0RAL PRESENTATIONS

Zinc binding to the Tyr402 and His402 allotypes of complement factor H: possible implications for age-related macular degeneration

Ruodan Nan,[1] Irene Farabella,[1] Felix F. Schumacher,[1] Ami Miller,[1] Jayesh Gor,[1] Andrew C.R. Martin,[1] David T. Jones,[1] Imre Lengyel[2] and Stephen J. Perkins[1]; [1]Department of Structural and Molecular Biology, Division of Biosciences Darwin Building, University College London, Gower Street, London WC1E 6BT, UK; [2] Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V 9EL, UK

In the innate immune system, Factor H (FH) with 20 short complement regulator (SCR) domains is a major regulator of the alternative pathway of complement activation. Polymorphisms of FH are associated with different disorders. In age-related macular degeneration a Tyr to His mutation is at position 402 is highly associated with the disease. In addition, pronounced FH immunostaining in sub-retinal pigment epithelial deposits suggestes that FH is causally associated with AMD. Apart from FH, high concentrations of zinc are found in the sub-RPE deposits in the back of the eye. We have shown that heterozygous FH is inhibited by zinc in the test tube which causes FH to aggregate strongly. In this study analytical ultracentrifugation showed that large amounts of oligomers are formed with both the native Tyr402 and the AMD-risk His402 homozygous allotypes of FH in the presence of zinc. X-ray scattering confirmed that both FH allotypes aggregated strongly at > 20 μM zinc. The functionally-important fragment of FH SCR-6/8 also aggregated with zinc while the other functionally-important SCR-1/5 and SCR-16/20 fragments were less likely to bind zinc. Starting from known zinc-binding sites, a total of 202 putative surface zinc binding sites were predicted in the 20 SCR domains of FH, but the most sites were predicted in SCR-6. Metal site prediction web servers confirmed this prediction and highlighted several other domains that may bind zinc. Predictions based on docked SCR-6/8 dimeric structures revealed potential zinc binding sites at the protein-protein interface that may lead to daisy-chained oligomers. We conclude that zinc binds weakly to FH at several surface locations, most probably within SCR-6/8, the domains that bind heparin, C3 and CRP. This may explains why zinc inhibits FH activity. In addition, large oligomers formation will render FH to be trapped in the Bruch's membrane and potentially provide a seeding point for sub-RPE-deposit formation, leading to AMD. Therefore, FH and zinc are potentially involved in AMD by contributing to deposit formation and inflammation.

Effect of Zinc ions on Hydroxyapatite Dissolution Kinetics Studied Using Scanning Microradiography

H. LINGAWI[1], M. BARBOUR[2], R.J.M. LYNCH[3] and P. ANDERSON[1]

[1] Queen Mary University of London, London, United Kingdom, [2]University of Bristol, Bristol, United Kingdom, [3]GlaxoSmithKline, Weybridge, United Kingdom.

Zinc is used in toothpaste for its anti-calculus properties and reducing oral malodour, but it may also have a role in inhibiting dissolution kinetics of enamel's principal inorganic component; hydroxyapatite (HAP).

The aim of this study was to investigate the effect of Zn^{+2} on surface physical chemistry influencing HAP dissolution by measuring the rate of HAP dissolution under strictly controlled thermodynamic conditions using scanning microradiography (SMR) at a range of Zn^{+2} concentrations at toothpaste concentrations.

Compressed sintered HAP discs (*Plasma-Biotal*, *UK*) were used. They were sterilised, coated with acid-resistant varnish on all surfaces except one, and located in an SMR cell. A bulk solution (6 litres) of 0.1% acetic acid pH4, divided into six (1 litre bottle) with the addition of 0, 5, 10, 15, 20 ppm of zinc acetate respectively was prepared. Same applied to 0.3% citric acid pH2.8. The demineralising solution was circulated at 0.80 $cm^3.min^{-1}$, and the rate of HAP dissolution (RD_{HAP}) was measured using SMR at a single centrally located point on each disc for periods of 24h at 22°C. Each experiment was repeated in triplicate.

The mean RD_{HAP} was 3.08E-03, 2.87E-03, 2.74E-03, 2.42E-03, and 2.04E-03$g.cm^{-2}.h^{-1}$ for 0.3% citric acid pH2.8 at zinc acetate concentration of 0, 5, 10, 15and 20ppm respectively and 4.7E-04, 4.0E-04, 3.13E-04, 2.91E-04 and 2.84E-04 $g.cm^{-2}.h^{-1}$ for 0.1% acetic acid pH4 with 0, 5, 10, 15and 20ppm zinc acetate concentration.

This study demonstrates that Zn^{+2} decreased RD_{HAP} under strictly controlled thermodynamic conditions relevant to tooth decay and erosion, possibly due to inhibition of dissolution nuclei on the HAP surfaces.

Endogenous zinc modulates feedback inhibition in dentate granule cells

ANTONIA GRAUERT[1], DOMINIQUE ENGEL[2], ARNAUD RUIZ[1]

[1]Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK, [2]GIGA Neurosciences, Electrophysiology Unit, University of Liege, Liege, Belgium

Introduction: Zinc is a common trace element in the brain and is particularly enriched in the hippocampus. Mossy fibres, the axons of granule cells of the dentate gyrus, provide a major excitatory input to the hippocampus and contain large amounts of endogenous zinc which can be released upon presynaptic depolarisation. It is well established that zinc ions inhibit NMDA and $GABA_A$ receptors at mossy fibre synapses made onto CA3 pyramidal cells, the principle cells of a sub-region of the hippocampus. However, zinc actions on hippocampal feed-back inhibition to the dentate gyrus are totally unknown.

Methods: In order to test the hypothesis that zinc modulates di-synaptic GABAergic feedback inhibition, brain slices were prepared from Sprague Dawley rats (p22-p40) and patch clamp experiments were performed from granule cells and dentate gyrus interneurones in voltage and current clamp modes. We positioned two tungsten electrodes in strata granulosum and lucidum to stimulate mossy fibres. $GABA_B$ receptors were routinely blocked with CGP-52432 (5 µM). The selective group II metabotropic receptor agonist DCG-IV was used to probe for a mossy fibre mediated input. Experiments were performed at room temperature. Data were expressed as mean ± standard error of the mean.

Results: DCG-IV depressed electrically-evoked postsynaptic currents in granule cells by 46.3% ± 8.1% (n = 9). These currents were abolished by the $GABA_A$ receptor antagonist bicuculline or the AMPA/kainate receptor blocker NBQX thus indicating an inhibitory polysynaptic feedback pathway from mossy fibres to the dentate gyrus.

Chelation of zinc with TPEN or Ca-EDTA reversibly increased the amplitude of inhibitory postsynaptic currents in granule cells (TPEN: 40 ± 18.5%, n = 10; Ca-EDTA: 103.1 ± 23.2%, n = 5; $p < 0.05$; t-test) whereas application of zinc chloride had the opposite effect (58.6 ± 5.5%, n = 5). To shed light onto the loci of actions of zinc, we also recorded from dentate gyrus basket cells since they receive zinc-containing mossy fibre inputs and provide most perisomatic inhibition onto neighbouring granule cells. Ca-EDTA increased the amplitude of NMDA-EPSCs in basket cells by 56.5 ± 11.2% (n = 5) indicating that endogenous zinc can modulate feedback inhibition in the dentate gyrus via indirect actions on synapses between mossy fibres and local interneurones.

To test the hypothesis that zinc may modulate excitability of mossy fibres and thereby affect transmitter release, we recorded from mossy fibre boutons and found that the spike half-width was unaltered, suggesting that there is no change in excitability. This was supported by extracellular recordings in the granule cell layer and granule cell whole-cell recordings, where we found no change in amplitude of the population spike and no change in the probability for evoking an antidromic spike, respectively. Ultimately, there was an increase in firing of granule cells with zinc chelation when the di-synaptic feed-back pathway was activated. These results indicate that zinc chelators affect granule cell firing without affecting mossy fibre excitability.

Conclusions: Our results provide the first evidence for a facilitation of feedback inhibition in granule cells by zinc chelators via an indirect mossy fibre mediated pathway. Because GABA has been shown to be excitatory in granule cells (Misgeld et al, 1986) and zinc is especially enriched in mossy fibres, which mostly target interneurones, this finding may have important consequences on information flow to the hippocampus.

References

Misgeld, U, Deisz, R.A., Dodt, H.U., Lux, H.D. (1986) *Science*, **232**, 1413-1415.

Gene expression changes in aged rat eyes following oral zinc supplementation

Neda Barzegar-Befroei , Jane Flinn, Grtechen Knaack, Christer Hogstrand and Imre Lengyel

Background: Zinc is a multifaceted trace element which plays a role in many metabolic pathways, either as a signalling molecule or as a component of proteins. It is involved in DNA replication, transcription, protein synthesis and signalling pathways, influencing cell division and differentiation. Zinc is found in unusually high concentrations in ocular tissues with the highest amount concentrated within the retinal pigment epithelium and choroid. As decreased zinc levels are associated with age related macular degeneration (AMD) supplementation with zinc is proposed to slow the progression of the disease with no clear understanding of how this might be beneficial at the cellular level. In the present study we supplemented the drinking water of aged rats with zinc and monitored changes in gene expression in the eye.

Method: Three groups of 12 months old female rats were given supplemented drinking water for 4 months as follows: 1) normal lab water; 2) +10 mg L^{-1} zinc sulphate; and +10 mg/L zinc sulphate + 0.2 mg/L copper sulphate. Following sacrifice, one eye of each animal was used to isolate RNA for microarray analysis. Gene expression (n=4 per group) was determined using GeneChip Rat Gene 1.0 ST (Affymetrix, CA) microarrays. Significant changes in gene expressions ($p<0.05$) were selected using *Qlucore* Omics Explorer and overrepresented pathways among regulated genes were selected using Ingenuity Pathway Analysis (IPA; Ingenuity Systems). For gene expression changes the control group was compared to the pooled supplementation groups. Differential gene expressions were validated by real-time quantitative polymerase chain reaction for 17 genes. The second eyes were fixed for assessing morphological changes.

Results: From the zinc affected genes 738 were represented in the Ingenuity database. Networks involved in oxidative stress, inflammatory response, DNA replication, recombination, and repair had high scores. Amongst the top molecular and cellular functions that were affected by zinc supplementation were: cell death, carbohydrate metabolism, small molecule biochemistry, cellular compromise and cell morphology. A number of interesting and significant molecules are being currently further investigated. There are no gross morphological changes observed following long term zinc supplementation but electron microscopy might highlight ultra structural changes.

Discussion: Oral zinc supplementation is a choice of treatment to slow the progression of AMD. Here we show the first time that oral zinc supplementation has the potential to modulate molecular level changes in the eye and provide evidence that zinc supplementation can modify essential processes within ocular tissues to combat the major cause of blindness in the elderly. It is especially important to emphasize that our supplementation study was carried out on aged animals and for an extended period of time, mimicking closely the use of zinc supplementation in AMD.

ZnT8 and pancreatic cells: effects of glucose on gene expression

Elisa A Bellomo, Gabriela da Silva Xavier, Fabrice Chimienti and Guy A Rutter

Background. The diverse role for Zn^{2+} in cellular processes requires that the Zn^{2+} homeostasis is tightly controlled by the coordinated action of Zn^{2+} transporters and metallothioneins. In pancreatic islets Zn^{2+} is an essential element for insulin maturation and processing and for its crystallization inside the β-cell granules. ZnT8 is strongly expressed in islets and exclusively localized to secretory granules membrane. Recent genome-wide studies have shown that a nonsynonymous SNP (rs13266634) in the SLC30A8 gene, resulting in the replacement of tryptophan-325 with arginine increases the risk of type 2 diabetes, possibly by decreasing insulin secretion and/or proinsulin processing.

Results. We demonstrate here that ZnT8 is highly expressed, at the mRNA level, in human and mouse pancreatic islets. In mouse ZnT4, 5 and 9 were also expressed while in human ZnT2, 4 and 9 were the other major Zn^{2+} transporters. We also demonstrate that treatment with high glucose concentrations (16.7 vs 3 mM) increases the expression level of ZnT8 in islets and clonal β-cells. We also describe the impact of the treatment with high glucose concentration on $[Zn2+]_{cyt}$ using a newly developed FRET-based sensor, eCALWY-4, which allows us to measure Zn^{2+} variation exclusively in the cytosol without contribution from other organelles.

Conclusions. ZnT8 expression is dynamically regulated in islet and we show here that this is likely to affect intracellular zinc homeostasis.

Two curious effects of dietary zinc supplementation in *Drosophila.*

Fanis Missirlis, School of Biological and Chemical Sciences, Queen Mary University of London

In my presentation I will describe new data from my lab regarding two interesting observations from the literature:

1) The Divalent Metal Transporter homologue in flies, encoded by the *Malvolio* (*Mvl)* gene, was originally identified in a mutagenesis screen designed to identify genes affecting taste behaviour in flies (Rodrigues et al., 1995). *Mvl* was implicated in metal transport when *Mvl* mutants reared in diets supplemented with Fe^{2+} or Mn^{2+} recovered their normal taste behaviour (Orgad et al., 1998). The study also demonstrated that rearing *Mvl* mutants in diets supplemented simultaneously with Zn^{2+} and Mn^{2+} did not recover normal taste behaviour, an intriguing observation that remains to be understood mechanistically (Orgad et al., 1998). Explaining the inhibitory effect of zinc on *Mvl* mutants' behaviour becomes even more exciting in view of the finding that similar dietary manipulations can affect honey bee division of labour (Ben-Shahar et al., 2004).

2) Metal Transcription Factor-1 (MTF-1) is a zinc responsive factor that mediates the cellular transcriptional response to zinc in both vertebrates (Hogstrand et al, 2008) and *Drosophila* (Yepiskoposyan et al, 2006). Key targets of MTF-1 include factors involved in zinc homeostasis and trafficking, yet two such targets have been identified that link MTF-1 to iron homeostasis. In humans, the iron hormone hepcidin is under the control of MTF-1, suggesting that MTF-1 mediates an interaction between zinc and iron levels (Balesaria et al, 2010). In *Drosophila*, the genes encoding for *ferritin heavy & light chain homologues* are direct MTF-1 targets and are induced following exposure of larvae to zinc (Yepiskoposyan et al, 2006). We have identified a new, MTF1-dependent expression site of zinc-induced (iron-poor) ferritin in the *Drosophila* larva. The function of this iron-poor ferritin expressed under conditions of zinc-overload in posterior midgut cells remains unclear.

Balesaria, S., Ramesh, B., McArdle, H., Bayele, H.K. and Srai, S.K. (2010). Divalent metal-dependent regulation of hepcidin expression by MTF-1. *FEBS Lett* **584**, 719-25.

Ben-Shahar, Y., Dudek, N. L. and Robinson, G. E. (2004). Phenotypic deconstruction reveals involvement of manganese transporter malvolio in honey bee division of labor. *J Exp Biol* **207**, 3281-8.

Hogstrand, C., Zheng, D., Feeney, G., Cunningham, P. and Kille, P. (2008). Zinc-controlled gene expression by metal-regulatory transcription factor 1 (MTF1) in a model vertebrate, the zebrafish. *Biochem Soc Trans* **36**, 1252-7.

Orgad, S., Nelson, H., Segal, D. and Nelson, N. (1998). Metal ions suppress the abnormal taste behavior of the Drosophila mutant malvolio. *J Exp Biol* **201**, 115-20.

Rodrigues, V., Cheah, P. Y., Ray, K. and Chia, W. (1995). malvolio, the Drosophila homologue of mouse NRAMP-1 (Bcg), is expressed in macrophages and in the nervous system and is required for normal taste behaviour. *EMBO J* **14**, 3007-20.

Yepiskoposyan, H., Egli, D., Fergestad, T., Selvaraj, A., Treiber, C., Multhaup, G., Georgiev, O. and Schaffner, W. (2006). Transcriptome response to heavy metal stress in *Drosophila* reveals a new zinc transporter that confers resistance to zinc. *Nucleic Acids Res* **34**, 4866-77.

Fabrice Chimienti

Over the last decade, remarkable progress has been made in our understanding of the molecular mechanisms contributing to intracellular zinc homeostasis. In particular, studies on signalling cascades triggered by zinc signals, as well as functional and structural studies on zinc transporters have highlighted the importance of intracellular zinc signalling for cellular functions and provided evidence for the mechanism of transport through zinc transporters. However, the molecular components of the zinc buffer/muffler remain elusive, and some important questions remain to be solved, e.g. how cytosolic zinc is supplied to transporters. In order to identify possible partner proteins of zinc transporters from the SLC30A family, we conducted a yeast two hybrid screening against a human pancreas cDNA library. Amongst possible positive hits, we identified a zinc-binding, actin-interacting adapter protein, PDLIM7, whose function might be relevant to the regulation of zinc transporters. Further, coimmunoprecipitation experiments confirmed a direct association with ZnT8, as indicated by the Y2H assay. Additionally, expression in epithelial cells was consistent with a localisation to cytoskeleton, and we showed that PDLIM7 partially colocalised with the zinc transporter ZnT8 in cultured beta cells. Our hypothesis is that this family of zinc-binding adapter proteins might participate in the control of cellular zinc homeostasis, possibly as a part of the zinc muffler system. At this stage we retain this novel concept as an hypothesis, and recognise that further work is required to confirm the real participation of PDLIM proteins in the control of zinc homeostasis.

POSTERS

Effect of zinc on fenestra formation in cultured endothelial cells

Bhavika Patel, Sabrina Cahyadi, Meihua Ju and Imre Lengyel, UCL Institute of Ophthalmology, 11-43 Bath Street, EC1V 9EL, UK

Purpose: The retinal pigment epithelium (RPE) contains the highest concentration of zinc/g of tissue in the body. We hypothesize that the release of zinc from RPE cells will affect neigbouring tissues including the choroidal microcapillaries. This, consequently, may influence the exchange of nutrients and waste material. To prove this hypothesis we monitored the effects of extracellular zinc on fenestrae formation in a cellular model.

Methods: For our experiments a murine endothelioma cell line, bEND5, was used. These cells have been shown to form fenestrae when treated with 1.5 uM Latrunculin A (LA), an actin depolymerising agent, for 3 h. Cells were seeded onto 1% gelatine coated covers slips and maintained in high-glucose DMEM including 10% FBS and antibiotics. Zinc treatment started following an overnight incubation to adhere cells to the cover slips for 20 hours. Results were compared to 3 hours treatment with LA. Rhodamine phalloidin was used to visualize F-actin rearrangement. Sieve-plate (fenestrae enriched areas) formation was monitored by the use of diaphragm protein PV1 specific immunostaining. Immunolabelling was visualized using Zeiss LSM700 confocal microscope. Transmission electron microscopy (TEM) was used to confirm the formation of fenestrae. For this cells were grown on formvar grids coated with 1% gelatine in PBS, postfixed and dehydrated for imaging using JEOL 1010 TEM. Biologically available zinc concentration in the culturing medium was determined by using a zinc selective fluorescence indicator (ZnAF2) and fluorometry.

Results: The cultur medium was able to fully buffer zinc to up to 75 uM external zinc. At 100 uM the available zinc concentration was 100 nM (3 order of magniture lower than that of added zinc!). Cells exposed to up to 150 uM extracellular zinc alone showed very minor F-actin rearrangements but clear signs of sieve-plate formation without LA. Cells exposed to concentrations of zinc higher than 175 uM showed signs zinc-induced toxicity perhaps related to the depletion of zinc buffering capacity of the culturing medium. TEM showed the presence of fenestrae in areas of sieve-plates.

Conclusions: Due to the high zinc binding capacity of components in culturing mediums it is essential to determine the concentration of bio-available zinc to assess its efficiency to modify any biological processes. Too high concentration of added zinc can be toxic, too little will be buffered by molecules like albumin. In support of our hypothesis, we found that nanomolar levels of bio-available zinc can induce the formation of fenestrae in vitro. Therefore, we propose that zinc might be involved in regulating fenestrae formation in vivo.

Acknowledgement: The research was supported by the Bill Brown Charitable Trust, Moorfields Eye Hospital Special Trustees, and Mercer Fund from Fight for Sight.

Bruch's membrane changes in the APP/PS1 transgenic mice model of Alzheimer's disease

Ashraf Gango[1], Sabrina Cahyadi[1], Zhi-Hong Chi[2], Zhan-You Wang[2] Tunde Peto[3] and Imre Lengyel[1], [1]UCL Institute of Ophthalmology, London, UK; [2]China Medical University, Shenyang, P.R. China; [3]Moorfields Eye Hospital, London, UK.

Purpose: Age-related macular degeneration (AMD) and Alzheimer's disease (AD) share many similarities. Almost identical genetic and environmental risk factors and similar molecular processes are involved in the resulting neurodegeneration. The purpose of this study was to examine whether deposit formation in the brain is associated with increased sub-RPE deposit formation in the Bruch's membrane in a transgenic animal model for AD and to assess whether excess zinc deposition is associated with Bruch's membrane (BM) changes.

Methods: The APP/PS1 transgenic mice model of AD were used to study ultra structural changes in the BM. Animals were sacrificed at 4 and 12 months of age and changes in BM thickness was compared to those of 4 and 12 months old wild type mice. Following terminal anaesthesia animals were injected with 10 mg/kg sodium selenide then the animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 20 min. Eyes were removed and processed for autometallography (Danscher, 1982) and electron microscopy. BM thickness were measured at segments of pictures where the choriocapillary lumen was both open and lined by a single layer of endothelium, avoiding the intercapillary pillars, far periphery and optic nerve. Ten measurements were made in segments of equal length form 10 pictures from each mouse. The number and size of zinc granules were measured using ImageJ 1.43 by cropping BM areas and using the semi-automated counting function.

Results: Our data confirms earlier reports showing a thickening of BM with normal ageing. At 4 month the average thickness of BM in wild type animals was 0.345 ± 0.047 μm that increased to 0.63 ± 0.12 μm by 12 months. However, in the transgenic groups BM was already thickened at 4 month (0.53 ± 0.04 μm). At 12 months there was a dramatic increase in BM thickenss (1.21 ± 0.07 μm) accompanied by the appearance of sub-RPE deposits. Preliminary data shows that there is substantially more zinc in BM in transgenic animals compared to wild type. Injection of DEDTC (a zinc selective chelator) completely abolished autometallographic labelling of zinc.

Conclusion: The APP/PS1 transgenic mice have an elevated level of amyloid beta and increased plaque formation through over production and altered processing. Simultaneously, thickening of Bruch's membrane and sub-RPE deposit formation is facilitated in the back of the eye. This thickening is associated with increased zinc deposition in the BM. These might be relevant to understand how sub-RPE deposits are formed but also important in strengthening further the potential association between AMD and AD. Importantly, this transgenic model for AD may represent a model to study early deposit formation leading to retinal degeneration and blindness in AMD.

Acknowledgement of funding, if applicable: We thank the Windayer Fundation, Mercer Fund, the Dorothy Hodgkin Postgraduate Award, the Special Trustees of Moorfields Eye Hospital, Lowy Medical Foundation and the Bill Brown Charitable Trust for support.

Eurreca tool kit for setting zinc dietary recommendations

Marisol Warthon-Medina, Anna Skinner, Victoria Moran, Fiona Dykes and Nicola Lowe, University of Central Lancashire, Preston, Lancashire, PR4 4AF. UK.

Zinc plays a central role in neurological function and in human tissue synthesis and structure; therefore adequate dietary intakes are essential for growth and repair, immune function and to maintain body zinc status across all populations groups. Dietary recommendations vary considerably across Europe and the rest of world. Many are based on a factorial approach, which for example, for pregnant women estimates the additional zinc required for the foetus and associated maternal tissues, as well as obligatory losses. Much of the variation can be explained by differences in expert views, research methodology and the data reviewed. The “EURRECA” (European micronutrient recommendations aligned) consortium is a European Commission funded Network of Excellence designed to systematically review and undertake meta- analyses of research data in order to create a database that includes “best practice” guidelines which can be used as a resource by future panels when setting micronutrient recommendations. Part of this process involves an analysis of the relationship between dietary intake, status and health outcomes for each life stage, such as growth and immune function in children and adolescents; foetal malformation, pre eclampsia and preterm delivery in pregnancy and lactating women; immune function, cognitive function, ischemic heart disease, diabetes mellitus and carcinogenesis in adults and elderly. This review will give an overview of the EURRECA approach and progress so far. This work is supported by EURRECA, European Commission Network of Excellence. http://www.eurreca.org/everyone.

Vascular targets for zinc deficiency

Ou Ou[1], Audrey Chandioux[1], Vivian Buchan[1], Henian Yang, Keith Allen-Redpath[2], Graeme Nixon[2], In-Sook Kwun[3] and John H. Beattie[1].

1 Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; 2 Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK; 3 Department of Food Science and Nutrition, Andong National University, Andong, Kyungbook, Republic of Korea

Zinc has multiple roles in maintaining physiological functions. Its deficiency influences the integrity of vascular cells and the expression and function of enzymes and hormones, which can be both direct and indirect risk factors of vascular disease. Using proteomic techniques, we have previously noted that zinc deficiency targets certain proteins in vascular smooth muscle including structural proteins which have been used as markers of differentiated smooth muscle. Some of these proteins were actually from “housekeeping genes” that are often used to normalise the expression of other genes of interest. We wanted to confirm that the structural proteins found previously by proteomics, including proteins from housekeeping genes, could indeed be regulated by zinc status and we used an independent technique (Western blotting) to demonstrate this. In a 2wk animal study, 8-wk old male rats were fed a zinc adequate (control) diet (C, 35mg/kg) or an acutely zinc deficient diet (AZD, <1mg/kg). Because acute zinc deficiency inhibits appetite and/or increases satiety, a third group of rats was included, and these animals (PF group) were pair-fed with those in the zinc-deficient group. Compared with the C group, the AZD group showed a significant decrease in food intake (22.8%), body weight (14.9%) as well as in plasma zinc (59.0%), while the PF group showed a 10.3% reduction in body weight but no significant decrease in plasma zinc ($p>0.05$). Several marker proteins for aorta vascular smooth muscle cell differentiation and proliferation were measured by Western blotting. Transgelin, calmodulin, calponin and GAPDH levels were decreased in AZD compared with C and PF. We conclude that zinc deficiency targets the smooth muscle cell differentiated phenotype and that related marker protein expression is therefore affected. We also conclude that low zinc status decreases the expression of a housekeeping protein (GAPDH) that is often used to normalise the levels of other proteins detected by Western blotting.

This study was funded by the Korea National Research Foundation, Global Research Network Program (KRF-2008-220-F00013).

Zinc as a regulator of development in the zebrafish embryo

Issa Muraina and Christer Hogstrand

King's College London

Several studies have highlighted multiple roles of zinc in biology and suggested that zinc signalling may be of particular importance during embryogenesis. We have exploited the tractability of the zebrafish for developmental studies. Preliminary results show that manipulation of zinc status and expression of zinc transporters in the zebrafish embryo has distinct effects on embryo development, affecting critical events such as gastrulation and hatching. A hypomorphic *znt1* mutation was shown to express a phenotype when available zinc was partially depleted from the embryo through chelation.

The potential role of extracellular zinc on RPE cell differentiation

Yemsrach Tadesse, Sabrina Cahyadi and Imre Lengyel, UCL Institute of Ophthalmology, 11-43 Bath Street, EC1V 9EL, UK

Purpose: Zinc has been found to be highly abundant in the retinal pigment epithelial (RPE)/Choroid complex suggesting its potential importance in normal RPE cell function. If zinc is important for RPE cells then we need to culture them in a zinc efficient environment. Foetal calf serum is important for RPE culturing, but it has proteins like serum albumin, that binds zinc and therefore the culturing mediums create a zinc deficient environment. We hypothesized that appropriate level of extracellular zinc is essential for appropriate differentiation and maturation of RPE cells.

Method: ARPE-19 cells were propagated using DMEM/F12, 10% FBS and 1% antibiotics. ARPE-19 cells were cultured using different compositions of culturing medium. These were DMEM-high glucose, DMEM-low glucose, DMEM/F12, DMEM/F12 + 50 uM zinc, DMEM/F12 + 100 uM zinc and DMEM/F12 + 150 uM zinc for a 5-week period. Every week we investigated the expression of the marker genes RPE65, RLBP1, SILV and BEST1 as well as measured transepithelial resistance (TER) and immune staining for ZO-1 a tight junction marker. The F-actin probe rhodamine was also used to demonstrate any changes in the cytoskeletal structures. Samples were also prepared for SEM, TEM and western blotting. Bio-available zinc concentration in all of the culturing conditions was determined using ZnAF2, a zinc selective fluorescence indicator.

Results: Transepithelial resistance and ZO-1 localization in the presence of zinc differed to the control (DMEM/F12). Prior to confluency, zinc appears to slow tight junction formation. Post-confluency, zinc appears to induce differential distribution of ZO-1. RPE65 gene expression appears to be slowed down in the presence of zinc whilst SILV gene expression appears to be stimulated in the presence of zinc. Zinc had no effect on RLBP1 and BEST1gene expression.

Conclusion: Extracellular zinc does appear to alter RPE cell differentiation. The exact mechanisms are, however, not understood. Overall, zinc has a varied effect on the different RPE cell markers, suggesting specificity in its effects. The increased SILV gene expression in the presence of zinc suggests that APRE-19 cells may develop better pigmentation when cultured in the presence of zinc and this might have implications for generating better RPE cells for transplanted into human eyes.

ICP-MS, LA-ICP-MS and GE-ICP-MS Based Strategies for the Investigation of the Role of Zinc in Age-related Macular Degeneration (AMD)

Syed R. Haider[1], Helen J. Reid[1], Neda F. Barzegar-Befroei[2], Imre Lengyel[2] and Barry L. Sharp[1]; 1- Centre of Analytical Science, Department of Chemistry, Loughborough University, Leicestershire, LE11 3TU, UK.; 2-UCL Institute of Ophthalmology, London, EC1V 9EL, UK.

This poster describes a brief study on the role of zinc (Zn) in age-related macular degeneration (AMD). Using inductively coupled plasma-mass spectrometry (ICP-MS), zinc and copper (Cu) were quantified with the help of the calibration curve in human serum obtained from the AMD patients. It was observed that zinc concentration varies in different age group people which can be an indication of different stages of the AMD. However, it is important to note that some of the comparatively younger people showed low Zn level of the AMD that leads to the finding that age is not always a contributing factor in progression of this disease. Further to this copper did not show any significant variations in the concentration. Similarly, females AMD serum were found lower zinc and higher copper concentration than males. The factors involved in this low concentration of zinc were also investigated and it was revealed that both serums did not show any significant difference in unbound zinc level. However, results showed that zinc in both serum samples is not firmly bound as compared to the copper which means more free zinc is available in both types of the serums. These findings lead to the mechanism involved and it could be suggested that the covalently protein-bound zinc in AMD serum chemically react with the free proteins or species present in the serum and further detach the zinc and form a Zn-protein or any other Zn-species complexes which migrate towards the eye and accumulate there. The presence of free Zn in AMD was confirmed by electrophoretic separation of serum proteins and detection of zinc and copper using laser ablation (LA)-ICP-MS. The cellulose acetate membrane was used instead of the gel because membranes have shown very low level of the metal and non-metal bound impurities which is very helpful in enhancing the LA-ICP-MS detection of the analytes. Gel electrophoresis (GE)-ICP-MS online coupling was carried out which further confirms the presence of free Zn in human serum.