Soluble curcumin ameliorates motility, adhesiveness and abrogate parthanatos in cadmium-exposed retinal pigment epithelial cells

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Introduction

Cadmium (Cd) is a nonessential transition metal and one of the most toxic environmental pollutants. Industrial, agricultural and urban activities are the main sources of Cd environmental contamination. Multiple deleterious effects of Cd exposure were reported for different cell types and living organisms in a great number of research papers. Cd bioaccumulation hazard is mediated by the relatively long half-life of this metal in an organism. For example, in mammals its half-life lasts for about 10–30 years. Cd exposure affects many tissues. However, some of them, including the central nervous system and sensory organs, are most susceptible to its toxicity. The harmful effects of Cd could be linked to oxidative stress generation and consequently intracellular signalling disruption. Since Cd induces redox imbalance the antioxidants could be a prospective tool to ameliorate Cd cytotoxicity. In present work, we have studied the protective efficacy of soluble curcumin on Cd-caused retinal pigment epithelium (RPE) cells viability, reactive oxygen species production, adhesive and extracellular matrix proteins expression, cell migration and parthanatos level. Low dose (5 μM) of soluble curcumin ameliorated all aforementioned indices of Cd-induced cytotoxicity. Curcumin has restored the RPE-cells motility as well as fibronectin and E-cadherin expression. Therefore, the modulation of RPE cells motility could as well as fibronectin and E-cadherin expression. Therefore, the modulation of RPE cells motility and adhesiveness could be regarded as a cytoprotective effect of curcumin. Furthermore, Cd-caused poly(ADP-ribose) polymerase-1 (PARP-1) suppression and cleaved PARP-1 upregulation were ameliorated by curcumin exposure. Therefore, the protective effect of soluble curcumin could be related, at least partially, to the modulation of PARP activity and inhibition of parthanatos flux. The observed results have demonstrated that low doses of soluble curcumin are a promising tool to protect RPE cells against Cd-caused retinal injury.

Keywords: heavy metal; ecotoxicology; biomarkers; PARP-1; Nrf2; fibronectin; E-cadherin; turmeric extract.
Curcumin exhibits multiple bioactive features including antioxidant (Ali-Jassabi et al., 2012; Mohajeri et al., 2017), antiviral (Khatayy et al., 2008), antimicrobial (Tajbakhsh et al., 2008), anti-inflammatory (Beresswil et al., 2010), and anti-tumour activities (Wang et al., 2009; Huang et al., 2013). The detected cytoprotective effect of curcumin is associated with the inhibition of lipid peroxidation and the maintenance of glutathione level (Strasser et al., 2005). In addition, curcumin activates antioxidant enzymes, prevents mitochondrial dysfunction and suppresses production of the pro-inflammatory cytokines through transcriptional control in both transformed and normal cell types (Wang et al., 2009; Huang et al., 2013). The detected cytoprotective effect of curcumin has been shown in liver cells damaged by hepatotoxic heavy metals including lead, mercury, cadmium, chromium, and copper (García-Niño & Pedraza-Chaverrí, 2008), anti-inflammatory (Bereswill et al., 2010), and anti-tumour (Fattori et al., 2015; Tong et al., 2016). Taking into account that Cd exposure as well as cytoprotective effect of curcumin against Cd cytotoxicity. The RPE cells (7×10⁵) were inoculated to 6-wells plates with DMEM/F12 medium containing 10% FBS. The cell treatment was removed and 180 µL PBS was added to each well for the cell viability and pro-inflammatory changes in the neural tissue cells (Phuagkhao pong et al., 2017).

Materials and methods

The human RPE cell line was obtained from American Type Culture Collection (Cat® CRL2302, ATCC, Rockville, MD). The RPE cells were cultivated in Dulbecco’s modified Eagle medium/Hams F-12 50/50 Mix (DMEM/F12) medium containing 10% foetal bovine serum (FBS), 1000 U/mL penicillin and streptomycin, 25 mM glucose, 1% L-glutamine, 1% sodium pyruvate. The cells were collected with trypsin/EDTA solution when the confluence was 80-90%. The cells (5×10⁵) were transferred to 6 cm Petri dish and growth-arrested with 0.2% FBS containing medium 24 hours before experiment. The RPE cells were subjected to 10 µM cadmium chloride (CdCl₂), water-soluble curcumin fraction in doses 2 and 5 µM and aforementioned doses of Cd with curcumin together for 48 h. When curcumin was used to treat Cd-exposed RPE cells, the cells were pre-incubated with curcumin for 1 h before the treatment with Cd.

The levels of cell viability was determined by measuring the capability of live cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay). The individual effects of Cd and curcumin as well as cytoprotective effect of curcumin in Cd-exposed RPE cells on cell viability were studied for 48 h treatment. Cells were gathered from 90% confluence flask using trypsin/EDTA solution, centrifuged and calculated. Cell pellet was suspended in DMEM containing 10% FBS and diluted to concentration 10⁵ cells/mL. The RPE cells (10⁵ cells/well) were inoculated into 96-well plates in DMEM/F12 medium containing 10% FBS and incubated for 12 hours at 37 °C in a humidified atmosphere with 5% CO₂. After cell attachment and the start of the growth, the medium was changed in every well and the cells were exposed to Cd (10 µM) and curcumin dissolved as described above. Control (untreated) cells were not exposed to any solution. Every control and Cd, curcumin and Cd + curcumin exposed concentration was represented by 3 wells and replicated twice. The medium was removed from plates after 48 h incubation and the cells were rinsed with PBS. After washing with the saline buffer PBS, the cells were incubated in the solution containing 180 µL PBS and 20 µL MTT reagent. The incubation with MTT reagent was carried out for 4 h at 37 °C in a humidified atmosphere with 5% CO₂. After incubation, MTT solution was removed and 180 µL DMSO was added to each well for the followed 10 min incubation. The absorbance level was measured at 570 nm length wave in the presence of 20 µL Soerensen’s buffer. The obtained data were presented as the percentage of control value.

The production of intracellular general forms of ROS was determined with 2,7’-dichlorofluorescin diacetate (DCFHDA). Control and exposed cells were rinsed with PBS, treated with 10 µM DCFHDA and incubated for 30 min at 37 °C. The ROS levels were determined using a SpectraMax Gemini EM spectrofluorometer with 485 nm wavelength excitation and 530 nm emission.

The control and curcumin cells exposed to Cd after 48 hour treatment were washed in Petri dishes trice with the cold PBS and collected by scratching without trypsinization. The collected cells were centrifuged and lysed in RIPa buffer containing proteinate and phosphatase inhibitor cocktail. The PRE cell proteins were extracted for 60 min at 4 °C. After

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The concentrations of the tested substances were selected based on the earlier published literature data (Kalariya et al., 2009; Morì et al., 2015; Lee et al., 2016). Stock solution of CdCl₂ was prepared by dissolving in DMEM at 100 mM and stored in ~20 °C. Curcumin stock solution was prepared by dissolving curcumin water-soluble fraction (Curcumin L-WS E-100, Sensient Technologies Corporation, the USA) in DMEM at 4 mM right before treatment. All chemicals except curcumin were obtained from Sigma-Aldrich. The migration activity of RPE cells was determined using wound-healing assay to clarify functional disorders induced by Cd exposure as well as cytoprotective effect of curcumin against Cd cytotoxicity. The RPE cells (7×10⁵) were inoculated into 96-well plates with DMEM/F12 medium containing 10% FBS. When the cell growth had achieved 100% confluence, the gap in their colony was made by scratching with a pipet’s 200-µL tip. The cells had been rinsed with phosphate buffer saline (PBS) to remove detached cells and were incubated with DMEM/F12 medium containing 0.2% FBS. Cell treatment with Cd or/and curcumin was performed in the same manner as mentioned above. The photographs were taken every 24 hours with invert microscope (Olympus CKX41, Olympus Corporation, Japan).

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lization, the cell extracts were centrifuged at 40,000 g for 20 min. The content of total protein in supernatants was measured with spectrophotometer by the Bradford method using BSA as the standard \( \text{Bradford, 1976} \). The supernatant of each protein extract was mixed with LaemmLI sample buffer containing 0.1 M dithiothreitol in ratio 1:1 and boiled for 5 min. Protein samples fixed with LaemmLI buffer were frozen and stored at \(-80^\circ\text{C}\) before the start of the western blot analysis.

The proteins were separated using polyacrylamide gel (PAGE) electrophoresis using 5-20% gradient of acrylamide and then the proteins were transferred from gel onto polyvinylidene fluoride (PVDF) membrane with application of the electric field \(10 \text{V/cm}\). After the transfer, PVDF membrane was rinsed and blocked in 1% bovine serum albumin (BSA) in PBS-Tween-20 solution. The blocked membrane was probed overnight at 4 \(^\circ\text{C}\) to primary antibodies anti-Nrf2 \(1:2000\), Abcam, ab137550), anti-PARP \(1:3000\), Abcam, ab32071), anti-fibronectin \(1:2000\), Abcam, ab199056), anti-E-cadherin \(1:2000\), Abcam, ab40772) and anti-GAPDH as a loading control \(1:2500\), Santa Cruz, sc-365062) antibodies. After the primary antibodies incubation, the membrane was washed and incubated with corresponding secondary either anti-rabbit or anti-mouse IgG antibodies conjugated with horseradish peroxidase \(1:20000\) Abcam, ab6721) were used to probe the membrane after primary antibodies. Immunostaining was developed with luminol-hydrogen peroxide solution by the enhanced chemiluminescence method with the use of X-ray films \(\text{Konica Minolta, Japan}\). Densitometric analysis of the immunostained polypeptide zones was performed using TotalLab TL120 software \(\text{USA}\). The intensity value obtained with scanning every individual band was normalized to the intensity in respect with correspondent actin band. Every track on the scanned picture was corrected to background level, which corresponds to nonreactive area on the X-ray film.

Statistical comparisons of the data were performed using a one-way analysis of variance \((\text{ANOVA})\) followed by Tukey’s post-hoc test using Origin, 2019 \(\text{OriginLab Corporation, the USA}\). The data were expressed as the mean \(\text{(M)}\) with standard error \(\text{(SEM)}\) of at least four independent experiments. \(P\) values less than 0.05 were considered statistically significant. The graphs were constructed using GraphPad Prism 9 \(\text{GraphPad Software, the USA}\).

**Results**

In order to detect the effects of Cd on cell viability, the RPE cells were exposed to 1, 2, 5 µM curcumin and 10 µM CdCl\(_2\) for 48 h. The progressive decline in cell viability was observed in the RPE cells treated with 5 and 10 µM CdCl\(_2\). These doses reduced cell viability by 82% \(P < 0.05\) and 64% \(P < 0.01\) respectively. The dose of 10 µM CdCl\(_2\) as a concentration, which initiates prominent cytotoxicity, was selected to investigate the cytoprotective effect of low doses of curcumin.

Curcumin slightly suppressed cell viability in the dose of 5 µM. On the other hand, the cytoprotective effect of curcumin was observed in Cd-exposed RPE cells. Cd-induced decline in cell viability was significantly \(P < 0.001\) prevented by 5 µM curcumin administration (Fig. 1).

Similar curcumin effect was detected for the suppression of ROS level in both 2 and 5 µM doses in Cd-exposed RPE cells. The exposure of untreated RPE cells to curcumin had no effect on the ROS production while the curcumin inhibited the ROS upregulation in Cd-exposed RPE cells in a dose-dependent manner (Fig. 2).

Cell migration plays a critical role in the repairing different damages to replace the injured cells. Cell migration is regulated by intercellular and cell-extracellular matrix (ECM) adhesion. The estimation of migration was studied to clarify a possible curcumin role in the transcriptional regulation of the RPE cell response against Cd cytotoxicity. The upregulation of Nrf-2 was observed in Cd-exposed RPE cells. The treatment with curcumin caused no changes in Nrf-2 content in the control untreated cells. However, the application of 5 µM curcumin significantly inhibited the increase in Nrf-2 expression (Fig. 4).

**Fig. 1.** Retinal pigment epithelium cells viability: Curc 1 – cells exposed to 1 µM curcumin, Curc 2 – exposed to 2 µM curcumin, Curc 5 – to 5 µM curcumin, Cd – to 10 µM CdCl\(_2\), Cd+C – 1 to 1 µM curcumin + 10 µM CdCl\(_2\), Cd+C 2 – to 2 µM curcumin + 10 µM CdCl\(_2\), Cd+C 5 – cells exposed to 5 µM curcumin + 10 µM CdCl\(_2\); data expressed as x ± SE; *** – P < 0.001 (compared with control group), ## – P < 0.01 (compared with Cd exposed group).

**Fig. 2.** Reactive oxygen species (ROS) level in retinal pigment epithelium cells: Curc 1 – cells exposed to 1 µM curcumin, Curc 2 – exposed to 2 µM curcumin, Curc 5 – to 5 µM curcumin, Cd – to 10 µM CdCl\(_2\), Cd+C 1 – to 1 µM curcumin + 10 µM CdCl\(_2\), Cd+C 2 – to 2 µM curcumin + 10 µM CdCl\(_2\), Cd+C 5 – cells exposed to 5 µM curcumin + 10 µM CdCl\(_2\); data expressed as x ± SE; *** – P < 0.001 (compared with control group), ## – P < 0.01 (compared with Cd exposed group).

The measuring of extracellular matrix (ECM) protein fibronectin and epithelial adhesive protein E-cadherin has shown a statistically significant decrease \(P < 0.01\) of them both in Cd-exposed RPE cells. Curcumin administration ameliorated a downregulation of both fibronectin and E-cadherin in a similar manner (Fig. 4).

The expression of PARP was studied in the present work to elucidate Cd-caused disturbance in the DNA repair mechanism depending on poly-ADP-ribose synthesis by this enzyme. The treatment with 10 µM CdCl\(_2\) induced statistically significant increase in 89 kDa PARP-cleaved subunit. At the same time, curcumin in the dose of 5 µM promoted restoration of the level of PARP initial content as in the intact cells (Fig. 4).

The results of western blot (Fig. 5) and wound healing assay suggest the involvement of adhesive protein E-cadherin and ECM protein fibronectin into the Cd-dependent downregulation of the cell migration.
Fig. 3. Wound healing assay of retinal pigment epithelium cells immediately (0 h) and 84 h after performing a scratch: Curcumin 5 – cells exposed to 5 µM curcumin, Cd 10 – cells exposed to 10 µM CdCl₂, Cd 10 + Curcumin 5 – cells exposed to 10 µM CdCl₂ and 5 µM curcumin; the gaps are indicated with white lines; the closed gaps are indicated with white arrows.
Fig. 4. The Nrf2 (a), E-cadherin (b), fibronectin (c) and PARP-1 (d) in retinal pigment epithelium cells: Curc 1 – cells exposed to 1 µM curcumin, Curc 2 – exposed to 2 µM curcumin, Curc 5 – to 5 µM curcumin, Cd – to 10 µM CdCl2, Cd+Cure 1 – to 1 µM curcumin + 10 µM CdCl2, Cd+Cure 2 – to 2 µM curcumin + 10 µM CdCl2, Cd+Cure 5 – cells exposed to 5 µM curcumin + 10 µM CdCl2; data expressed as x ± SE; ** – P < 0.01, *** – P < 0.001 (compared with control group); # – P < 0.05, ### – P < 0.01 (compared with Cd exposed group)

Fig. 5. Western blot analysis of Nrf2, E-cadherin, fibronectin, PARP-1 and GAPDH: Contr – control group, Curc 1 – cells exposed to 1 µM curcumin, Curc 2 – exposed to 2 µM curcumin, Curc 5 – to 5 µM curcumin, Cd – to 10 µM CdCl2, Cd+Cure 1 – to 1 µM curcumin + 10 µM CdCl2, Cd+Cure 2 – to 2 µM curcumin + 10 µM CdCl2, Cd+Cure 5 – cells exposed to 5 µM curcumin + 10 µM CdCl2
The data obtained bear evidence for the prospective potential of curcumin administration for the amelioration of Cd-caused disturbance in E-cadherin and fibronectin expression and protection of the migration function.

Discussion

Molecular mechanisms of curcumin cytoprotection. The mechanisms of curcumin cytoprotection were reported in several models of heavy metal toxicity including aluminium, Cd and lead (García-Niño & Pedraza-Chaverri, 2014; Mohajeri et al., 2017; Kar et al., 2019). Recently, protective antioxidant effect of curcumin was detected in serum of mice treated with cadmium (Morneni & Eskandari, 2020). In addition, the efficacy of curcumin against Cd toxicity was shown in several reports. For instance, the neuroprotection and hippocampal neurogenesis promotion were demonstrated in mice CNS (Narayagil et al., 2020). Furthermore, the glioprotection in cultured primary astrocytes was confirmed in respect to glial cytokeratin and energy metabolism (Nedevtsky et al., 2018; Narayagil et al., 2020). Nevertheless, the cytoprotective effect of soluble curcumin on Cd-induced retinal damages remains unknown.

Therapeutic efficacy of curcumin was verified in a number of studies and is related to the control molecular signaling pathways, which in turn modulate cell survival and cellular response to cytotoxic agents (Hatcher et al., 2008). Natural curcumin has a low bioavailability and exhibits anti-proliferative activity in high (10–50 M) doses towards malignant cells. Besides, 10 M dose was reported as cytoprotective of retinal neurons (Wang et al., 2011). However, several cell types including RPE cells are susceptible to the effects of curcumin. A decrease was determined in cell viability and VEGF secretion in RPE cells exposed to curcumin in doses above 10 μM (Hollborn et al., 2013). Therefore, the curcumin doses that are effective in inhibiting the neuronal cell death have adverse effects on RPE cells. In the present work, we have studied respectively low doses (1–5 M) of soluble curcumin to protect RPE cells against Cd cytotoxicity.

The effect of curcumin on Cd-caused oxidative stress, cell viability and the transcriptional factor Nr2 expression. There is limited data on Cd toxicity effects in the retina. The recent findings on heavy metal content in the human retina have shown that Cd is one of the prior candidates to be an inducer of retinal pathogenesis in the age-related macular degeneration (AMD) (Pamphlett et al., 2020). Oxidative stress and apoptosis were confirmed as undoubted Cd-caused abnormalities in retinal cells (Kalaraygi et al., 2009; Kletner et al., 2012). Cd-induced apoptosis in human ARPE-19 cells is initiated with the caspase 3 activation and causes the loss of cell viability (Chen et al., 2008). However, the exact mechanisms of Cd-caused cytotoxicity remain poorly understood. The activation of mito-gem activated protein kinase (MAPK) and inhibition of protein phosphatases were reported as the disturbances, accompanied by neuronal apoptosis and oxidative stress (Chen et al., 2008). There was seen a link between Cd accumulation in human retina and progress in AMD (Wills et al., 2009). Recently, endoplasmic reticulum (ER) stress and autophagy flux were detected in RPE cells exposed to Cd (Zhang et al., 2019). The authors concluded that these abnormalities play a pivotal role in AMD and blindness.

Cd is confirmed as a ROS upregulating agent in various tissues including the retina (Kletner, 2012; López-Malo et al., 2020). Oxidative stress is one of the most studied initiators of the progress in retinal pathogenic changes, in which both destructive and repairing molecular mechanisms are involved. In conjunction with molecular damage, oxidative stress generates the signalling of cellular response aimed at supporting the cell survival. For instance, ROS-dependent phosphorylinoitride 3-kinase (PI3K) activation is a part of intracellular signalling and leads to the initiation of cellular response against toxic agents via PI3K/Nr2/PHO-1 pathway (Wang et al., 2018). Furthermore, the role of many factors involved in this response to oxidative stress and associated complications are diverse and conflicting. Several protein kinases may conduct both protective and apoptotic pathways. The decline in RPE cells viability and upregulation of ROS production, which we observed in our study, are coherent with earlier published results on the dose-dependent manner of Cd toxicity (Kalaraygi et al., 2009; Zhang et al., 2019). Furthermore, the role of oxidative stress in retinal pathology was confirmed by applying N-acetylcystei-ne as an antioxidant to prevent Cd-caused retinal damage (Zhang et al., 2019). Another aspect of oxidative stress impact in retinal pathogenesis was studied in the retina of human donor eyes. The decrease in Nr2 and hemoxigenase-1 (HO-1) transcript levels was observed in the retina of human donor eyes with AMD associated changes (Aberamni et al., 2019). Nr2 and HO-1 are the parts of antioxidant defence and their depletion leads to increase in oxidative stress.

The mild increase in Nr2 expression was confirmed in our study can be related to the low dose and short-term Cd exposure. Moreover, some data evidence that Cd low doses activate the cell response and metabolic activity as was demonstrated in astrocytes and neuronal cells (Gulisano et al., 2009; Phaugkhuapong et al., 2017). Therefore, we may assume that the results observed in Cd-exposed RPE cells upregulation of Nr2 are a part of defensive response against increased ROS production and multifaceted Cd toxicity. In the present study, we have shown that Cd-caused retinal cell death exhibits a dose-dependent manner accompanied by defensive response initiation. Our results are consistent with Cd-dependent disturbances that were reported for the neuronal cells (Chen et al., 2008).

Curcumin administration had no changes in ROS level, cell viability and Nr2 content in untreated control cells. However, in Cd-exposed RPE cells curcumin in the dose of 5 μM inhibited both the ROS upregulation and the cell viability decrease. Furthermore, curcumin induced a significant increase in Nr2 expression. Nr2 controls HO-1 expression transcriptionally and in this manner mediated cell response against toxicity. Thus, one of possible mechanisms of the protective effect of curcumin could be mediated with Nr2-dependent transcriptional regulation of antioxidant defence including HO-1.

The effect of curcumin on Cd-caused cell motility and adhesion. Cell migration is a fundamental process to repair damaged areas and support tissue functions. The suppression of RPE cells migration can suspend the retinal repair as well as the vision function. The disruption of the mechanisms that provide cell migration was studied in TM4 Sertoli cells treated with 1 and 12 μM CdCl2 for 4 h (Egbowon et al., 2016) and in endothelial ECV-304 cell line exposed to 1–5 μM CdCl2 for 24 h (Kolluru et al., 2006). Also, Liu and co-authors reported that Cd does not affect proliferation and migration of lung cancer A549 cells at concentrations of 0.1–10 μM (Liu et al., 2015). On the other hand, there was shown the Cd-dependent activation of migration capability of breast cancer MDA-MB-231 cells (Wei & Sheikh, 2017). The similar effect of chronic Cd exposure was observed in the breast non-cancer epithelial cell lines (MCF10A) and in the hTERT gene containing human pancreatic nestin expressing cells (hTERTHPNE) (Vanlaey et al., 2020). Therefore, contradictory effect of Cd depends on the cell type as well as the total duration and dosage.

The inhibitory effect of Cd on RPE cell migration observed in the present study could reflect Cd-caused multiple harmful effect. A lack of migration ability as a result of Cd exposure was recognized as a function of cytokeratin disturbance (Egbowon et al., 2016). However, in the present work, we detected that the decrease in RPE cells motility was accompanied by downregulation of fibronectin and E-cadherin in RPE cells. ECM and cell adhesion proteins provide both intercellular communication and cell migration. The imbalance in the location and content of these proteins leads to disturbance in the cell behaviour including their motility. Thus, Cd-caused decrease in fibronectin and E-cadherin contents could be, at least partially, a cause of the decrease in RPE cells motility. Our results are consistent with the data that Cd can disrupt cadherin-mediated cell-cell adhesion in endothelial cells (Woods et al., 2008). Moreover, the link between E-cadherin expression and inhibition of RPE cells migration in the course of Cd exposure is presented for the first time. The current study confirmed that curcumin ameliorated the RPE cells motility as well as fibronectin and E-cadherin expression. Therefore, the modulation of RPE adhesiveness could be considered a cytoprotective effect of curcumin.

The effect of curcumin on Cd-caused PARP alteration and parthanatos flux. Genotoxicity of Cd was studied on various tissues and is well known. However, the detrimental effect of Cd on repairing cellular systems is poorly understood. In the present study, we examined DNA repair enzyme poly(ADP-ribose) polymerase 1 (PARP-1), one of wide spread enzymes among eukaryotic cells. The results we obtained in our study indicated that the exposure to 10 μM Cd induced the PARP upregu-
ulation. On the other hand, curcumin application inhibited Cd-caused PARP increase. Our results are consistent with earlier presented data on the Cd effect in the renal epithelial cells and astrocytes (Kushwaha et al., 2018; Luo et al., 2020). PARP can be activated by several genotoxic compounds to bind DNA strand breaks. Activated PARP catalyzes the poly-ADP-ribosylation of nuclear proteins, which control the chromatin structure and DNA replication. In the recent years, there has been a large amount of data to display the relation of PARP activities with the regulation of cellular response. PARP proteins family also plays important roles in the regulation of transcription, the cellular stress response, mRNA stability, cell division, and protein degradation (Bai, 2015). Furthermore, parthanatos, which is a PARP-1-dependent form of programmed cell death, was discovered in cancer and non-transformed cells (Ma et al., 2016). The upregulation in cleaved-PARP content is the main index of the parthanatos progress. The genotoxic effect of Cd on PARP-1-dependent parthanatos was determined in osteoblast MC3T3-E1 cell line, renal tubular epithelial NRK-52E cell line and cultured rat astrocytes (Kushwaha et al., 2018; Luo et al., 2020; Ou et al., 2021).

Increasing cleaved-PARP/PARP ratio is considered as a process accompanied by apoptotic changes (Kushwaha et al., 2018). Together with parthanatos, multiple kinases and antioxidants are involved in a response to Cd cytotoxicity while balance in apoptosis, autophagy and parthanatos is extremely instable (So & Oh, 2016). Furthermore, Cd can initiate the process of cell death through mitochondrial damage and synergistic effect caused by the positive feedback between machinery of parthanatos and oxidative stress (Luo et al., 2017). The results we obtained demonstrate that low doses of curcumin are potent to ameliorate PARP activity and prevent harmful effect of PARP overactivation. PARP-1 plays a dual role in genotoxicity. PARP-1 mediates DNA repair when DNA damage is low. However, if DNA damage is severe, PARP can aggravate multiple cell damage and initiate cell death (So & Oh, 2016; Luo et al., 2017). Thus, the results presented in our study are evidence that Cd-caused decrease in RPE cells viability could be at least partially initiated with synergistic interaction of parthanatos and oxidative stress.

The effects of curcumin as anti-inflammatory, anti-proliferative and apoptosis inhibition agent in malignant cells have been documented over the recent years. However, the efficacy of curcumin to prevent over-reactivity of normal glial cells remains unstudied. Exposures to 25–100 μM doses of curcumin are efficient to induce the cell death in various cancer cell types. For instance, curcumin was observed as an apoptosis inducer through cleaved PARP-1 upregulation and caspase-3 dependent pathway in lymphoblastic leukaemia cell lines (Mishra et al., 2016). Despite this, similar high doses of curcumin are cytotoxic to most normal cell types. On the contrary, low doses of curcumin administration can stimulate the protective mechanisms in damaged cells (Nedevzsky et al., 2017, 2018; Ağca, 2019). It was reported that curcumin prevents the apoptosis initiation as well as cleaved PARP production in osteoblasts and adipocytes (Zhu et al., 2015; Chen et al., 2016). Besides, low doses of curcumin exerted cytoprotection in the diabetic retina model while PARP is involved in retinal abnormalities (Platania et al., 2018). Therefore, the protective effect of curcumin could be related, at least partially, to the regulation of PARP activity.

Several reports have confirmed the efficacy of curcumin in retinal cytoprotection. However, the protective effect of curcumin was confirmed for retinal cells in respect with its antioxidant features (Park et al., 2017; Muangnoi et al., 2019; López-Malo et al., 2020). On the other hand, curcumin is potent to affect different vital pathways including autophagy and apoptosis initiation. PARP also causes cell death and plays a dual role in apoptosis through the effects on the intrinsic (mitochondrial-dependent) and extrinsic (death-receptor-dependent) pathways (Zhang et al., 2012). In spite of a number of reports, the mechanism of curcumin-caused PARP inhibition is poorly understood. Curcumin was reported to enhance the susceptibility of poly(ADP-ribos)e polymerase to its inhibitors (Choi & Park, 2015). It is hypothesized that curcumin can regulate the induction of cell death by expression of the autophagy markers Becl-2 and Becl-1, which mediate the functional crosstalk between autophagy and apoptosis (Anto et al., 2002).

Moreover, curcumin is well characterized as an NF-κB inhibitor (Schwertheim et al., 2017). The direct and indirect PARP and NF-κB interactions could be promising means to mediate the PARP-dependent cytoprotective effect of curcumin as was demonstrated on astrocytes (Nedevzsky et al., 2018; 2019; Kirci et al., 2019).

The number of proposed effective strategies to prevent Cd-caused retinal damages is extremely limited. Taking together the results obtained in our work, the administration of low doses of soluble curcumin exhibits multiple cytoprotective effects on RPE cells exposed to Cd. In addition, the observed protective effect of curcumin is mediated at least with modulation of PARP-1 activity, E-cadherin, fibronectin and Nrf2 expressions.

**Conclusion**

Cd that is considered a widespread dangerous ecotoxicant decreases viability of RPE cells, which is accompanied by the oxidative stress, depletion of migration, parthanatos, imbalance in adhesiveness, and ECM proteins expression. The administration of soluble curcumin to Cd-stressed RPE cells ameliorated all aforementioned disturbances. Therefore, low doses of soluble curcumin are potent to ameliorate cytotoxic effect of Cd exposure through the suppression of ROS production, parthanatos and restoring RPE cells adhesiveness. The presented results demonstrate that soluble curcumin can be considered a promising protector against Cd-caused retinal injury.

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