From metabolic neurotoxicity to hypoxic neuroprotection: an MRS study of cyclosporine role in rat brain.

Natalie SERKOVA Dr1, Paul Donohoe Dr2, Sven Gottschalk3, Carsten Hainz4, Uwe Christians Dr5, Dieter LEIBFRITZ6

1University of Bremen, Dept. Chemistry/ Biology, Leobener Str. NW2, Bremen, Germany; 2University of California, San Francisco, Anesthesiology Dept.; 3University of Bremen, Germany; 4University of Bremen, Dept. Chemistry/ Biology, Germany; 5University of California, San Francisco, School of Pharmacy, San Francisco, CA USA; 6University of Bremen, Bremen, Germany;

Introduction

Cyclosporine (CsA) is a major immunosuppressant after organ transplantation. To date, two controversial facts about CsA have been reported. Paradoxically, on one hand, CsA causes neurotoxicity via unknown mechanisms in up to 60% of transplant patients, which requires the withdrawal of CsA therapy. On the other hand, in contrast to its neurotoxic potential, significant neuroprotective effects against ischemia have been reported for CsA recently. It was speculated that whether CsA is neurotoxic or neuroprotective is concentration-dependent: high concentrations of CsA cause neurotoxicity, while concentrations below the therapeutic range are neuroprotective. Thus, it was our goal to evaluate and to compare the dose-dependent neuroprotective effects of CsA on rat brain metabolism in normoxia as well as its neuroprotective biochemical mechanisms under hypoxia.

Methods

To assess in vivo CsA toxicity, rats were orally treated with 10 mg/kg/day CsA. After 12 hours of the last dose, brains were removed and their perchloric acid (PCA) extracts were analyzed at Bruker 360 MHz high-resolution spectrometer. To evaluate in vitro CsA effects, cerebral brain slices (350 µm thick, from 8-day-old rats) were treated with CsA when perfused with 5mM [1-13C] glucose for 4 hours. Ex vivo 31P- and 13C-MRS of perfused rat brain slices was carried out using a Nalorac 4.7 Tesla animal scanner or a Bruker 600 MHz spectrometer, respectively.

For experiments with brain slices under hypoxia (<5 mmHg O2), oxygen in the perfusion medium was switched to nitrogen. Neuroprotective effects of CsA were evaluated by 31P-MRS during 30 min of hypoxia and subsequent reperfusion. Slices were pre-treated with CsA for 4 hours. After 31P-MRS, brain slices were quickly frozen, extracted with PCA and analyzed using a Bruker 360 MHz spectrometer.

In addition, to study changes in intracellular calcium, rat hippocampal neurons were loaded with the fluorescent agent Fura 2-AM. The enzyme activities were measured using specific enzyme assays, and the generation of reactive oxygen species (ROS) was assessed by monitoring fluorescence of DCF-diacetate. CsA distribution into rat brain as well as into brain slices was measured using a sensitive LC/MS assay.

Results

I. Normoxic neurotoxicity: After 6 days of treatment, CsA reduced oxidative glucose metabolism (glutamate: 78% of control; GABA: 67%; NAD+: 76%; n=5; P<0.05). The same MRS-detectable changes were found in perfused rat brain slices (500 µg/l CsA: ATP: 84% of control, phosphocreatine: 73%; n=6; P<0.01) via inhibition of the Krebs cycle (decrease of glutamate) and oxidative phosphorylation (decrease of NAD+). CsA interfered with glucose metabolism at its entrance since low fluxes through pyruvate dehydrogenase (PDH) were detected. The MRS-detectable decrease of NAD+ concentration was confirmed by LC/MS analysis.

II. Hypoxic neuroprotection: However, during and after 30 min hypoxia, the same CsA concentration (500 µg/l) almost completely prevented the increase of intracellular calcium: 4.1-fold [Ca2+] increase without CsA vs. 1.4-fold with CsA; n=12; P<0.001. CsA (500 µg/l) protected high-energy phosphate metabolism as well as the intracellular pH (6.2 without CsA vs. 6.6 with CsA; n=4; P<0.02) in perfused rat brain slices during 30 min of hypoxia and reperfusion. Although in the presence of CsA the ATP concentrations in brain slices prior to the induction of hypoxia were only 80% of the controls without CsA (which corresponds to CsA neurotoxicity after 4 hours of pre-treatment), the energy state remained in the better condition in CsA-treated slices during and after 30 min hypoxia: (11% without CsA vs. 35% with CsA; n=4; P<0.05).

Discussion

Our results showed that CsA has the potential to cause neurotoxicity and neuroprotection simultaneously and the effective concentration ranges for toxicity and protection significantly overlap. The net effect, however, depends on the concentration of oxygen: in the presence of oxygen the negative effects of CsA on brain energy metabolism are more important, during hypoxia, the protective effects. Based on our results, we hypothesize that CsA toxicity and protection against ischemic and reperfusion injury are based on the same mechanism. CsA binds with the high affinity at its cellular immunophilin, cyclophilin D. Cyclophilin D is largely distributed in brain mitochondria and has been shown to be involved in the regulation of Ca2+ efflux pores as well as the mitochondrial permeability transition (MPT) pore resulting in activating of mitochondrial transition. Our results and those from the literature provide strong evidence that under normoxic conditions CsA, probably through inhibition of cyclophilin-regulated Ca2+ efflux, leads to Ca2+ accumulation in mitochondria with the subsequent generation of ROS and inhibition of TCA cycle and energy production. In contrast, during hypoxia and reperfusion, CsA inhibits opening of the MPT pore by inactivating cyclophilin D, prevents Ca2+ efflux into the cytosol and ultimately maintains energy homeostasis. Thus, hypoxic neuroprotection and normoxic metabolic neurotoxicity by CsA arise primarily from its interaction with mitochondria.

References