Brain Research 1676 (2017) 57–68

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Uridine treatment protects against neonatal brain damage and long-term cognitive deficits caused by hyperoxia

Bulent Goren [a], Aysen Cakir [a], Cansu Sevinc [b], Sema Serter Kocoglu [c], Busra Ocalan [a], Ceren Oy [c], Zehra Minbay [c], Nevzat Kahveci [a], Tulin Alkan [a,*], Mehmet Cansev [b]

[a] Uludag University School of Medicine, Department of Physiology, Bursa, Turkey
[b] Uludag University School of Medicine, Department of Pharmacology, Bursa, Turkey
[c] Uludag University School of Medicine, Department of Histology and Embryology, Bursa, Turkey

ARTICLE INFO

Article history:
Received 26 May 2017
Received in revised form 7 September 2017
Accepted 8 September 2017
Available online 15 September 2017

Keywords:
Hyperoxia
Brain injury
Uridine
Neonatal rat
Behavior
Learning and memory

ABSTRACT

Exposure to excessive oxygen in survivors of preterm birth is one of the factors that underlie the adverse neurological outcome in later life. Various pathological changes including enhanced apoptotic activity, oxidative stress and inflammation as well as decreased neuronal survival has been demonstrated in animal models of neonatal hyperoxia. The aim of the present study was to investigate the effect of administering uridine, an anti-apoptotic agent, on cellular, molecular and behavioral consequences of hyperoxia-induced brain damage in a neonatal rat model. For five days from birth, rat pups were either subjected continuously to room air (21% oxygen) or hyperoxia (80% oxygen) and received daily intraperitoneal (i.p.) injections of saline (0.9% NaCl) or uridine (500 mg/kg). Two-thirds of all pups were sacrificed on postnatal day 5 (P5) in order to investigate apoptotic cell death, myelination and number of surviving neurons. One-thirds of pups were raised through P40 in order to evaluate early reflexes, sensorimotor coordination and cognitive functions followed by investigation of neuron count and myelination. We show that uridine treatment reduces apoptotic cell death and hypomyelination while increasing the number of surviving neurons in hyperoxic pups on P5. In addition, uridine enhances learning and memory performances in periadolescent rats on P40. These data suggest that uridine administered during the course of hyperoxic insult enhances cognitive functions at periadolescent period probably by reducing apoptotic cell death and preventing hypomyelination during the neonatal period in a rat model of hyperoxia-induced brain injury.

1. Introduction

Premature birth is a major problem all over the world and preterm infants have increased risk of mortality and morbidity despite recent advances in neonatal medicine (Manuck et al., 2016). Preterm infants are vulnerable to several complications including respiratory distress syndrome, bronchopulmonary dysplasia and chronic lung disease, intestinal injury, compromised immune system, cardiovascular disorders, as well as hearing/vision and neurological problems (Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes, 2007). Up to 50% of surviving extremely preterm infants show cognitive deficits or behavioral problems during the later stages of development (Volpe, 2001).

Most preterm infants are exposed to supraphysiological oxygen therapy during perinatal period in the neonatal intensive care unit (NICU). Although oxygen treatment is required for several purposes including resuscitation, pulmonary hypertension and respiratory distress, hyperoxia itself exhibits toxic effects on premature infants such as bronchopulmonary dysplasia (BPD) (Gien and Kinsella, 2011), retinopathy of prematurity (ROP) (Saugstad, 2006) and white and grey matter damage in the brain (Collins et al., 2001; Felderhoff-Mueser et al., 2004; Reich et al., 2016).

The pathological consequences of supraphysiological oxygen in the brain include enhanced oxidative stress, inflammation and matrix metalloproteinase activity accompanied by increased apoptotic cell death and reduced neuro-glial development (Sifringer et al., 2009, 2010, 2015; Brehmer et al., 2012; Endesfelder et al., 2017). Various treatment approaches have been tested experimentally in order to ameliorate hyperoxia-induced brain damage in neonates including erythropoietin (Yis et al., 2008b; Sifringer

* Corresponding author at: Uludag University School of Medicine, Department of Physiology, Gorukle Campus, Bursa 16059, Turkey.
E-mail address: talkan@uludag.edu.tr (T. Alkan).

http://dx.doi.org/10.1016/j.brainres.2017.09.010
0006-8993/

et al., 2009, 2010), caffeine (Endesfelder et al., 2017), acetylcholinesterase (AChE) inhibitors (Sifringer et al., 2013), dexmedetomidine (Sifringer et al., 2015), topiramate (Kurul et al., 2009) and, recently, fingolimod (Serdar et al., 2016) and mesenchymal stem cells (Kim et al., 2016) in order to reduce oxidative stress, inflammation and apoptosis, thereby providing neuroprotection.

Uridine is the principal pyrimidine nucleoside in human blood circulation (Wurtman et al., 2000; Cansev, 2006) and a building block of nucleic acids which also exhibits beneficial effects in the brain when given exogenously. We previously showed that uridine provides neuroprotection in a neonatal rat model of hypoxic-ischemic encephalopathy (HIE) by reducing apoptotic cell death (Cansev et al., 2013) and inhibiting histone deacetylase (HDAC) activity (Koyuncuoglu et al., 2015). We further showed that, uridine treatment during neonatal term ameliorates the cognitive impairment during periadolescent period in the same rat model (Goren et al., 2017). Since cell death is the predominant form of injury following hyperoxia exposure (Brehmer et al., 2012), the aim of the present study was to investigate the effect of treatment with uridine, an anti-apoptotic agent, in a rat model of hyperoxic brain damage.

We show in the present study that, intraperitoneal (i.p.) uridine administration for five consecutive days during hyperoxic insult significantly decreases apoptotic cell death, prevents hypomyelination and increases the number of surviving neurons in CA1 and CA3 regions of the hippocampus in 5 days-old rat pups. Moreover, treatment with uridine enhances learning and memory performances of hyperoxic rats during periadolescent period. Apparently, the beneficial effect of uridine with regard to providing neuroprotection is probably mediated by preventing apoptosis and hypomyelination in the brain during neonatal period which is reflected by amelioration of cognitive impairment during periadolescent period. These data suggest that uridine might confer benefit in treatment of hyperoxic brain damage in human neonates.

2. Results

2.1. Effects of uridine treatment on apoptotic cell death on P5

In order to evaluate apoptotic cell death in the brain, the expression of cleaved Caspase-3 protein in brain homogenates as well as DNA fragmentation in brain sections of P5 pups were analyzed using western blotting and terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) assay, respectively.

Number of TUNEL(+) cells per unit area were significantly increased in pups in Hyperoxia + Saline group in CA1 (22.9 ± 0.9 vs. 14.8 ± 1.3; $p < 0.001$) and CA3 (16.6 ± 0.9 vs. 11.9 ± 0.5; $p < 0.01$) regions of hippocampi compared to those in Normoxia + Saline group while uridine (500 mg/kg; i.p.) administration for 5 consecutive days decreased the number of TUNEL(+) cells to 15.3 ± 1 ($p < 0.001$) and 12.5 ± 1.3 ($p < 0.05$) in CA1 and CA3 regions, respectively (Fig. 1B and C).

Compared to Normoxia + Saline group, cleaved Caspase-3/β-tubulin ratio was increased in brains of P5 pups that were subjected to hyperoxic insult (Hyperoxia + Saline group) by 46.7% ($p < 0.001$), while uridine administration reduced this ratio, approaching to control levels, significantly ($p < 0.01$) (Fig. 1D).

2.2. Effects of uridine treatment on hippocampal neuron count in P5 pups

Mean number of neurons counted by cresyl violet staining were decreased significantly from 85.5 ± 1.4 or 70.4 ± 1.4 in Normoxia + Saline group to 47.6 ± 0.8 ($p < 0.001$) or 43.8 ± 1.1 ($p < 0.001$) in Hyperoxia + Saline group in CA1 or CA3 region of hippocampi in P5 pups, respectively (Fig. 2). Uridine treatment increased mean number of surviving neurons to 81.7 ± 1.9 ($p < 0.001$) or 69.9 ± 1.6 ($p < 0.001$) in CA1 or CA3 region, respectively (Fig. 2).

2.3. Effects of uridine treatment on myelination in P5 pups

Myelination in P5 pups was evaluated by both measuring corpus callosum thickness with myelin basic protein (MBP) immunohistochemistry and western blotting for MBP expression. Thickness of corpus callosum was reduced from 382.9 ± 15.4 μm in Normoxia + Saline group to 283.8 ± 16.1 μm ($p < 0.001$) in Hyperoxia + Saline group, while the thickness of corpus callosum was significantly increased to 365.7 ± 10.3 μm ($p < 0.001$) in P5 pups receiving uridine (Fig. 3A and B).

In accordance, the expression of MBP in whole brain homogenates of P5 pups were reduced by about 31.5% ($p < 0.001$) in Hyperoxia + Saline group while it was increased significantly by 22% ($p < 0.001$) approaching to control levels following uridine administration (Fig. 3C).

2.4. Effects of uridine treatment on behavioral parameters on P10-P40

2.4.1. Negative geotaxis

Compared to the first trial day (P10), early reflexes of rat pups were gradually and significantly improved through P20 in all groups (Fig. 4). Time to reach (in seconds) the upper top edge of the board was reduced from 53.2 ± 1.9 to 29.3 ± 3.2 ($p < 0.001$) in Normoxia + Saline group, from 54.7 ± 1.8 to 31.7 ± 1.5 ($p < 0.001$) in Normoxia + Uridine group, from 57 ± 1.3 to 33 ± 2.1 ($p < 0.001$) in Hyperoxia + Saline group and from 54.8 ± 1.1 to 31.5 ± 2 ($p < 0.001$) in Hyperoxia + Uridine group from P10 through P20. Two-Way Repeated Measures (RM) ANOVA revealed a significant effect of time ([$F(5,3) = 112.224$, $p < 0.001$]), but not treatment ([$F(5,3) = 2.793$, $p = 0.076$]) or time x treatment interaction ([$F(5,9) = 0.0556$, $p = 1.000$]).

2.4.2. Beam walking

Sensorimotor coordination of animals was evaluated by analyzing average percentage of pups in each group on three trials at each time point (P16, P18, P20, and P25) scoring 0, 1 or 2, as has been reported previously (Karalis et al., 2011; Goren et al., 2017). Percentage of animals scoring 0 (falling off the beam) in each group reduced gradually from P16 through P25, becoming statistically significant on P20 in Normoxia + Saline and Hyperoxia + Uridine groups and on P25 in all groups except for Normoxia + Uridine group (Fig. 5A). Percentage of animals scoring 1 (staying on the beam but not reaching the platform) only tended to increase in pups in all groups from P16 through P25 (Fig. 5B). Likewise, percentage of animals scoring 2 (escaping to one of the platforms) tended to increase within all groups from P16 through P25, while statistical significance was detected only in Normoxia + Saline group between P25 and P16 test days (Fig. 5C). No significant difference was detected between groups on a given trial day for percentage of animals scoring 0, 1 or 2 (Fig. 5). It is worthwhile to underline that no significant effect of uridine on post-hyperoxia beam walking was found and the significant differences detected within groups in beam walking task are unrelated to uridine treatment.

2.4.3. Morris water maze

Learning and memory performances of the periadolescent rats were evaluated by Morris Water Maze (MWM) task on days P35 through P39 (Fig. 6). Latency to reach the platform decreased gradually and significantly in Normoxia + Saline (from 35.6 ± 3.5 s on the first trial day to 4.4 ± 0.8 s on the fourth trial day; $p < 0.001$),