

Research article

Lens epithelial cell apoptosis and intracellular Ca^{2+} increase in the presence of xanthurenic acid

Halina Malina*^{1,2}, Christoph Richter², Beatrice Frueh³ and Otto M Hess¹

Address: ¹Swiss Cardiovascular Research Center, Inselspital, CH-3010 Bern, Switzerland, ²Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), Universitaetstrasse 16, CH-8092 Zuerich, Switzerland and ³Department of Ophthalmology, Inselspital, CH-3010 Bern, Switzerland

E-mail: Halina Malina* - halina.malina@dkf2.unibe.ch; Christoph Richter - richter@bc.biol.ethz.ch; Beatrice Frueh - beatrice.frueh@insel.ch; Otto M Hess - otto.martin.hess@insel.ch

*Corresponding author

Published: 5 April 2002

Received: 5 December 2001

BMC Ophthalmology 2002, 2:1

Accepted: 5 April 2002

This article is available from: <http://www.biomedcentral.com/1471-2415/2/1>

© 2002 Malina et al; licensee BioMed Central Ltd. Verbatim copying and redistribution of this article are permitted in any medium for any purpose, provided this notice is preserved along with the article's original URL.

Abstract

Background: Xanthurenic acid is an endogenous product of tryptophan degradation by indoleamine 2,3-dioxygenase (IDO). We have previously reported that IDO is present in mammalian lenses, and xanthurenic acid is accumulated in the lenses with aging. Here, we studied the involvement of xanthurenic acid in the human lens epithelial cell physiology.

Methods: Human lens epithelial cells primary cultures were used. Control cells, and cells in the presence of xanthurenic acid grow in the dark. Western blot analysis and immunofluorescence studies were performed.

Results: In the presence of xanthurenic acid human lens epithelial cells undergo apoptosis-like cell death. In the control cells gelsolin stained the perinuclear region, whereas in the presence of 10 μM xanthurenic acid gelsolin is translocated to the cytoskeleton, but does not lead to cytoskeleton breakdown. In the same condition caspase-3 activation, and DNA fragmentation was observed. At low (5 to 10 μM) of xanthurenic acid concentration, the elongation of the cytoskeleton was associated with migration of mitochondria and cytochrome c release. At higher concentrations xanthurenic acid (20 μM and 40 μM) damaged mitochondria were observed in the perinuclear region, and nuclear DNA cleavage was observed. We observed an induction of calpain Lp 82 and an increase of free Ca^{2+} in the cells in a xanthurenic acid concentration-dependent manner.

Conclusions: The results show that xanthurenic acid accumulation in human lens epithelial cells disturbs the normal cell physiology and leads to a cascade of pathological events. Xanthurenic acid induces calpain Lp82 and caspases in the cells growing in the dark and can be involved in senile cataract development.

Background

The primary cause of senile cataract development is still unclear. To date the involvement of α -crystallin, a molecular chaperone for β - and γ -crystallin, has principally been

considered in senile cataract development [1,2], the decrease in the chaperone ability of α -crystallin with age being implicated. Xanthurenic acid is produced from a metabolite of tryptophan (3-hydroxy-kynurenine) [3] in

the presence of 2,3-dioxygenase [4–7]. While 3-hydroxykynurenine [3] is photochemically inert [8] and serves as a protective UV filter of retina, xanthurenic acid is a photosensitizer [9,10]. Xanthurenic acid accumulates with aging in mammalian lenses [11,12] and is involved in the increased fluorescence of the lens with aging [13]. The glucoside of xanthurenic acid is also present in aged lenses [14]. Xanthurenic acid is an example of an endogenous ER stressor provoking an accumulation of unfolded proteins which in turn leads to an overexpression of Grp 94 and calreticulin in the lens epithelial cells of young mammals [15]. We have previously reported that porcine lens epithelial cells in culture respond to xanthurenic acid exposure by an overexpression of stress chaperone proteins, Grp 94 and calreticulin, in [15]. Here, we report that xanthurenic acid leads to human lens epithelial cells (HuLEC) death associated with caspase-3 activation, intracellular Ca^{2+} increase and calpain Lp82 induction. Previously, lens epithelial cell apoptosis was observed in an *in vitro* model of the cataract [16–18].

Materials and Methods

Reagents

We used the following polyclonal antibodies from Santa Cruz Biotechnology Inc. CA, USA: antibody against cytochrome c, secondary antibodies IgG-fluoresceine (FITC)-conjugated. Primary antibody against active caspase-3 p17 was from Promega, Madison, USA. Secondary IgG-Texas Red-conjugated antibodies and Mitotracker CMXRos, DiOC₁₈, Calcium Orange™, Hoechst 33342, propidium iodide were from Molecular Probes, Leiden, The Netherlands. Antibody against GPIP1 peptide of gelsolin was prepared as described previously [24]. Other reagents were from Sigma if not specified. Antibody against calpain Lp82 was from Dr. T. R. Shearer (University of Oregon, Oregon, USA).

Preparation of human lens epithelial cells primary culture

Lenses were obtained after transplantation of cornea from 58, 59, and 63 years old donors from Eye Bank University Hospital, Bern. The primary cells cultures were prepared separately from every donor using two lens capsules. The lens capsules from one donor were treated with 1.5 mg/ml of collagenase 1A and 4 overnight at 37°C. Thereafter, 1 ml of MEM medium with 10% FCS was added, and cells were centrifuged for 10 min at 300 g. The supernatant was discarded and the cells were re-suspended in 1.5 ml the growth medium, as described below, for 14 days in one well of 12-well plate. The cells were cultivated in the dark, in Minimal Essential Medium (MEM) with Earle's salts (Gibco BRL). Cells were grown under a humidified atmosphere of 5% CO₂ in air at 37°C in MEM supplemented with 10% fetal bovine serum, penicillin (10 U/ml), streptomycin (10 µg/ml) and fungizone (250 ng/ml). When confluent, they were incubated in MEM or MEM supple-

mented with xanthurenic acid. A 20 mM stock solution of xanthurenic acid was prepared in 0.5 M NaHCO₃, and diluted in 0.01 M PBS pH 7.4.

Cytotoxicity and apoptosis assay

Cells were observed with differential interference contrast and phase contrast optics on a Zeiss Avionert 405 M inverted microscope, and images recorded with a Matsumoto 3-chip CCD cooled camera with images stored using Adobe Photoshop 4. Cell viability was determined by staining the cells with Hoechst 33342 and propidium iodide (PI) (Juro, Switzerland) using 50 µg/ml of each dye. Fragmented, apoptotic, nuclei were observed with excitation at 350 nm, and necrotic nuclei at 530 nm.

Cell lysis and immunoblotting

Cells were washed twice with cold 0.01 M PBS, pH 7.4. For Western blotting, cells were lysed in buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1 % Triton X-100, and the following protease inhibitors: 1 mM phenyl-methylsulfonyl fluoride, and leupeptin, aprotinin, and pepstatin, each at 1 µg/ml. The concentration of proteins was calculated from the absorption maximum at 280 nm, as described previously [15], and the concentration of xanthurenic acid from its absorption maximum at 342 nm (ϵ_M 6500). The lysate was centrifuged for 10 min at 14 000 g, and the supernatant was boiled in loading-buffer for 5 min. Proteins (50 µg per lane) were separated by SDS-PAGE containing 10 or 12.5% acrylamide. After transfer to Hybond ECL membrane (Amersham Pharmacia Biotech AB, Uppsala, Sweden) the proteins were probed with the appropriate antibodies. Chemiluminescence ECL system (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used for the detection of peroxidase-conjugated secondary antibody.

Immunofluorescence studies

Cells grown on glass coverslips were fixed for 10 min at room temperature in 4% paraformaldehyde in 0.1 M PIPES, pH 6.8, washed in PBS and permeabilized for 5 min in PIPES containing 0.05% saponin (65 µl per coverslip), washed in PBS, incubated for 10 min in cold acetone for additional fixing and permeabilisation, and again washed in PBS. Cells were incubated for 1.5 hour with the first antibody diluted in PBS containing 1% bovine serum albumine, and after washing incubated for 1.5 hour with the secondary antibody. The coverslips were then washed in PBS and incubated for 10 min with 65 µl of solution containing 1 µl of Hoechst 33342 dye (1 mg/ml), washed in PBS, and incubated with Antifade Kits (Molecular Probes, Leiden, The Netherlands) according to the supplier's instruction. Staining of mitochondria was performed using Mitotracker CMXRos, as follows: confluent cells cultures were pre-incubated without or with xanthurenic acid in MEM medium for 72 hours. The medium was removed

and replace with medium containing 100 nM Mitotracker CMXRos. After an incubation for 1 hour Mitotracker CMXRos was removed, coverslips were washed twice with PBS, and mounted on the slides using as antioxidative solution 9% w/v of Mowiol (Calbiochem) in 22% glycerol buffered with 0.2 mM Tris/HCl to pH containing 3.5% (w/v) of 1,4-diazabicyclo (2.2.2) octane (Sigma). Membranes were stained using overnight incubation in DiOC₁₈ at concentration of 12 μM in MEM medium.

Results

Xanthurenic acid activates caspase-3 and translocates gelsolin from the mitochondrial region to the cytoskeleton

We observed that 10 μM xanthurenic acid in the HuLEC cell culture medium activate caspase-3 (Fig. 1B). In the same cells the translocation of gelsolin from perinuclear region to cytoskeleton was observed (Fig. 1C, 1D). Previously it was reported that caspase-3 and -9 are associated with mitochondrial membranes [19]. Gelsolin is the cytoskeletal protein responsible for the disintegration of F actin during apoptosis induced by Fas [20]. It was also suggested that gelsolin keeps caspases in the inactive state [21]. We reported previously that gelsolin is abnormally cleaved during apoptosis induced by xanthurenic acid. This abnormal gelsolin cleavage could be a reason of absence of cytoskeleton breakdown in the presence of active caspase-3 (Fig. 1B, 1D).

Xanthurenic acid leads to mitochondrial migration, cytochrome c release, and destruction of mitochondrial structure

In the control cells mitochondria occupy the perinuclear region (Fig. 2A). In the presence of 10 μM xanthurenic acid mitochondrial migration was observed (Fig. 2B). However, at higher concentrations (20 and 40 μM) xanthurenic acid led to the destruction of mitochondria. An intrinsic apoptotic pathway is activated by cytochrome c release and apoptosome formation with APAF-1 and ATP [22]. The apoptosome leads to activation of caspase-9, which activates caspase-3. We observed that in the presence of 10 μM xanthurenic acid cytochrome c was release from mitochondria (Fig. 3). APAF-1 is present in the HuLEC and its level is independent from xanthurenic acid concentration (not shown). Thus, release of cytochrome c is responsible for the observed caspase-3 activation, and nucleus cleavage.

Mitochondrial damage in the presence of xanthurenic acid is associated with nuclear cleavage

In control cells about 5 percent are apoptotic (Fig. 4A, Fig. 5). In the presence of 10 μM xanthurenic acid condensed and/or cleaved nuclei were observed, which a mostly stained only with Hoechst 33342, and not with propidium iodide. This indicated that the observed cell death was apoptotic and not necrotic (Fig. 4B, 4C). With con-

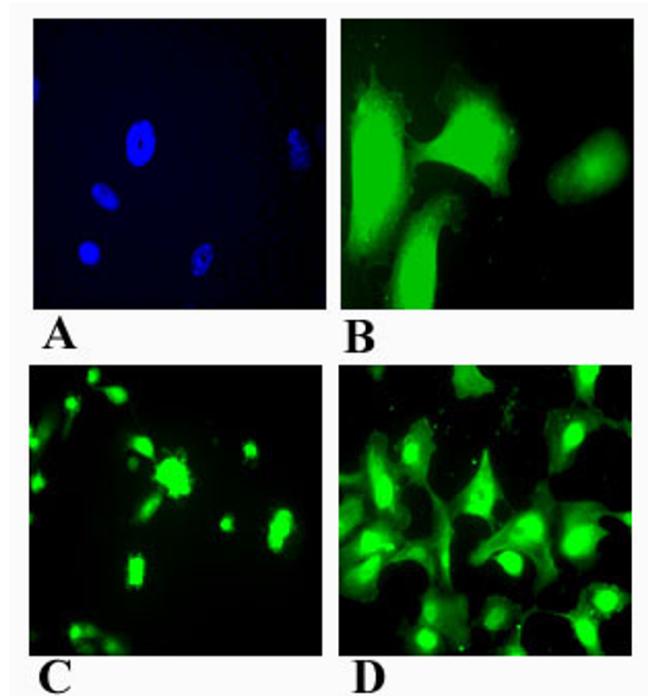


Figure 1

Caspase-3 activation and gelsolin translocation in HuLEC in the presence of xanthurenic acid for 96 hours. Cells stained for active caspase-3 (A, B), and gelsolin (C, D): (A) control cells stained with Hoechst 33342 and for cleaved caspase-3, (B) cells exposed to 10 μM xanthurenic acid and stained for cleaved caspase-3, (C) control cells, (D) in the presence of 10 μM xanthurenic acid.

centrations of xanthurenic acid of 20 μM (Fig. 4D,4E,4F) and 40 μM (Fig. 4G,4H,4I) the destruction of the mitochondrial structure, was associated with nuclear cleavage. Cell death depended on the xanthurenic acid concentration (Fig. 5). In the presence of 10 μM xanthurenic acid about 40 percent of cells were dead, and an increase of xanthurenic acid to 20 μM provoked about 70 percent of cell death.

Xanthurenic acid leads to damage of the cell membrane

The cell membranes were stained with DiOC₁₈ and co-stained with Mito-Tracker Red CMXRos, and the nucleus was stained with Hoechst 33342 (Fig. 6). In the control cell mitochondria were in the perinuclear region and the cell membranes were uniformly stained with DiOC₁₈ (Fig. 6A,6B,6C) In the presence of 10 μM xanthurenic acid the mitochondria migrated to the cell periphery and the cell membranes were not uniformly stained (Fig. 6D,6E,6F). In the presence of 20 μM of xanthurenic acid the mitochondrial structure was destroyed, nuclear DNA was degraded and membranes were not stained with DiOC₁₈ (Fig. 6G,6H,6I).

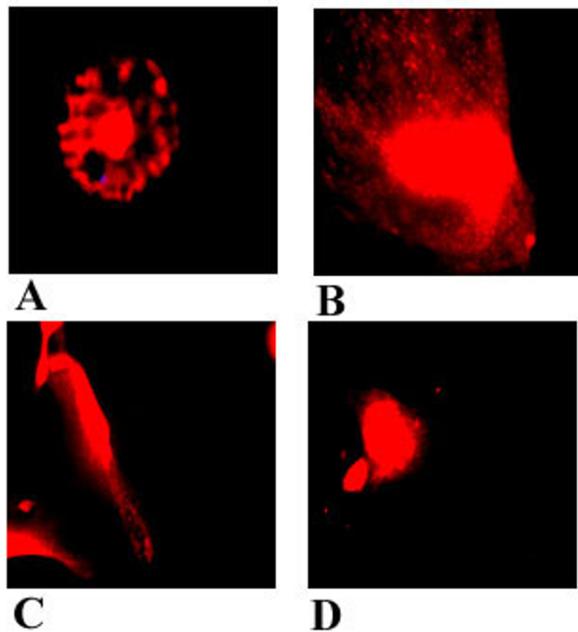


Figure 2
HuLEC, grown in the presence of xanthurenic acid for 96 hours, stained with Mito-Tracker Red CMXRos: (A) control cells; (B-D) in the presence of xanthurenic acid: (B) 10 μ M, (C) 20 μ M, (D) 40 μ M.

Xanthurenic acid causes an increase of free intracellular Ca^{2+} and an induction of the lens calpain Lp82

Ca^{2+} increases are associated with cataract development. We investigated intracellular Ca^{2+} by loading the cells with acetomethyl ester of Calcium Orange™. This dye becomes fluorescent when hydrolysed in the cell by esterases and conjugated with free Ca^{2+} . Cells were incubated without xanthurenic acid or with xanthurenic acid at concentration of 0.125; 0.25, 0.5; 1; 2; 5, 10, and 20 μ M. A presence of xanthurenic acid in the cell culture medium higher than 2 μ M provokes an increase of intracellular Ca^{2+} in comparison with control. In the presence of xanthurenic acid at concentration of 10 μ M and 20 μ M an intensive staining with Calcium Orange™ was observed indicating an increase of free Ca^{2+} in the cell in a xanthurenic acid concentration-dependent manner (Fig. 7).

The lens specific calpain Lp82 [23] was not detectable using the Western blot analysis in the lens epithelial cell culture cultivated in the absence of xanthurenic acid (Fig. 8, lane 1). In the presence of xanthurenic acid the calpain Lp82 was induced (Fig. 8, lanes 2-4).

Discussion

Our previous studies in cell culture showed that xanthurenic acid is a potent endogenous pathological substance in retinal pigment epithelium and smooth muscle

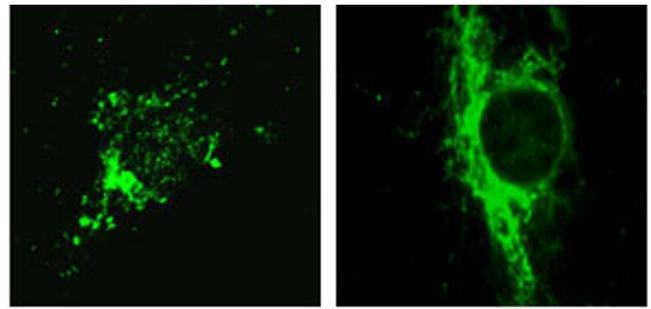


Figure 3
Cytochrome c release in HuLEC grown in the presence of xanthurenic acid for 72 hours. (A) control cells, (B) cells grown in the presence of 10 μ M xanthurenic acid.

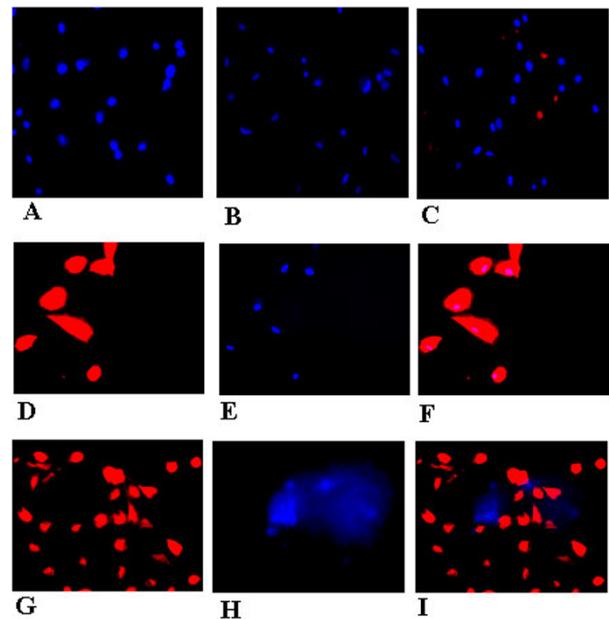


Figure 4
Nuclear DNA degradation in HuLEC in the presence of xanthurenic acid. (A) control cells stained with Hoechst; (B) cells grown in the presence of 10 μ M xanthurenic acid for 96 h and stained with Hoechst; (C) as (B) but stained with Hoechst and propidium iodide; (D-F) cells in the presence of 20 μ M xanthurenic acid: (D) stained with Mitotracker, (E) stained with Hoechst, (F) merge of (D and E); (G-I) cells in the presence of 40 μ M xanthurenic acid: (G) stained with Mitotracker, (H) stained with Hoechst, (I) merge of (G) and (H).

cells [24]. In this study, we observed that xanthurenic acid leads to lens epithelial cells death associated with an induction of caspase-3 and calpain Lp82. Apoptosis was observed in models of cataract upon lens treatment with

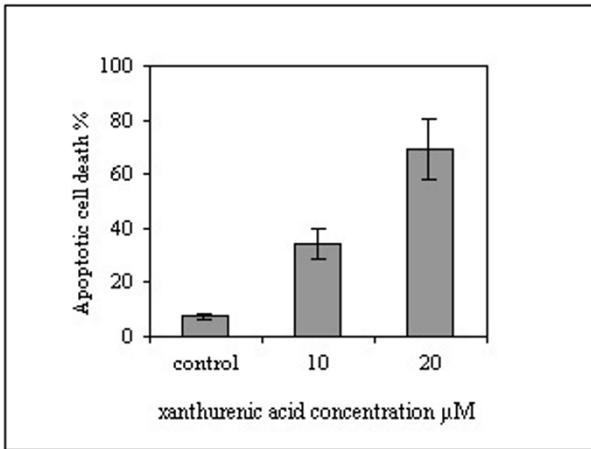


Figure 5
Apoptotic death of HuLEC in the presence of xanthurenic acid.

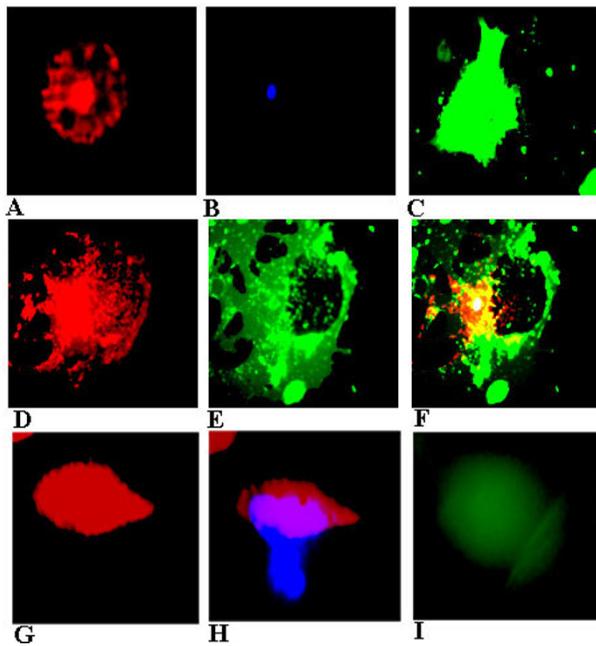


Figure 6
Staining of the mitochondria (Mito-Tracker Red CMXRos), nucleus (Hoechst) and cell membranes (DiOC_{18}) in HuLEC grown in the presence of xanthurenic acid for 96 hours: (A-C), control cells: (A) mitochondria, (B) nucleus, (C) membranes; (D-F) with 10 μM xanthurenic acid: (D) mitochondria, (E) membranes, (F) merge of D, E, and nucleus stained by Hoechst; (G-I) with 20 μM xanthurenic acid: (G) mitochondria, (H) merge of (G) and Hoechst staining, (I) membranes

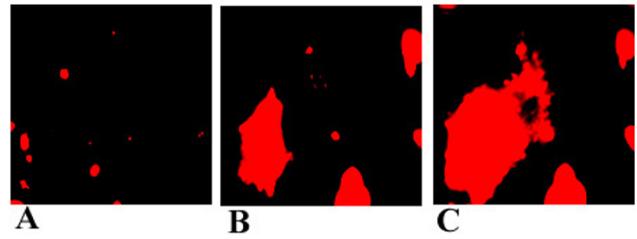


Figure 7
Increase of free Ca^{2+} in HuLEC after growth in the presence of xanthurenic acid for 96 hours. HuLEC were stained with Calcium Orange™: (A) control cells, (B) in the presence of 10 μM xanthurenic acid, (C) in the presence of 20 μM xanthurenic acid.

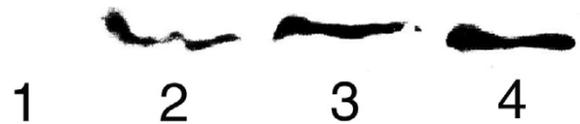


Figure 8
Induction of calpain Lp82 in HuLEC growing 24 hours in controlled cells (lane-1) and in the presence of 1,2,4 mM xanthurenic acid (lanes-2,3,4, respectively).

staurosporine, diamide, and ionophore [16–18]. In the selenite cataract model caspase-3 and calpain were induced [25]. In the normal apoptosis, such as observed with development, cells disappear because of caspase-3-dependent cleavage of DNA and the cytoskeleton [26,27]. The caspase remodeling of the cytoskeleton was indicated as a possible mechanism leading to the aging of lenses [28,29]. Apoptosis is considered as a common cellular basis for non-congenital cataract in mammals [30]. Recent data indicate that senile cataract is a result of proteolysis of crystalins by calpains [31]. Calpains are activated by Ca^{2+} [32].

Previously, it was reported that ER Ca^{2+} homeostasis affects the cells' sensitivity to apoptosis [33]. Lens epithelial cells overloaded by Ca^{2+} showed vimentin cleavage and opacification [34] Thapsigargin, a plant alkaloid, which depletes Ca^{2+} from the ER, was used to stop lens epithelial cell growth [35]. Here, we show that xanthurenic acid, an endogenous molecule, lead to induction of calpain Lp82 and caspase-3 activation. The simultaneous activation of caspase and calpain may lead to an abnormality of apoptosis because calpain cleaves caspases [36]. The calpain Lp82 is involved in cataract formation in connexin α -3 knockout mice [37]. The induction of calpain Lp82 leads to the cleavage of crystalins in the lenses and is involved in the senile cataract development [38]. Xanthurenic acid leads to formation of unfolded proteins [15].

An accumulation of unfolded protein can lead to Ca^{2+} release from intracellular stores as well to caspase induction [39,40]. The observed death of the lens epithelial cells has apoptotic characteristics because release of cytochrome c and caspase-3 activation were observed. However, the apoptosis-like process does not lead to a collapse of the cytoskeleton driven by caspase-3 cleaved gelsolin in the presence of Fas-induced apoptosis [20]. In our study, in the presence of xanthurenic acid cells look normal when observed by the light microscopy. However, when visualized by fluorescence microscopy with Hoechst and propidium iodide a nuclear dysfunction is evident. We have previously reported that xanthurenic acid leads to an abnormal cleavage of gelsolin. The gelsolin stains cytoskeleton of the dead cells. Ca^{2+} and bis-phosphatidylinositol (PPI2) regulate, respectively, association and dissociation of gelsolin to actin [41]. Thus, also the increase in Ca^{2+} may play a role in the association of gelsolin to cytoskeleton.

The mitochondrial damage observed in the presence of xanthurenic acid associated with cytochrome c release could lower energy necessary for the lens enzymes activation observed in senile cataract development.

In this study we observed that xanthurenic acid accumulation can be an upstream event leading to an induction of the lens proteases: caspase and calpain. An accumulation of xanthurenic acid in the human lenses with aging can change the intracellular Ca^{2+} homeostasis. In summary, xanthurenic acid can induce the pathology of the lens epithelial cells without participation of light.

Author contributions

All authors contributed equally to realize this work

Competing interests

None declared

Acknowledgements

This work was supported by grant awarded to H. Z. M. by the Swiss National Foundation (32-59183.99) and MSE-Pharmaceutika GMBH, Bad Homburg, Germany. We thank Dr. T. R. Shearer for the kind gift of the antibody against calpain Lp82, Mr. R. Fischer and Mrs A. Marrero Nodarse for cell culture facilities, and Mrs D. Zuercher for lens epithelial cell culture preparation.

References

- Andley UP, Song Z, Wawrousek EF, Fleming TP, Bassnett S: **Differential protective activity of alpha A- and alphaB-crystallin in lens epithelial cells.** *J Biol Chem* 2000, **275**:36823-36831
- Horwitz J, Huang QL, Ding L, Bova MP: **Lens alpha-crystallin: chaperone-like properties.** *Methods Enzymol* 1998, **290**:365-383
- Heyningen R: **Assay of fluorescent glucosides in human lens.** *Exp. Eye Res.* 1973, **15**:121-126
- Carlin JM, Ozaki Y, Byrne GI, Brown RR, Borden EC: **Interferons and indoleamine 2,3-dioxygenase: role in antimicrobial and antitumor effects.** *Experientia* 1989, **45**:535-541
- Malina HZ, Martin XD: **Indoleamine 2,3-dioxygenase activity in the aqueous humor, iris/ciliary body, and retina of the bovine eye.** *Graefes Arch Clin Exp Ophthalmol* 1993, **23**:1:482-486
- Malina HZ, Martin XD: **Indoleamine 2,3-dioxygenase: antioxidant enzyme in the human eye.** *Graefes Arch Clin Exp Ophthalmol* 1996, **234**:457-462
- Takikawa O, Littlejohn TK, Truscott RJ: **Indoleamine 2,3-dioxygenase in the human lens, the first enzyme in the synthesis of UV filters.** *Exp Eye Res* 2001, **72**:271-277
- Dillon J: **Photophysics and photobiology of the eye.** *J Photochem. Photobiol. B: Biol.* 1991, **10**:23-40
- Roberts JE, Finley EL, Patat SA, Schey KL: **Photooxidation of lens proteins with xanthurenic acid: a putative chromophore for cataractogenesis.** *Photochem Photobiol* 2001, **74**:740-744
- Roberts JE, Wishart JF, Martinez L, Chignell CF: **Photochemical studies on xanthurenic acid.** *Photochem Photobiol* 2000, **72**:467-471
- Malina HZ, Martin XD: **Deamination of 3-hydroxykynurenine in bovine lenses: a possible mechanism of cataract formation in general.** *Graefes Arch Clin Exp Ophthalmol* 1995, **233**:38-44
- Malina HZ, Martin XD: **Xanthurenic acid derivative formation in the lens is responsible for senile cataract in humans.** *Graefes Arch Clin Exp Ophthalmol* 1996, **234**:723-730
- Malina HZ, Martin XD: **3-hydroxykynurenine transamination leads to the formation of the fluorescent substances in human lenses.** *Eur J Ophthalmol* 1996, **6**:250-256
- Shirao E, Ando K, Inoue A, Shirao Y, Balasubramanian D: **Identification of a novel fluorophore, xanthurenic acid 8-O- β -D-glucoside in human brunescant cataract.** *Exp Eye Res* 2001, **73**:421-431
- Malina HZ: **Xanthurenic acid provokes formation of unfolded proteins in endoplasmic reticulum of the lens epithelial cells.** *Biochem Biophys Res Commun* 1999, **265**:600-605
- Andersson M, Sjostrand J, Petersen A, Honarvar AK, Karlsson JO: **Caspase and proteasome activity during staurosporin-induced apoptosis in lens epithelial cells.** *Invest Ophthalmol Vis Sci* 2000, **41**:2623-2632
- Azuma M, Shearer TR: **Involvement of calpain in diamide-induced cataract in cultured lenses.** *FEBS Lett* 1992, **307**:313-317
- Sanderson J, Marcantonio JM, Duncan G: **Calcium ionophore induced proteolysis and cataract: inhibition by cell permeable calpain antagonists.** *Biochem Biophys Res Commun* 1996, **218**:893-901
- Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, et al: **Mitochondrial release of caspase-2 and -9 during the apoptotic process.** *J Exp Med* 1999, **189**:381-394
- Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, et al: **Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis.** *Science* 1997, **278**:294-298
- Azuma T, Koths K, Flanagan L, Kwiatkowski D: **Gelsolin in complex with phosphatidylinositol 4,5-bisphosphate inhibits caspase-3 and -9 to retard apoptotic progression.** *J Biol Chem* 2000, **275**:3761-3766
- Zou H, Li Y, Liu X, Wang X: **An APAF-1-cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9.** *J Biol Chem* 1999, **274**:11549-11556
- Nakamura Y, Fukiage C, Ma H, Shih M, Azuma M, Shearer TR: **Decreased sensitivity of lens-specific calpain Lp82 to calpastatin inhibitor.** *Exp. Eye Res.* 1999, **69**:155-162
- Malina HZ, Richter C, Mehl M, Hess OM: **Pathological apoptosis by xanthurenic acid, a tryptophan metabolite: activation of cell caspases but not cytoskeleton breakdown.** *BMC Physiol* 2001, **1**:7
- Tamada Y, Fukiage C, Nakamura Y, Azuma M, Kim YH, Shearer TR: **Evidence for apoptosis in the selenite rat model of cataract.** *Biochem Biophys Res Commun* 2000, **275**:300-306
- Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S: **A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD.** *Nature* 1998, **391**:43-50
- Geng YJ, Azuma T, Tang JX, Hartwig JH, Muszynski M, Wu Q, et al: **Caspase-3-induced gelsolin fragmentation contributes to actin cytoskeletal collapse, nucleolysis, and apoptosis of vascular smooth muscle cells exposed to proinflammatory cytokines.** *Eur J Cell Biol* 1998, **77**:294-302
- Kilic F, Trevithick JR: **Modelling cortical cataractogenesis. XX-IX. Calpain proteolysis of lens fodrin in cataract.** *Biochem Mol Biol Int* 1998, **45**:963-978

29. Lee A, Morrow JS, Fowler VM: **Caspase remodeling of the spectrin membrane skeleton during lens development and aging.** *J Biol Chem* 2001, **276**:20735-20742
30. Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, et al: **Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals.** *J Cell Biol* 1995, **130**:169-181
31. David LL, Azuma M, Shearer TR: **Cataract and the acceleration of calpain-induced beta-crystallin insolubilization occurring during normal maturation of rat lens.** *Invest Ophthalmol Vis Sci* 1994, **35**:785-793
32. Strobi S, Fernandez-Catalan C, Braun M, Huber R, Masumoto H, Nakagawa K, et al: **The crystal structure of calcium-free human m-calpain suggests an electrostatic switch mechanism for activation by calcium.** *Proc Natl Acad Sci USA* 2000, **97**:588-592
33. Nakamura K, Bossy-Wetzel E, Burns K, Fadel MP, Lozyk M., Goping IS, et al: **Changes in endoplasmic reticulum luminal environment affect cell sensitivity to apoptosis.** *J Cell Biol* 2000, **150**:731-740
34. Sanderson J, Marcantonio JM, Duncan G: **A human lens model of cortical cataract: Ca²⁺-induced protein loss, vimentin cleavage and opacification.** *Invest Ophthalmol Vis Sci* 2000, **41**:2255-2261
35. Duncan G, Wormstone IM, Liu CS, Marcantonio JM, Davies PD: **Thapsigargin-coated intraocular lenses inhibit human lens cell growth.** *Nat Med* 1997, **3**:1026-1028
36. Chua BT, Guo K, Li P: **Direct cleavage by the calcium-activated protease calpain can lead to inactivation of caspases.** *J Biol Chem* 2000, **275**:5131-5135
37. Baruch A, Greenbaum D, Levy ET, Nielsen PA, Gilula NB, Kumar NM, et al: **Defining a Link between Gap Junction Communication, Proteolysis, and Cataract Formation.** *J Biol Chem* 2001, **276**:28999-29006
38. Shearer TR, Ma H, Shih M, Hata I, Fukiage C, Nakamura Y, et al: **Lp82 calpain during rat lens maturation and cataract formation.** *Curr Eye Res* 1998, **17**:1037-1043
39. Wang KK, Posmantur R, Nadimpalli R, Nath R, Mohan P, Nixon RA, et al: **Caspase-mediated fragmentation of calpain inhibitor protein calpastatin during apoptosis.** *Arch Biochem Biophys* 1998, **356**:187-196
40. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, et al: **Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress.** *J Biol Chem* 2001, **276**:13935-13940
41. Sun HQ, Yamamoto M, Mejillano M, Yin HL: **Gelsolin, a multifunctional actin regulatory protein.** *J Biol Chem* 1999, **274**:33179-33182

Pre-publication history

The pre-publication history for this paper can be accessed here:

<http://www.biomedcentral.com/1471-2415/2/1/prepub>

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright



Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>

editorial@biomedcentral.com