; ATP, adenosine-5'-triphosphate; CCD, charge-coupled device; CH, convex hull; ChAT, choline acetyltransferase; CNS, central nervous system; DAB, diaminobenzidine tetrahydrochloride; DMSO, dimethyl sulfoxide; DZX, diazoxide; Iba1, ionized calcium-binding adaptor molecule 1; mK_{ATP}, mitochondrial adenosine-5'-triphosphate dependent potassium; PBS, phosphate buffered saline; RTV, real-time viewing; sCMOS, scientific complementary metal-oxide semiconductor; s.e.m., standard error of the mean; TPBS, phosphate buffered saline with 0.2% triton x-100; VEH, vehicle (dimethyl sulfoxide (DMSO))

* Corresponding author at: Institute of Biophysics, Biological Research Centre, Szeged, Temesvári krt. 62, P.O. Box 521, 6726 Szeged, Hungary.

E-mail address: siklos.laszlo@brc.hu (L. Siklos).

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