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RESEARCH ARTICLE

The protective effect of selenium in cisplatin-related retinotoxicity

Alper Yazici[1], Esin Sogutlu-Sari[1], Arzu Yay[2], Hasan Aksit[3], Adil Kilic[1], Dilek Aksit[4], Onur Yildiz[3], and Sitki Samet Ermis[1]

[1]*Department of Ophthalmology, Faculty of Medicine, Balikesir University, Balikesir, Turkey,* [2]*Department of Histology and Embryology, Faculty of Medicine, Erciyes University, Kayseri, Turkey,* [3]*Department of Biochemistry, Faculty of Veterinary, Balikesir University, Balikesir, Turkey, and* [4]*Department of Pharmacology, Faculty of Veterinary, Balikesir University, Balikesir, Turkey*

Abstract

Purpose: The aim of this study is to evaluate the retinal toxicity of cisplatin and neuroprotective effect of selenium in cisplatin-related retinal toxicity.

Methods: Eighteen adult Wistar-Albino rats were divided into three groups. Group 1 ($n = 6$) received intraperitoneal (i.p.) injection of 2.5 ml physiologic saline for three days, group 2 ($n = 6$) received i.p. 16 mg/kg cisplatin for three days and group 3 ($n = 6$) received i.p. 16 mg/kg cisplatin for three days and 1.5 mg/kg twice daily selenium *via* gavage five days prior to cisplatin injection and for three days concomitantly with cisplatin injections. The total retinal thickness, outer nuclear layer (ONL), inner nuclear layer (INL) and inner plexiform layer (IPL) thicknesses were measured in hematoxylin/eosin and apoptotic index (AI) of ganglion cell layer (GCL) and INL was evaluated in TdT-mediated dUTP-biotin nick end labeling (TUNEL)-stained retina sections.

Results: Selenium statistically succeeded to reduce total retinal thickness in cisplatin-toxicated retinas: from 210.17 ± 23.40 to 173.55 ± 20.43, ONL: 49.79 ± 5.32 to 41.87 ± 6.30, INL: 33.72 ± 7.93 to 25.06 ± 5.73 and IPL: 53.61 ± 8.63 to 45.61 ± 6.92 μm in hematoxylin/eosin-stained retina sections. The AI was also reduced in INL (30.10 ± 12.02 to 19.48 ± 12.99) and in GCL (37.59 ± 17.70 to 33.15 ± 13.78). However, statistical significance was present in only AI values of INL.

Conclusions: Selenium limited edema due to the toxicity and reduced the retinal thickness and showed neuroprotection in cisplatin-induced retinotoxicity.

Keywords

Antioxidant, cisplatin, inflammation, neuroprotection, retina, selenium, toxicity

History

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Introduction

Cisplatin (cis-diamminedichloroplatinum) is an alkylating chemotherapeutic agent used in many types of cancers like sarcoma, gonad, breast, lung, bladder and lymphoma[1]. Cisplatin achieves its anti-cancer effect through formation of DNA adducts and production of reactive oxygen species. Its use is limited due to the side effects like ototoxicity, gonadotoxicity, nephrotoxicity, neurotoxicity and marrow suppression[2,3]. Although the exact mechanisms of side effects are not known, lipid peroxidation, mitochondrial dysfunction, inhibition of protein synthesis and DNA injury are blamed[4–6]. Retina is a sensory organ in which photoreceptors with rich polyunsaturated fatty acids in its membranes turn photon energy into electrical impulse, and the axons of ganglions (uniting to form optic nerve) transmit this electrical energy to brain. This function needs a continuous oxidant–antioxidant buffer to scavenge the free radicals that are produced in this continuous turnover. Cisplatin creating an additional oxidant stress on retina might disturb this critical oxidant–antioxidant buffer and harm retinal tissues. Retinal toxicity of cisplatin was also mentioned in different studies[7–10]. Cisplatin and carboplatin are platinum-based agents, and the platinum might act as a heavy metal and be toxic to the retina. The platinum-related toxicity might be in the form of pigmentary maculopathy[11], altered color perception due to possible cone dysfunction[12] and ischemic retinal changes[13].

Selenium is an essential trace element that is needed to be consumed in the diet. It has antioxidant and neuroprotective features and is an important component of several antioxidant enzymes like glutathione peroxidase, thioredoxin reductase and selenoproteins[14]. It was demonstrated to be effective in cisplatin-related toxicities[14,15], other heavy metal-related toxicities[16,17] and ischemia reperfusion (IR) injuries[18–22].

In this article, we aimed to investigate the possible protective effect of selenium in an animal model of cisplatin-related retinotoxicity. To the best of our knowledge, this is the first study to evaluate the effect of selenium in cisplatin-related retinal toxicity.

Materials and methods

Institutional ethics committee approval for animal studies was obtained prior to the study. All animals used in the study received care in compliance with the guidelines established

Address for correspondence: Alper Yazici, Balikesir University, Balikesir, Turkey. E-mail: lpryzc@yahoo.com

by the committee. All experiments were conducted in accordance with the Animal Care and Use Committee and The Association for Research in Vision and Ophthalmology guidelines.

Animals

Eighteen healthy adult Wistar rats weighing 250–300 g were housed in 14/10 h light/dark cycle with free access to food and water. Rats were assigned to three groups. Group 1 ($n = 6$) received intraperitoneal (i.p.) injection of 2.5 ml physiologic saline for three days, group 2 ($n = 6$) received i.p. injection of 16 mg/kg cisplatin (Eczacibasi, İstanbul, Turkey) for three days, group 3 ($n = 6$) received not only i.p. injections of 16 mg/kg cisplatin (Eczacibasi) for three days but also 1.5 mg/kg twice daily selenium *via* gavage (sodium selenite 98% powder, Sigma S5261, Saint Louis, MO) five days prior to cisplatin injection and for three days concomitantly with cisplatin injections. At the end of the study period, the animals were anesthetized with 30 mg/kg of ketamine (Ketalar®, Eczacibasi) and 4 mg/kg xyzaline (Rompun®, Bayer, Leverkusen, Germany), and right eye of each rat was enucleated.

Histopathologic evaluation

The study included paraffin wax embedded formaldehyde-fixed eye tissues from each group. Routine paraffin wax embedding procedures were used for histopathologic evaluation. In brief, the tissue samples were fixed in 10% formalin solution, alcohol dehydrated and paraffinized. After paraffin embedding, 5 μm thickness retinal cross sections 1–2 mm away from optic disc were cut and followed by Hematoxylin/eosin (H&E) staining. Images were captured by using an Olympus BX51 microscope for morphological observation of the retinal layers.

TdT-mediated dUTP-biotin nick end labeling staining

Analysis of retinal thickness and percentage of TUNEL-positive cells

The thickness was measured on the magnified images (×400). Three visual fields under a light microscope (Olympus BX51, Tokyo, Japan) per section were randomly chosen to measure the thickness of the total retina and retinal sub-layers (outer nuclear layer (ONL), inner nuclear layer (INL) and inner plexiform layer (IPL)) thickness. Quantitative histomorphometry was performed with Image J software (National Institutes of Health, Bethesda, MD) in the carefully defined reference fields.

To evaluate percentage of TdT-mediated dUTP-biotin nick end labeling (TUNEL)-positive cells, three visual fields per section were randomly chosen, and the number of TUNEL-positive cell nuclei were counted in the INL and ganglion cell layer (GCL) with the same magnification (×400). The apoptotic index (AI) was calculated using the following formula: AI = apoptotic nuclei/total nuclei × 100%.

Statistical analysis

The results were statistically analyzed with SPSS version 15.0 (SPSS Inc., Chicago, IL). Shapiro–Wilk test was used to evaluate the normality of distribution of the data and one-way ANOVA and Post-hoc Tukey HSD tests were used for statistical comparison since the data was normally distributed. All data were given as the mean ± SD (standard deviation), and $p < 0.05$ was considered as statistically significant.

Results

Histologic findings

To determine the retinal thickness changes that cisplatin induce, we measured the thickness of the retinal layers. The morphology of the retina, including measurements of the thickness of the total retina, ONL, INL and IPL, was determined with H&E staining of retinal sections (Figure 1). Three micrographs of each group were analyzed, and the data was expressed as mean ± SD. The overall retinal thickness values were 155.81 ± 22.55 μm, 210.17 ± 23.40 μm and 173.55 ± 20.43 μm for groups 1, 2 and 3, respectively. The total retinal, ONL, INL and IPL thicknesses in the cisplatin group was increased compared to the control group and selenium-treated group ($p < 0.05$). Histological measurements of H&E-stained retinas showed that the thickness of the retinal layers due to the edema induced by cisplatin toxicity was reduced with the selenium treatment. The change in total retinal, ONL, INL and IPL thicknesses among groups are presented in Figure 2.

Selenium inhibited apoptosis of the neural cells in the cisplatin-injured retina

We investigated the influence of selenium on the AI in each experimental group and evaluated the apoptotic cell number with TUNEL method. TUNEL-positive cells were distributed mainly in the INL and GCL of the retinas from experimental group (see Figure 3). The mean AI values of groups 1, 2 and 3 in INL were 7.01 ± 2.12, 30.10 ± 12.02 and 19.48 ± 12.99 and in GCL were 12.28 ± 6.91, 37.59 ± 17.70 and 33.15 ± 13.78, respectively (see Figure 4). Although AI in both INL and GCL was lower in selenium-treated groups compared to cisplatin toxicity group, the statistical significance was present only for INL ($p < 0.05$ and $p = 0.16$, respectively). The retinal thicknesses, AI values and statistical comparison results are summarized in Table 1.

Discussion

Cisplatin is an important chemotherapeutic widely used in the treatment of many cancers. It shows its affect in various ways such as formation of DNA adducts, production of reactive oxygen species, increased lipid peroxidation and increased mitochondrial stress[2,23]. When it affects the normal tissue metabolism, side effects like ototoxicity, gonadotoxicity, nephrotoxicity, neurotoxicity and marrow suppression appears[3,23]. Many agents like pomegranate, mirtazapine, resveratrol and selenium was used to prevent cisplatin toxicity in different organs[2,15,23,24]. The retinotoxicity of cisplatin is not studied extensively and mostly reported as case reports with even loss of vision at the end[8,9,17]. The reported toxicities in retina are hypothesized to be due to pigmentary maculopathy, cone dysfunction and retinal ischemia[9]. The literature grants studies dealing with cisplatin toxicities and

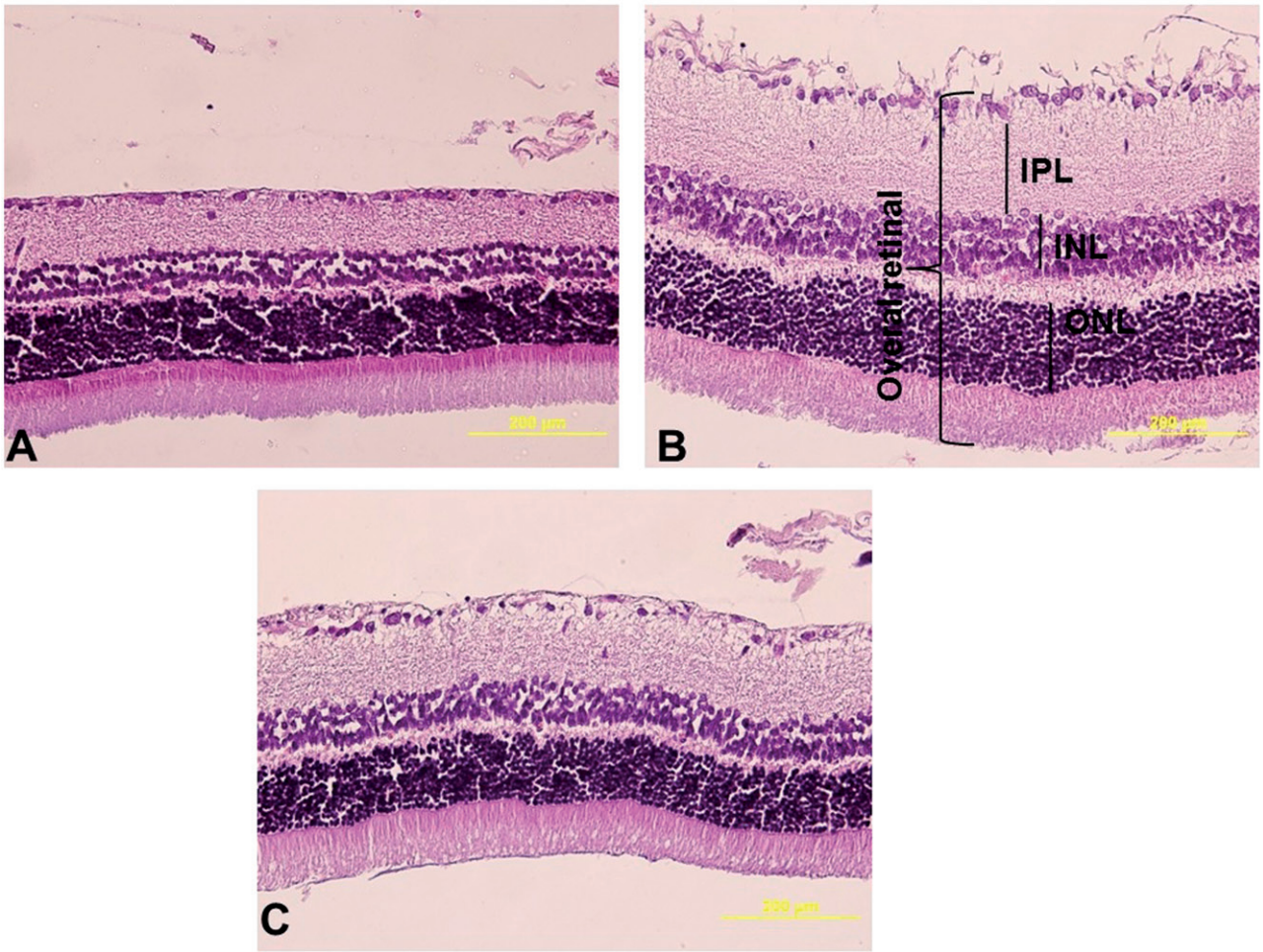

Figure 1. The morphology of the retina. The images were representatives of the H&E-stained sections of retina from experimental groups. (A) Sagittal sections of the rat retina showing the structure of layers in control group. (B) The retinal image of cisplatin only group. (C) The retinal image from cisplatin + selenium group. Original magnification, 40 ×.

efforts to limit or alleviate the toxic side effects with different chemicals for this purpose, including selenium[2,15,23]. Selenium is an essential trace element that has direct or indirect antioxidant, neuroprotective effects and reported to be effective in cisplatin-related toxicities. We tried to express the toxic effects of cisplatin and possible protective effect of selenium in the rat retina.

Cisplatin had resulted to an increase in total retinal, ONL, INL and IPL thickness when compared to control and selenium-treated group. Mukhopadhyay et al.[25] observed that cisplatin caused an oxidative stress in mitochondria followed by a reactive oxygen and nitrogen species generation, intense inflammatory response and histopathological injury. The continuous hit of the light photons creates heat, which is relieved by the blood flow of the underlying choroid that constitutes 20% of the cardiac output. This high oxygen necessitation, high metabolism rate and the higher density of polyunsaturated fatty acids in cell membranes in the retina requires a perfect oxidative balance. Cisplatin causes an imbalance in favor of oxidative stress and consequent inflammatory response and edema. The retina being thickened in all segments of the retina shows us that the toxicity is throughout the whole retina. The selenium-treated group showed a statistically significant reduction in all thickness values, which means that it has an effective antagonism to cisplatin. As known, selenium is an important part of antioxidant enzymes that buffers the oxidant and antioxidant status. It reduces the mitochondrial stress, protects DNA, lipids and proteins *via* the action through glutathione peroxidase-like activity and reduction of reactive oxygen and nitrogen species[18,26,27]. Apart from these effects, selenium also has anti-inflammatory properties that involve the cyclooxygenase and lipoxygenase pathways and affect cytokine and chemokine expression[28]. Retina has glial cells such as microglia, Müller cell and astrocytes, all having specific actions. Especially when stimulated with trauma (toxic, ischemic, etc.), the number of microglial cells increases at the initial phases of the inflammatory response and phagocyte the damaged neurons. Selenium was speculated to limit the microglial action at these initial phases of inflammation[29]. Thru the above-mentioned anti-inflammatory, antioxidant and possible unknown mode of actions, inflammatory changes and eventual edema development is limited. According to the method described by Hughes[30], selenium had a statistically significant protection in our study since it reduced the thickness of IPL statistically. This method was also used in a similar study that evaluated the protective effects of vitamin E forms in an IR model of retinal injury. They also found that

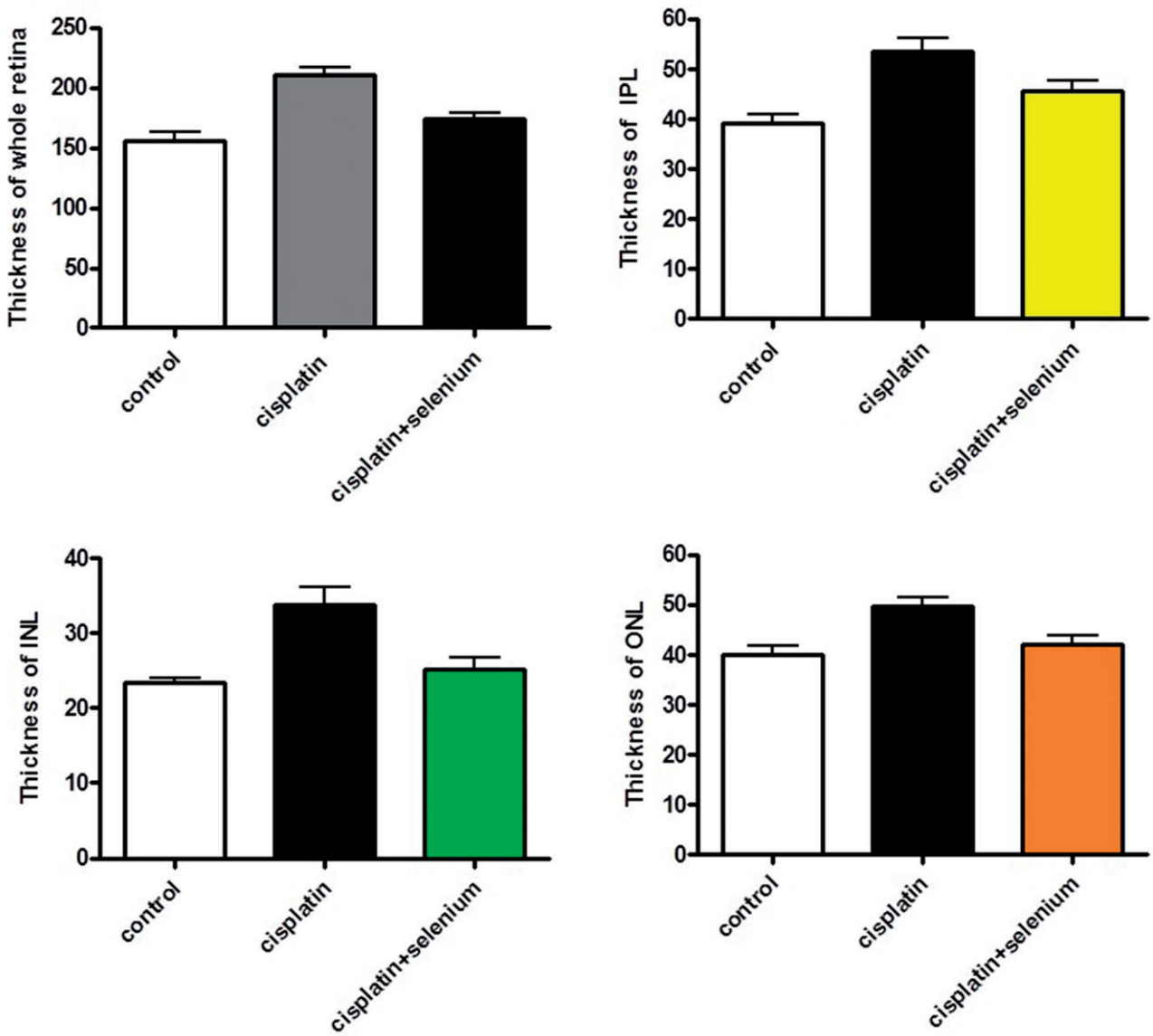

Figure 2. The graph shows the comparison of total retinal thickness, inner plexiform layer (IPL), inner nuclear layer (INL) and outer nuclear layer (ONL) thicknesses among groups.

vitamin E was very efficient in decreasing the retinal thickness due to IR injury and considered vitamin E as a neuroprotective agent[31]. The significant reduction in retinal thickness in our study showed that selenium is a very potent anti-inflammatory and neuroprotective agent and successfully limits inflammation, edema and tissue damage.

Selenium treatment achieved a significant success in limitation of inflammatory response and edema in cisplatin-related retinotoxicity. The TUNEL is an immunohistochemical staining method to investigate the apoptosis in tissues and gives clinicians a chance to quantify the cells with fragmented DNAs[18]. The TUNEL-positive cells, consistent with the knowledge that the inner layers were affected more than the outer layers of the retina, were distributed mainly in GCL and INL[30]. When AI was evaluated, selenium showed protection in both GCL and INL, although statistical significance was present only for INL. Beyond having neuroprotective effect due to the antioxidant action, selenium is also believed to increase *de novo* protein synthesis for neuroprotection[29]. Authors reached this conclusion from the fact that selenium preserves this protective effect even in glutathione depletion. Selenium failed to show statistically significant protection in GCL in our study. The number of cells in the INL is more numerous compared to ganglion cells. So the sampling of the retina might not be enough to show changes in GCL since it is much easier to show cell changes in larger scale samples than the samples containing limited number of cells as in the case of INL and GCL. Besides sampling error, other possibilities, such as selenium efficiency in protecting INL better than GCL and cisplatin being more toxic to GCL than INL, should be kept in mind.

IR injury similar to cisplatin toxicity also harms tissues *via* ischemia, mitochondrial stress and imbalance between oxidant and antioxidant stress. They also cause tissue inflammatory response, capillary hyperpermeability, leakage and eventual tissue edema. Diseases like proliferative retinopathies, diabetic retinopathies, retina vascular occlusions and premature retinopathy all damage retina with IR-based

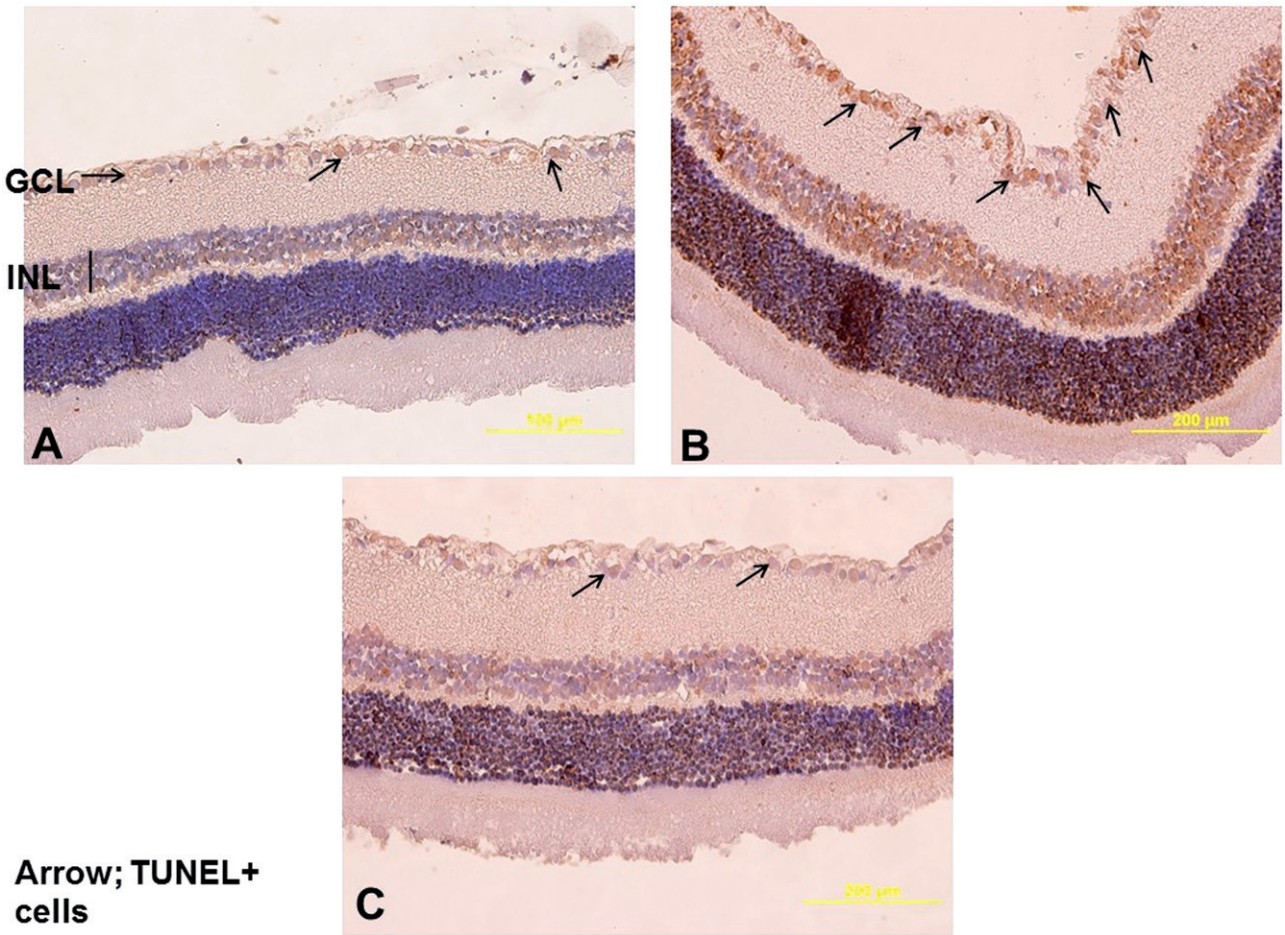

Figure 3. Representative images of TUNEL assay of the rat retina of experimental groups (original magnification, 40 ×). (A) TUNEL assay of retina in control group. (B) TUNEL + apoptotic cells increased in GCL and INL layers of cisplatin only group. (C) Treatment with selenium-attenuated apoptosis of neural cells in cisplatin + selenium group. Arrow: TUNEL + cell. AI: apoptotic index; GCL: ganglion cell layer; and INL: inner nuclear layer.

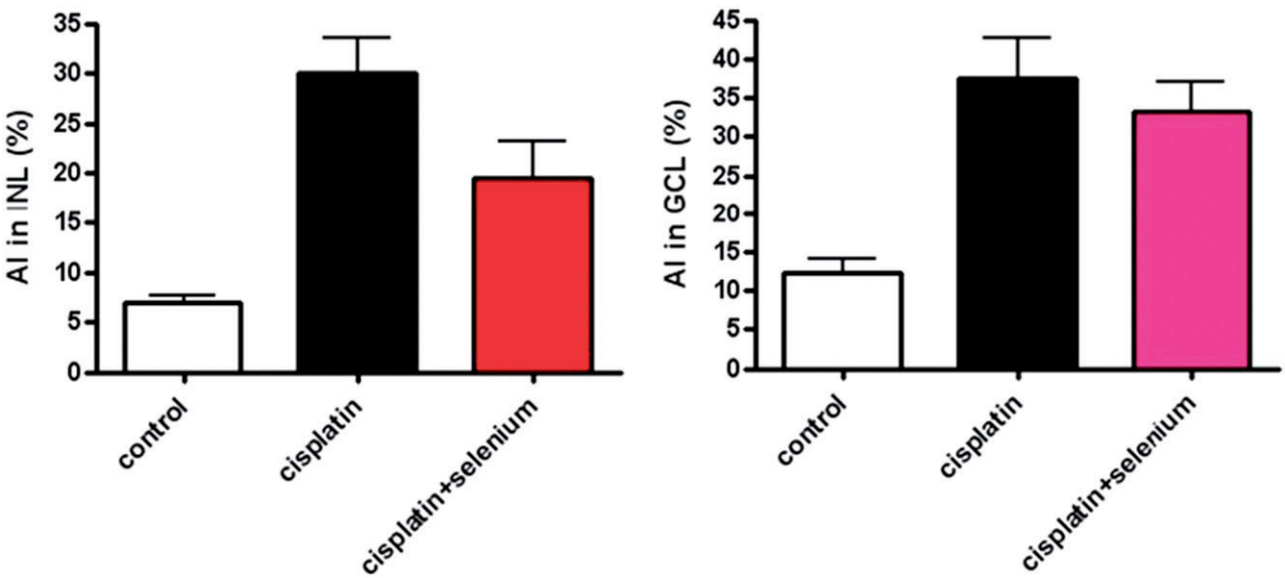

Figure 4. The graph shows the comparison of AI in inner nuclear layer (INL) and ganglion cell layer (GCL) among groups.

Table 1. Summary of retinal thicknesses and AI values among groups.

	Total retinal thickness (μm)	ONL (μm)	INL (μm)	IPL (μm)	AI (INL)	AI (GCL)
Group 1	155.81 ± 22.55	39.97 ± 5.59	23.33 ± 1.99	39.28 ± 4.56	7.01 ± 2.12	12.28 ± 6.91
Group 2	210.17 ± 23.40	49.79 ± 5.32	33.72 ± 7.93	53.61 ± 8.63	30.10 ± 12.02	37.59 ± 17.70
Group 3	173.55 ± 20.43	41.87 ± 6.30	25.06 ± 5.73	45.61 ± 6.92	19.48 ± 12.99	33.15 ± 13.78
p[a]	0.013	0.030	0.038	0.008	0.009	0.16

[a]Comparison of group 2 and 3 (Post-hoc Tukey HSD test).

pathophysiology. When we apply our result to clinical practice, selenium can be successfully used in reduction of retinal thickness and neuroprotection also in IR injuries. However, to reach a definitive conclusion, studies conducted on IR retinal injuries should be performed.

Declaration of interest

The authors report no declarations of interest

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