

Protective effect of L-carnitine in a rat model of retinopathy of prematurity

Sadullah KELEŞ^{1*}, İbrahim CANER², Orhan ATEŞ¹, Özgür ÇAKICI³, Fatih SARUHAN⁴, Uğur Yılmaz MUMCU⁵, Deniz ÜNAL⁶, Kadir Şerafettin TEKGÜNDÜZ², Ayhan TAŞTEKİN⁷, Ahmet HACİMÜFTÜOĞLU⁴, Nesrin GÜRSAN⁸, Hamit Hakan ALP⁹

¹Department of Ophthalmology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

²Department of Pediatrics, Faculty of Medicine, Atatürk University, Erzurum, Turkey

³Department of Ophthalmology, Faculty of Medicine, Muğla University, Muğla, Turkey

⁴Department of Pharmacology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

⁵Department of Ophthalmology, Tunceli Government Hospital, Tunceli, Turkey

⁶Department of Histology & Embriology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

⁷Department of Pediatrics, Selçuklu Faculty of Medicine, Selçuk University, Konya, Turkey

⁸Department of Pathology, Faculty of Medicine, Atatürk University, Erzurum, Turkey

⁹Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Turkey

Received: 03.01.2013 • Accepted: 06.05.2013 • Published Online: 31.03.2014 • Printed: 30.04.2014

Aim: To investigate the effects of L-carnitine (LC) on rats with oxygen-induced retinopathy.

Materials and methods: The study was conducted on 40 Sprague Dawley rat pups. The rat pups were randomly divided into 4 groups: group 1 (n = 10), the healthy control group with intraperitoneal 0.1 mL/day physiological saline injection; group 2 (n = 10), exposed to hyperoxygen, did not receive LC but received 0.1 mL/day physiological saline intraperitoneally; group 3 (n = 10), exposed to hyperoxygen and received 100 mg/kg/day LC intraperitoneally; group 4 (n = 10), exposed to hyperoxygen and received 200 mg/kg/day LC intraperitoneally. After postnatal day 20, the rat pups were killed and an histological examination was performed on the eyes, in addition to the detection of plasma malondialdehyde (MDA) levels.

Results: The retinal and choroidal histopathological changes due to hyperoxygen were less in group 3 and minimal in group 4 compared with group 2. Compared with the healthy control group, the increase in the MDA levels in group 2 was significant (P < 0.05). Compared with group 2 there was a significant (P < 0.05) decrease in the MDA levels in groups 3 and 4.

Conclusion: LC has beneficial effects on oxygen-induced retinopathy in rats in terms of histopathological changes and MDA levels.

Key words: L-carnitine, malondialdehyde, oxygen toxicity, retinopathy of prematurity

1. Introduction

Retinopathy of prematurity (ROP) in preterm infants is a leading cause of blindness in childhood. Factors such as premature birth, low birth weight, blood transfusion, sepsis, intraventricular hemorrhage, light exposure, mechanical ventilation, and oxygen therapy increase the risk of ROP development (1,2).

ROP is a typical and original sign of oxygen toxicity in advanced premature infants. Supplementary oxygen therapy contributes to ROP development with toxic effects in the retina (3–5). Although there is not yet a clear explanation for their mechanisms, reactive oxygen species (ROS), generated by higher oxygen concentrations in tissues, result in oxidative damage to cellular components. Higher levels of ROS generated by over production or

insufficient elimination of ROS results in damage to cellular proteins, nucleic acids, and membrane lipids (6,7). ROS-induced oxidation in membrane lipids results in the production of lipid peroxidation products. Lipid peroxidation is a well-defined cause of cellular damage in human beings, and the existence of lipid peroxidation products is a sign of oxidative damage to cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, among which malondialdehyde (MDA) is the most abundant (8,9).

The antiperoxidative effect of L-carnitine (LC) on different tissues has been proposed (10,11). LC protects tissues with some mechanisms against peroxidative stress; LC prevents ROS formation generated by the

* Correspondence: opdr_sadullah@yahoo.com

xanthine/xanthine oxidase system and reduces possible damage in the cell membrane (12,13). In addition, LC has been reported to have a scavenging effect on ROS and a stabilizing impact on damaged cell membranes (13).

We aimed to determine the choroidal and retinal histopathological changes and the levels of MDA in rats with oxygen-induced retinopathy (OIR) that were treated with LC.

2. Materials and methods

In this experiment 40 Sprague Dawley rat pups were used. The rat pups were randomly divided into 4 groups: group 1, (n = 10), the healthy control; group 2 (n = 10), exposed to hyperoxygen and did not receive LC but did receive 0.1 mL physiological saline intraperitoneally; group 3 (n = 10), exposed to hyperoxygen and received 100 mg/kg LC intraperitoneally; group 4 (n = 10), exposed to hyperoxygen and received 200 mg/kg LC intraperitoneally.

A relative hypoxic condition state in the OIR rat model has been previously described (14). Together with their mothers, newborn rat pups were exposed to hyperoxygen ($80 \pm 1.3\% O_2$) in Plexiglas chambers (Natus Oxydome II, Seattle, WA, USA) from postnatal (P) day 2 to P14 at 2 h/day in room air. They were then returned to normal ambient air conditions (room air, $21 \pm 1.5\% O_2$) from P14 to P20. Control rat pups were kept in normal room air. All rat pups were killed on P20.

LC was injected (Carnitine ampule, Sigma-Tau, Rome, Italy) intraperitoneally in both groups 3 and 4 every day, starting 1 day before (P1) hyperoxia exposure and ending 14 days after (P14) (total of 15 days). Both the control group and group 2 were administered daily 0.1 mL of physiological saline intraperitoneally starting from P1 and ending at P14 (total of 15 days).

The animals were housed in facilities accredited by international guidelines and the studies were approved by and conducted in accordance with the Institutional Animal Care and Use Committee of Atatürk University.

2.1. Tissue preparation

At the end of the experiment, all the animals were killed by intraperitoneal injections of pentobarbital (200 mg/kg). The eyes of the rat pups were removed. Each eye was fixed in a 10% formalin solution for 48–55 h, dehydrated in a graded alcohol series, embedded in paraffin wax, and sectioned using a microtome (Leica RM2125RT). Sections of 5 µm were mounted onto glass slides and stained with hematoxylin-eosin for routine histological examination. All sections were studied and photographed by using a light photomicroscope (Olympus BH40).

Analysis of plasma for MDA levels was determined in supernatants spectrophotometrically (CE 3041, Cecil,

Cambridge, UK) (15). Its level was expressed as nmol/mg protein.

Statistical analyses were done using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The statistical significance was calculated using chi-square and one-way ANOVA tests. The results are presented as mean \pm standard deviation and the significance level was set at $P < 0.05$.

3. Results

All ROP models (groups 2, 3, 4) had a characteristic appearance of severe ROP with peripheral avascular retina similar to zone II ROP at P14. Inner retinal plexus vascularization had extended to the ora serrata in the control group.

Histopathological results in group 1 (healthy control group): Parts of the tunica vasculosa such as the choroid and the ciliary body were found to be normal. In both low and high magnifications of the sections, the neural tunic was clearly detected.

Histopathological results in group 2: Hyperchromasia in ciliary epithelial cells, edema under the ciliary epithelium, and inflammatory cell infiltration under the ciliary epithelium were detected in all rats. Intense cell degeneration in the inner nuclear layer and ganglion cells layer were observed. There was intense degeneration in nerve fiber and edema among fibers in the inner plexiform layer. Many necrotic cells and large amounts of cell debris were detected.

Histopathological results in group 3: Hyperchromasia in ciliary epithelial cells, a few inflammatory cell infiltration, and edema under the ciliary epithelium were determined in the choroid of group 3. Cell degeneration in the inner nuclear layer and the ganglion cells layer was less than in group 2. Degeneration in nerve fiber and edema among fibers in the inner plexiform layer was less than in group 2. Sparse necrotic cells and cell debris were also determined.

Histopathological results in group 4: There was no hyperchromasia in the ciliary epithelial cells. Edema and inflammatory cell infiltration were not detected under the ciliary epithelium. There was no degeneration in the inner nuclear layer and ganglion cells. Degeneration in nerve fiber and edema among fibers in the inner plexiform layer was not detected. There were no necrotic cells.

All rats had choroidal changes (hyperchromasia in ciliary epithelial cells, edema under ciliary epithelium, and inflammatory cell infiltration under ciliary epithelium) in groups 2 and 3. All rats had retinal changes (intense cell degeneration in inner nuclear layer and ganglion cells layer, degeneration in nerve fibers and edema among

fibers in inner plexiform layer, many necrotic cells, and large amount of cell debris) in groups 2 and 3. No rats had choroidal or retinal changes in groups 1 and 4. Retinal and choroidal change levels were found to be increased in groups 2 and 3 compared with the control and group 4 rats.

The levels of MDA in the samples are all presented in the Table. Compared with the healthy control group, increases in the MDA level as an end product of lipid peroxidation in group 2 were significant ($P < 0.05$). Compared with group 2, LC administration in groups 3 and 4 resulted in a significant decrease in MDA levels ($P < 0.05$).

4. Discussion

The retina is one of the organs exposed to high oxygen tension. Retinal over-oxygenation can play an important role in ROP pathogenesis with rising ROS levels (16,17). ROS results in more oxidative damage through lipid peroxidation by invading the double bonds of polyunsaturated fatty acids. Cell membrane oxidation due to ROS results in products of lipid peroxidation such as MDA (18,19). The present study found high levels of MDA in rats with ROP compared to those without ROP, as in previous research (19,20).

Some agents were reported in the literature for protecting the retina in animal models of OIR. In a previous study, Kaya et al. (21) demonstrated that octreotide acetate inhibited endothelial cell proliferation in an OIR model of mouse. They also showed that intravitreal octreotide acetate decreased apoptotic cell death.

LC is a carrier of long-chain fatty acids and plays an important role in branched-chain amino acid metabolism, ketone body utilization, peroxisomal oxidation, and erythrocyte membrane phospholipid turnover (22). Carnitine can also act as a chelator by decreasing the concentration of cytosolic iron, which plays a very important role in free radical chemistry (23).

The present study, in parallel with other studies, suggests that LC plays a role against oxidative damage,

preventing lipid peroxidation and supporting the antioxidant system (24). Other research has shown that LC reduces acetaminophen-induced high aspartate transaminase, alanine transaminase, total sialic acid, and MDA concentrations (25).

Shamsi et al. (26) examined the effect of LC on oxidative changes in the retinal pigment epithelium (RPE). They identified a protective effect of LC against oxidative stress on the RPE. They pointed out that micronutrients could play a crucial role in oxidation-induced eye diseases based on the protective effect of LC against H_2O_2 -induced oxidative damage.

Alagoz et al. (27) examined the efficiency of LC on ischemia-reperfusion injury in guinea pigs. They found that the mean MDA value and the tissue thickness of the LC-treated group were statistically insignificant versus those of the control group. However, these values were significantly different in the group receiving saline versus the control group and that receiving LC. At the end of study, they concluded that LC might be an alternative drug for ischemia-reperfusion injury of the retina.

Ates et al. (28) determined the antioxidant properties of LC in patients with age-related macular degeneration (AMD). Their study involved 60 patients diagnosed with early AMD. The patients received LC supplementation for 3 months. At the end of the 3-month period, the MDA level was significantly reduced in patients treated with LC.

Likewise, Kocer et al. (29) studied the efficiency of LC on retinal ischemia-reperfusion injury in guinea pigs. In their study, TBARS levels in retinal tissue were found lower in the LC group than in the control group and mean retinal thickness was found to be increased in the control group when compared to the LC group. They concluded that LC is effective in preventing retinal injury followed by ischemia-reperfusion.

Park et al. (30) investigated the effect of triamcinolone acetonide (TA) on retinal expression of decorin in a rat model of OIR. The results of their study showed that neuronal cell death was increased in OIR rats relative to

Table. Levels of MDA in all groups.

Groups	Healthy control group	Hyperoxia-exposed group	Group exposed to hyperoxia and treated with 100 mg/kg LC	Group exposed to hyperoxia and treated with 200 mg/kg LC
	Group 1	Group 2	Group 3	Group 4
Levels of MDA in plasma (nmol/mg)	13.13 ± 1.02	24.44 ± 1.75	13.86 ± 1.00	12.28 ± 1.24

LC: L-carnitine.

controls. However, treatment with TA restored neuronal cell death in OIR rats. They suggested that decorin is involved in hypoxic retinal damage and that TA protects retinal neurons damaged by relative hypoxia.

In conclusion, LC plays an important role in OIR in rats, reducing lipid peroxidation and minimizing histopathological changes. Therefore, it might be used as an alternative drug with other treatments in OIR.

References

1. Yin H, Li XX, Li HL, Zhang W. Incidence and risk factor analysis of retinopathy of prematurity. *Zhonghua Yan Ke Za Zhi* 2005; 41: 295–9.
2. Knezevic S, Stojanović N, Oros A, Knezević J. The importance of timely ophthalmologic examination in preterm infants at risk of retinopathy occurrence. *Vojnosanit Pregl* 2012; 69: 765–70.
3. Araz-Ersan B, Kir N, Akarcay K, Aydinoglu-Candan O, Sahinoglu-Keskek N, Demirel A, Akdogan B, Coban A. Epidemiological analysis of retinopathy of prematurity in a referral centre in Turkey. *Br J Ophthalmol* 2013; 97: 15–7.
4. Auld PA. Oxygen therapy for premature infants. *J Pediatr* 1971; 78: 705–9.
5. Urrets-Zavalía JA, Crim N, Knoll EG, Esposito FA, Collino E, Urrets-Zavalía ME, Saenz-de-Tejada G, Torrealday JJ, Serra HM, Gilbert C. Impact of changing oxygenation policies on retinopathy of prematurity in a neonatal unit in Argentina. *Br J Ophthalmol* 2012; 96: 1456–61.
6. Kelly FJ. Free radical disorders of preterm infants. *Br Med Bull* 1993; 49: 668–78.
7. Rao NA, Wu GS. Oxygen free radicals and retinopathy of prematurity. *Br J Ophthalmol* 1996; 80: 387.
8. Kunitomo M. Oxidative stress and atherosclerosis. Review. *Yakugaku Zasshi* 2007; 127: 1997–2014 (article in Japanese with English abstract).
9. Nanetti L, Raffaelli F, Vignini A, Perozzi C, Silvestrini M, Bartolini M, Provinciali L, Mazzanti L. Oxidative stress in ischaemic stroke. *Eur J Clin Invest* 2011; 41: 1318–22.
10. Ribas GS, Biancini GB, Mescka C, Wayhs CY, Sitta A, Wajner M, Vargas CR. Oxidative stress parameters in urine from patients with disorders of propionate metabolism: a beneficial effect of L-carnitine supplementation. *Cell Mol Neurobiol* 2012; 32: 77–82.
11. Dokmeci D, Akpolat M, Aydogdu N, Doganay L, Turan FN. L-Carnitine inhibits ethanol-induced gastric mucosal injury in rats. *Pharmacol Rep* 2005; 57: 481–8.
12. Ribas GS, Biancini GB, Mescka C, Wayhs CY, Sitta A, Wajner M, Vargas CR. Oxidative stress parameters in urine from patients with disorders of propionate metabolism: a beneficial effect of L-carnitine supplementation. *Cell Mol Neurobiol* 2012; 32: 77–82.
13. Izzüt-Uysal VN, Ağaç A, Derin N. Effect of carnitine on stress-induced lipid peroxidation in rat gastric mucosa. *J Gastroenterol* 2001; 36: 231–6.
14. Chung IY, Kim YH, Park JM, Seo SW, Choi WS, Cho GJ, Yoo JM. Protective effects of triamcinolone acetonide upon the upregulation and phosphorylation of GAP 43 in an animal model of retinopathy of prematurity. *Acta Ophthalmol* 2010; 88: 217–21.
15. Czauderna M, Kowalczyk J, Marounek M. The simple and sensitive measurement of malondialdehyde in selected specimens of biological origin and some feed by reversed phase high performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011; 879: 2251–8.
16. Grimm C, Willmann G. Hypoxia in the eye: a two-sided coin. *High Alt Med Biol* 2012; 13: 169–75.
17. Shahidi M, Wanek J, Blair NP, Little DM, Wu T. Retinal tissue oxygen tension imaging in the rat. *Invest Ophthalmol Vis Sci* 2010; 51: 4766–70.
18. Pifferi F, Perret M, Guesnet P, Aujard F, Alessandri JM. Lipids. Fatty acid composition of the brain, retina, liver and adipose tissue of the grey mouse lemur (*Microcebus murinus*, primate). *Lipids* 2012; 47: 793–801.
19. Ates O, Alp HH, Caner I, Yildirim A, Tastekin A, Kocer I, Baykal O. Oxidative DNA damage in retinopathy of prematurity. *Eur J Ophthalmol* 2009; 19: 80–5.
20. Weinberger B, Anwar M, Henien S, Sosnovsky A, Hiatt M, Jochnowitz N, Witz G, Hegyi T. Association of lipid peroxidation with antenatal betamethasone and oxygen radial disorders in preterm infants. *Biol Neonate* 2004; 85: 121–7.
21. Kaya FS, Akkoyun İ, Haberal Reyhan AN, Dağdeviren A, Yılmaz G, Oto S, Akova Y. Effect of intravitreal octreotide acetate injection on retinal neovascularization, morphology, and apoptotic cell death in an oxygen-induced retinopathy mouse model. *Turk J Med Sci* 2012; 42 (Suppl. 2): 1519–27.
22. Şıktar E, Ekinci D, Şıktar E, Beydemir Ş, Gülçin İ, Günay M. Protective role of L-carnitine supplementation against exhaustive exercise induced oxidative stress in rats. *Eur J Pharmacol* 2011; 668: 407–13.
23. Panjwani U, Thakur L, Anand JP, Singh SN, Amitabh, Singh SB, Banerjee PK. Effect of L-carnitine supplementation on endurance exercise in normobaric/normoxic and hypobaric/hypoxic conditions. *Wilderness Environ Med* 2007; 18: 169–76.
24. Karanth J, Jeevaratnam K. Oxidative stress and antioxidant status in rat blood, liver and muscle: effect of dietary lipid, carnitine and exercise. *Int J Vitam Nutr Res* 2005; 75: 333–9.

25. Yapar K, Kart A, Karapehlivan M, Atakisi O, Tunca R, Erginsoy S, Cital M. Hepatoprotective effect of L-carnitine against acute acetaminophen toxicity in mice. *Exp Toxicol Pathol* 2007; 59: 121–8.
26. Shamsi FA, Chaudhry IA, Boulton ME, Al-Rajhi AA. L-Carnitine protects human retinal pigment epithelial cells from oxidative damage. *Curr Eye Res* 2007; 32: 575–84.
27. Alagoz G, Celiker U, Ilhan N, Yekeler H, Demir T, Celiker H. L-carnitine in experimental retinal ischemia-reperfusion injury. *Ophthalmologica* 2002; 216: 144–50.
28. Ates O, H. Alp HH, Mumcu U, Azizi S, Cinici E, Kiziltunc A, Baykal O. The effect of L-carnitine treatment on levels of malondialdehyde and glutathione in patients with age related macular degeneration. *Eurasian J Med* 2008; 40: 1–5.
29. Kocer I, Taysi S, Ertekin MV, Karslioglu I, Gepdiremen A, Sezen O, Serifoglu K. The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model. *Graefes Arch Clin Exp Ophthalmol* 2007; 245: 588–94.
30. Park YJ, Kim YH, Choi WS, Chung IY, Yoo JM. Treatment with triamcinolone acetonide prevents decreased retinal levels of decorin in a rat model of oxygen-induced retinopathy. *Curr Eye Res* 2010; 35: 657–63.