Neuroscience 253 (2013) 380–388

TAUROURSODEOXYCHOLIC ACID PROTECTS RETINAL NEURAL CELLS FROM CELL DEATH INDUCED BY PROLONGED EXPOSURE TO ELEVATED GLUCOSE

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Abstract—Diabetic retinopathy is one of the most frequent causes of blindness in adults in the Western countries. Although diabetic retinopathy is considered a vascular disease, several reports demonstrate that retinal neurons are also affected, leading to vision loss. Tauroursodeoxycholic acid (TUDCA), an endogenous bile acid, has proven to be neuroprotective in several models of neurodegenerative diseases, including models of retinal degeneration. Since hyperglycemia is considered to play a central role in retinal cell dysfunction and degeneration, underlying the progression of diabetic retinopathy, the purpose of this study was to investigate the neuroprotective effects of TUDCA in rat retinal neurons exposed to elevated glucose concentration. We found that TUDCA markedly decreased cell death in cultured retinal neural cells induced by exposure to elevated glucose concentration. In addition, TUDCA partially prevented the release of apoptosis-inducing factor (AIF) from the mitochondria, as well as the subsequent accumulation of AIF in the nucleus. Biomarkers of oxidative stress, such as protein carbonyl groups and reactive oxygen species production, were markedly decreased after TUDCA treatment as compared to cells exposed to elevated glucose concentration alone. In conclusion, TUDCA protected retinal neural cell cultures from cell death induced by elevated glucose concentration, decreasing mito-nuclear translocation of AIF. The antioxidant properties of TUDCA might explain its cytoprotection. These findings may have relevance in the treatment of diabetic retinopathy patients.

Key words: retina, diabetes, diabetic retinopathy, TUDCA, neural apoptosis.

INTRODUCTION

Diabetic retinopathy is a leading cause of vision loss and blindness among working age adults. Diabetic retinopathy has been considered a microvascular disease characterized by increased vascular permeability, appearance of macular edema, and neovascularization, which usually occurs at the later stages of the disease and can lead to blindness (Antonetti et al., 2006). However, increasing evidence has shown that the neural components of the retina are also affected, even before the detection of microvascular changes. Alterations in electroretinograms in diabetic patients and animals, and loss of color and contrast sensitivity are early signs of neuronal dysfunction in the retina (Roy et al., 1986; Daley et al., 1987; Sakai et al., 1995; Kohzaki et al., 2008; Aung et al., 2013). Apoptosis and increased caspase activation in retinal neurons, as well as retinal thinning have been noted in diabetic animals (Barber et al., 1998, 2005; Lieth et al., 2000; Krady et al., 2005; Gastinger et al., 2006; Whitmire et al., 2011; Yang et al., 2012). In postmortem human retinas, an increase in the levels of apoptosis-inducing factor (AIF) was also reported (Abu El-Asrar et al., 2007). Moreover, long-term exposure (7 days) of primary retinal neural cell cultures to elevated glucose concentration, to mimic chronic elevated hyperglycemia, increases cell death by a caspase-independent mechanism, mediated by the translocation of AIF from the mitochondria to the nucleus (Santiago et al., 2007).

Tauroursodeoxycholic acid (TUDCA) is an endogenous hydrophilic bile acid that is produced at very low levels in humans. TUDCA is formed by the conjugation of ursodeoxycholic acid (UDCA) with taurine. Several reports have demonstrated the hepatic cytoprotective effects of UDCA and TUDCA, with UDCA

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Abbreviations: AGEs, advanced glycation end products; AIF, apoptosis-inducing factor; BBS, Bardet–Biedl syndrome; DCF, dichlorodihydrofluorescein; DNPH, 2,4-dinitrophenylhydrazine; DPBS, Dulbecco's PBS solution; ECF, enhanced chemifluorescence; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; FITC, fluorescein isothiocyanate; H_2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; PS, phosphatidylserine; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; TUDCA, tauroursodeoxycholic acid; TUNEL, terminal transferase dUTP nick end labeling; UDCA, ursodeoxycholic acid.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.08.053

being widely used in clinical applications such as for the treatment of primary biliary cirrhosis and various other cholestatic disorders (Maillette de Buy Wenniger and Beuers, 2010). Interestingly, it has been proven that TUDCA is neuroprotective in a variety of experimental systems including models of neurodegenerative disorders such as Alzheimer's and Huntington's, as well as against damage induced by ischemia and hemorrhagic stroke (McLean, 1997; Keene et al., 2002; Rodrigues et al., 2003; Ramalho et al., 2008; Viana et al., 2009; Fernandez-Sanchez et al., 2011). The protective effects of TUDCA are considered to be mainly due to its ability to inhibit apoptosis by reducing Bax translocation to mitochondrial membrane, cytochrome c release from mitochondria to cytoplasm, caspase activation and DNA and nuclear fragmentation (Amaral et al., 2009). Besides these antiapoptotic properties, TUDCA can also function as an anti-inflammatory, immunomodulatory and antioxidant agent (Amaral et al., 2009), and activates the PI3-K/Akt-dependent survival pathway (Sola et al., 2003; Amaral et al., 2009). The molecular mechanisms underlying TUDCA neuroprotective properties appear to be complex and may engage a number of different molecular targets, possibly involving gene regulation, which result in strong anti-apoptotic, anti-inflammatory, immunomodulatory and antioxidant properties (Amaral et al., 2009).

Regarding retinal diseases, TUDCA greatly slowed retinal degeneration in light-induced retinal degeneration and rd10 mice. Retinal function was improved in mice treated with TUDCA, and retinas of TUDCA-treated mice had thicker outer nuclear layers, more photoreceptor cells, and more fully-developed photoreceptor outer segments. TUDCA effects were explained by dramatic suppression of apoptosis in both models (Boatright et al., 2006). Subsequent studies, confirmed that TUDCA protects retinal damage induced by light and oxidative stress in animal models of retinitis pigmentosa (Phillips et al., 2008; Fernandez-Sanchez et al., 2011; Oveson et al., 2011). Finally, TUDCA showed neuroprotective effects on photoreceptor cells in an experimental retinal detachment model, which were associated with decreased oxidative stress and caspase activity (Mantopoulos et al., 2011). Recently, TUDCA was shown to reduce endoplasmic reticulum stress, prevented apoptosis, and reduced cone degeneration in a mouse model of Leber congenital amaurosis (Zhang et al., 2000). Interestingly, TUDCA ameliorated the obesity that accompanies retinal degeneration in a mouse model of Bardet–Biedl syndrome (BBS) type 1, an autosomal recessive ciliopathy that causes severe retinal degeneration in humans (Drack et al., 2011).

Since hyperglycemia, the hallmark of diabetes, is considered to play a central role in retinal cell dysfunction and degeneration, underlying the progression of diabetic retinopathy, the purpose of this study was to investigate for the first time the potential neuroprotective effects of TUDCA in rat retinal neurons against cell death induced by elevated glucose concentration.

EXPERIMENTAL PROCEDURES

Primary cultures of rat retinal neural cells

All procedures involving animals were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

Retinal cell cultures were prepared as previously described (Santiago et al., 2006a). Briefly, 3- to 4-day-old Wistar rat pups were decapitated, and the retinas were dissected, using a light microscope, in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (in mM: 137 NaCl, 5.4 KCl, 0.45 KH_2PO_4, 0.34 Na_2HPO_4, 4 $NaHCO_3$, 5 glucose; pH 7.4). The retinas were digested with 0.05% trypsin (w/v) for 10 min at 37 °C. After dissociation, the cells were pelleted by centrifugation and resuspended in Eagle's minimum essential medium (MEM) supplemented with 26 mM $NaHCO_3$, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were plated at a density of 2×10^6 cell/cm^2 on poly-D-lysine (0.1 mg/ml)-coated plates. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2/air. After 2 days in culture, the cells were incubated with 25 mM D-glucose (yielding a total 30 mM glucose) or with 25 mM D-mannitol (plus 5 mM glucose), which was used as an osmotic control, and maintained for further 7 days. The concentration of glucose in control conditions was 5 mM (control). These conditions are identical to those described previously (Santiago et al., 2007). TUDCA (100 μM) was added at culture day two, and every other day.

Annexin-V fluorescein isothiocyanate (FITC) staining

The loss of plasma membrane asymmetry is one of the earliest features in the apoptotic program. In apoptotic cells, phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane. Annexin V is a Ca^{2+} dependent phospholipid-binding protein with high affinity for PS. Annexin V staining was performed using a kit from BD Biosciences Clontech (San Jose, CA, USA) following the instructions provided by the manufacturer. Briefly, cells were rinsed and then incubated with Annexin V-FITC (20 μg/ml in Tris-NaCl) for 10 min in the dark at room temperature. Cells were washed and mounted on a glass slide. The preparations were visualized immediately with a Zeiss Axioshop 2 Plus microscope. For each experimental condition, at least five random fields were counted in each coverslip.

Terminal transferase dUTP nick end labeling (TUNEL) staining

TUNEL, with fluorescein detection, was performed in cultured retinal neural cells, following the instructions provided by the manufacturer (Promega, Madison, WI, USA). Briefly, cells were washed in PBS and fixed in 4% paraformaldehyde for 20 min at room temperature. After washing, the cells were permeabilized with 0.2% Triton X-100 in PBS for 5 min. The cells were rinsed and