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The Effect of Aluminium on the Morphologic Appearance, Viability and Phagocytic Activity of ARPE-19 Cells

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Abstract

Purpose: Aluminium is known to have toxic effects on the central nervous system. We wanted to explore the effects of aluminium on cultured ARPE-19 cells, in particular, changes in the morphologic appearance, viability and in the phagocytic activity of these cells.

Methods: After addition of different concentrations of aluminium to the cell cultures, cellular morphology was evaluated by photomicrographs; viability was determined by mitochondrial activity measurement and phagocytosis by uptake of europium-labeled FluoSpheres.

Results: Pretreatment of the cells with aluminium led to the formation of clots in the cell culture and there was a relative weak dose-dependent decrease in viability. However, phagocytic activity was severely impaired at each concentration with a peak decrease of 92.45% (± 8.21) at 1000 µmol.

Conclusions: Exposure to aluminium occurs mainly through contaminated food and beverages. Given that sufficient concentrations accumulate in the RPE, inhibition of the phagocytic activity of RPE cells might represent a novel important side effect of this metal. Although no conclusions can be drawn from *in vitro* results on the effect in vivo, it seems that caution is recommended with consumption of food with high concentrations of aluminium. The reduced viability of RPE cells, however, is clinically less relevant since the effect was relative weak in vitro.

Keywords: Retinal pigment epithelium; Aluminium; Morphologic appearance; Viability; Phagocytosis

Introduction

The retinal pigment epithelium (RPE) is a monolayer of cells located immediately adjacent to the photoreceptor layer of the neural retina. These cells are highly specialized and exert a variety of functions which are essential for the maintenance of retinal homeostasis and visual function. Their manifold functions have been reviewed elsewhere [1]. In brief, they are responsible for the absorption of light at the beginning of the cascade of visual processing; they transport ions, water and metabolic end products from the subretinal space to the blood, take up nutrients such as glucose, retinol and fatty acids from the blood and deliver these nutrients to the photoreceptors. Furthermore, the RPE is able to secrete a variety of growth factors helping to maintain the structural integrity of the choriocappillaris endothelium and photoreceptors. These cells are mitotically quiescent under physiological conditions in the eye, but undergo dedifferentiation and proliferation when the neural retina suffers traumatic injury leading to the development of proliferative vitreoretinopathy. This disease is characterized by the formation of epiretinal membranes which in the worst case results in traction retinal detachment (review, see [2]). But apart from these effects, the main function of RPE cells, however, is their phagocytic activity. In particular, they contribute to disc shedding and phagocytose more material over a lifetime than any other cell type in the body [3]. Consequently, impairment of the phagocytic activities of these cells may result in the development of retinal disorders, such as accumulation of debris clinically manifesting as appearance of drusen [3].

Aluminium is a toxic metal which is present ubiquitously on earth. Whereas it has no biological role to play in the body, adverse physiological effects have been observed in mammals. There are neurotoxic effects of this metal in the central nervous system, and impaired mitochondrial function and oxidative stress have been implicated in aluminium- induced neurodegeneration (review, see [4]). Aluminium was found to induce retinal changes in the rat indicating that it is toxic to the retina [5]. We investigated the effect of this metal on RPE cells that exert many crucial effects. In the present study, we aimed to find out whether (i) pretreatment with aluminium leads to morphological changes in cultures of ARPE-19 cells, (ii) exposure of cultured ARPE-19 cells to aluminium is toxic resulting in reduced viability of these cells, and finally (iii) exposure of cultured ARPE-19 cells to this metal leads to impairment of their phagocytic activity.

Materials and Methods

Cell culture

Human ARPE-19 pigment epithelial cells (American Type Culture Collection, CRL-2302) were cultured in a RPMI 1640 medium (Gibco, UK) including L-glutamine and HEPES. An antibiotic-antimycotic mixture (Gibco, UK:100 units/ml penicillin, 100 µg/ml streptomycin,

250 ng/ml amphotericin B) with 5% fetal bovine serum (Gibco, UK) was added at 37°C. In this study, only cells from passages 52–58 were used. The cells were cultured until reaching confluence and they were then sub-cultured on 96-well assay plates (Becton Dickinson, Belgium) for the experiments. Aluminum chloride (Roth, Germany) was dissolved in RPMI 1640 at a pH of 7.4, autoclaved, and a 0.1 mol stock solution was prepared which was diluted to different final concentrations for the experiments.

Mitochondrial activity measurement

The cells were seeded at a density of 3.13×10^5cells per cm^2 in 100 µl medium into each well of a 96-flat-well microtiter plate. The cells were allowed to grow for two days until confluent. Then, an additional 100 µl of the medium with different concentrations of aluminum was added (effective concentrations from 15 µMol to 1000 µMol). The same amount of RPMI 1640 without aluminum was added and served as a positive control. After an exposure time of 48 h, cell viability was assayed with the EZ4U test (Biomedica, Austria) according to manufacturer´s instructions. Briefly, after washing with RPMI, cells were incubated with 200 µl of medium containing 10% of the substrate-activator reagent. In the test the colorless substrate is converted in the dye XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide by the activity of mitochondrial enzymes. In every experiment a positive control without aluminum and a blank of the substrate only was done in parallel. The concentrations were assayed eight times in parallel in three independent experiments. The cells were incubated with the reagent for approximately 60-90 min. After gentle shaking the red formazan dye was formed and was measured at the wavelength of 450 nm using a Multiskan MS microplate reader (Labsystems, Finland). The results were related to control values from cells treated without aluminum. The OD values of the controls were set as 100% viability and were calculated to the percentage of viability of the aluminum concentrations.

Phagocytosis of europium-labeled microspheres by RPE cells

For determining the phagocytic activities of RPE cells, europium-labeled Fluospheres® (Molecular Probes, Eugene, Oreg., USA) were used. ARPE-19 cells were seeded at a density of 3.13×105 cells per cm^2 in 100 µl medium in a 96-flat-well microtiter plate. All cells were cultured for 3 days until confluence was reached. Then, a further 100 µl of medium containing 1:0.008% of 0.2 µm diameter particles and different aluminum concentrations (effective concentrations from 15 µMol to 1000 µMol) were added. The ARPE-19 cells were allowed to phagocytose the microspheres for 48 hrs, after which the wells were washed 3 times with 37°C warm RPMI 1640 to remove non-phagocytosed particles. The adherent cells were then lysed by adding 100 µl of 3% Triton-X 100 (Sigma-Aldrich, St. Louis, Mo., USA) to each well under continuous shaking with an orbital shaker (Janke &Kunkel-IKA-Labortechnik, Staufen, Germany) for 30 min. Uptake of europium-labeled microspheres was quantified by lanthanide-specific time-resolved fluorometry using a 1234 Delfia Research Fluorometer (Wallac, Turku, Finland). All concentrations were assayed eight times in parallel in three independent experiments.

Photographs

Photographs were taken after 48-h incubation with aluminum with an inverted phase contrast microscope (Olympus, Japan).

Statistics

Statistical calculation of differences between the data on controls versus aluminium pretreatment was performed by the Mann-Whitney U test ($^{**}p<0.01$; $^{***}p< 0.001$).

Results

The morphologic appearance of the ARPE-19 cells in culture both in controls and after aluminium pretreatment is illustrated in Figure 1. In controls, the typical spindle-shaped morphology of the cells developed in confluent cultures (Figure 1a). Exposure of cells to aluminium led to a change in their arrangement, in particular, it led to the formation of clots. This rearrangement was dose-dependent, with fewer clots at low concentrations (150 µmol, Figure 1b) but more clots at medium (250 µmol, Figure 1c) and much more at high concentrations, with the maximum seen at the highest concentration tested (1000 µmol, Figure 1d).

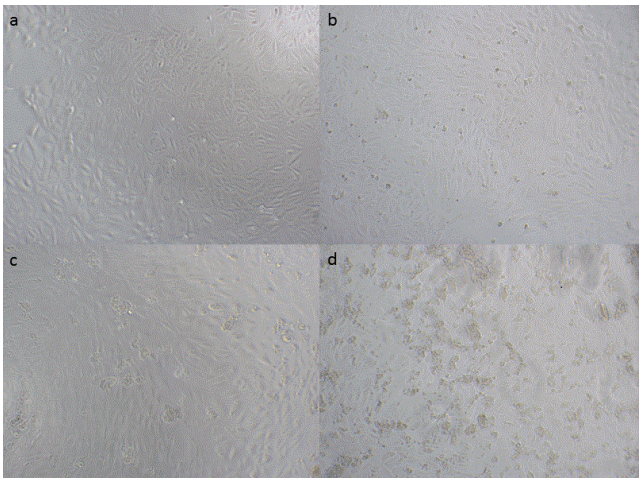

Figure 1: Morphologic appearance of ARPE-19 cells in culture. In confluent cultures in controls, cells are typically spindle-shaped (a) whereas clots are increasingly formed when cells are exposed to 150 µmol (b), 250 µmol (c) and 1000 µmol aluminium (d).

Next, the dose-dependence of the effect of aluminium on the viability of ARPE-19 cells was evaluated and the outcomes are documented in Figure 2. There was a slight dose-dependent decrease which averaged 95.62% (± 1.86), 94.67% (± 2.10), 92.57% (± 3.35), 86.20% (± 2.50), 80.99% (± 2.63) and 83.56% (± 2.91) of controls at 4, 15, 62.5, 250, 500 and 1000 µmol, respectively. Statistically significant effects on viability were observed both at 4 and 15 µmol ($^{**}p<0.01$) and at 62.5, 250, 500 and 1000 µmol concentrations ($^{***}p< 0.001$).

Finally, the effect of aluminium on the phagocytic activity of ARPE-19 cells has been explored and the results are shown in Figure 3. There was pronounced phagocytic activity of ARPE-19 cells in controls under normal conditions whereas aluminium dose-dependently decreased the phagocytic activity. In particular, 4 µmol aluminium led to a 26.69% (± 3.39) decrease, 15 µmol to a 74.25% (± 7.08) decrease, 62.5 µmol to a 84.67% (± 9.97) decrease, 250 µmol to a 85.6% (± 6.53) decrease and 1000 µmol to a 92.45% (± 8.21) decrease. Statistical calculation of differences between the values of controls vs. cells pretreated with aluminium revealed a highly significant reduction

of phagocytic activity in the latter at each concentration tested (***p<0.001).

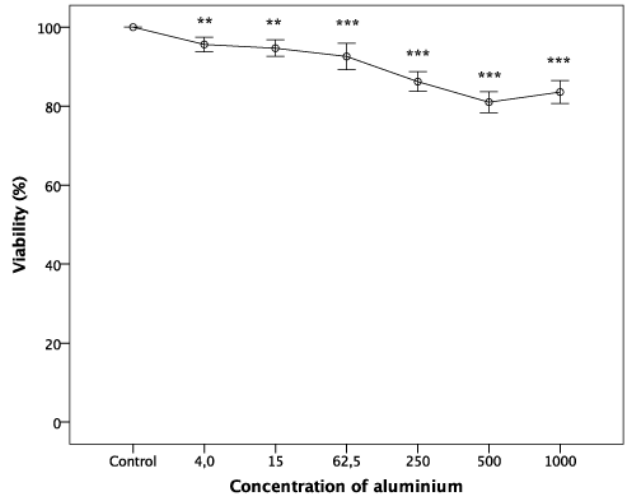

Figure 2: Effect of various concentrations of aluminium on the viability of ARPE-19 cells. Different concentrations of aluminium were added to the culture medium indicated horizontally as µmol. There was only a weak decrease of viability but was statistically significant at each concentration. Values represent means ± S.E.M. (**p<0.01; ***p<0,001; n=24 for each group).

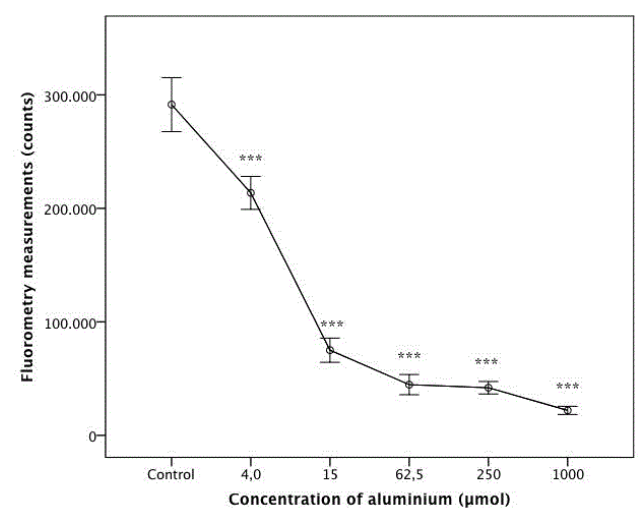

Figure 3: Effect of various concentrations of aluminium on the phagocytic activity of ARPE-19 cells. There was statistically significant reduction in the phagocytic activity at each concentration indicated horizontally as µmol which featured a peak decrease of 92,45% (± 8,21) at 1000 µmol. Values represent means ± S.E.M. (n=36 for each group; ***p<0.001, Mann- Whitney U test).

Discussion

In this study, the effect of various concentrations of aluminium on the viability and changes in phagocytic activity of ARPE-19 cells in culture was evaluated. We found a weak dose-dependent impairment in viability but pronounced statistically significant impairment in phagocytic activity. Furthermore, RPE cells are usually spindle-shaped but exposure of ARPE-19 cells to aluminium leads to formation of numerous clots. As far as we know, such a phenomenon has not been observed in other cells exposed to this metal. There was only a relative weak effect of not more than a 19% decrease on cell viability at a high concentration and statistically significant differences were seen only because of the high number of single experiments performed under each concentration. This finding is in agreement with previous studies reporting that only very high concentrations of aluminium exerted an effect in vitro not only on RPE cells but also on SH-SY5Y neuroblastoma and U 373MG glioblastoma cells suggesting a limited vulnerability of these cells towards this metal [6,7]. In contrast, more pronounced reductions of cell viability resulted from exposure to mercuric mercury and methylmercury, suggesting that the cells are more susceptible to these metals [7]. By contrast, certain alterations in the brains of patients with neurodegenerative diseases, in particular with Alzheimer´s disease, amyotrophic lateral sclerosis, Parkinson´s disease or dialysis dementia was found to be associated with an accumulation of aluminium [8-11]. But it is not clear whether the accumulation of aluminium is the cause or a consequence of these diseases. It has been proposed that glial cells might accumulate neurotoxic metals leading to apoptosis which subsequently results in neuronal degeneration [12-14].

On the other hand, exposure of cultures of ARPE-19 cells to aluminium led to reductions in the phagocytic activity of these cells as consequence. RPE cells are the most actively phagocytic cells in the body. Phagocytic processes comprise the three distinct phases of recognition/binding, internalization and digestion [15]. Phagocytosis by RPE cells is important for the maintenance of retinal homeostasis and inhibition of this function disturbs retinal homeostasis [15]. A similar effect of aluminium on phagocytosis by other tissues and structures of the body has not been described so far, and thus the effect we observed on the phagocytic activity of RPE cells appears to be unique. The mechanisms which are responsible for this particular function of RPE cells are not fully understood but it seems that two pathways are involved, namely the receptor tyrosine kinase MerTK and its secreted ligands Gas6 and protein S, and the integrin receptor αvß5 and its secreted ligand MFG-E8 [16-18]. Whether one or both of these mechanisms are affected by this metal remains to be elucidated.

In humans, exposure to aluminium occurs primarily through contaminated food and water or airbone particles. In workers in the aluminium industry and miners, exposure is the result of breathing aluminium-contaminated dust [19]. The most important aluminium exposure is dietary, mainly from food additives [20-22]. The main dietary sources of aluminium in the US are foods and beverages but coffee, wine, black and green tea and drinking water contain aluminium as well [23]. On the other hand, a diet study in France reported that the main contributors to aluminium exposure are hot beverages other than coffee and vegetables excluding potatoes [24]. It is not known whether consumption of food high in aluminium results in accumulation of this metal in the RPE in concentrations sufficient for inhibiting the phagocytic activity of these cells. The results of our study, however, suggest that exercising caution with consumption of food with significant aluminium concentrations may be recommended in the future. The inhibition of phagocytic activity of RPE cells might be a potential novel important side effect of dietary aluminium. It must be emphasized that such a side effect may indeed appear in vivo and might thus consequently be clinically relevant since there was a

reduction of the phagocytic activity evident already at minimal concentrations in vitro.

In conclusion, our novel finding is that pretreatment of cultured ARPE-19 cells with aluminium had only minor effects on the viability of the cells but dose-dependently impaired their phagocytic activity. Since oral or inhalative aluminium uptake is the major source of a possible enrichment of this metal in the cells, and their phagocytic activity is essential for maintaining retinal homeostasis, caution is recommended in consumption of food with high aluminium concentrations and care should be taken to avoid exposure to aluminium-laden dust.

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