connection, Fe accumulation in several brain regions, and specifically in the *substantia nigra* has been reported in PD patients. We have previously demonstrated that dopaminergic neurons exposed to α-synuclein overexpression and Fe overload display lipid dyshomeostasis that results in triacylglycerol accumulation and exacerbated phospholipid hydrolysis. In this work, our goal was to characterize the brain lipid profile in an *in vivo* model of ferroptosis. For this purpose, C57BL/6 mice were subjected to Fe overload by performing a four-doses scheme of intraperitoneal administration (Fe-saccharate -800 or 1332 mg/kg- or vehicle). During treatment (16 days), animal welfare and locomotor activity were periodically evaluated. After sacrifice, biochemical parameters were determined in several organs (brain, liver and kidney). Motor skills were assessed by using open field and footprint tests. Mice exposed to Fe overload (1332 mg/kg) showed a 60% diminution of total distance traveled, associated with a greater thigmotaxis (20%; $p < 0.05$) and a slightly delayed right footprint. These alterations in motor skills were related to increased α-synuclein expression. A buildup of oxidative stress markers associated with ferroptosis, such as lipid peroxide levels and conjugated dienes and trienes products derived from fatty acid oxidation (200% and 500%, respectively), was detected in the brain of Fe-treated animals compared to controls ($p<0.001$). Liver and kidney presented a similar profile of oxidative stress markers. Brain lipid content was altered in Fe-treated mice. Whereas increased cholesterol ($p< 0.05$) and diacylglycerol ($p<0.001$) levels were detected, their acylated forms were decreased ($p<0.05$). Total brain phospholipid levels remained unaltered in the ferroptosis model. Changes in neutral lipid profile were paradoxically associated with diminished expression of lipases such as calcium-independent phospholipase A2 and adipose-triacylglycerol lipase. Our results demonstrate that lipid cacostasis is associated with brain Fe accumulation, ferroptosis and motor impairment. The imbalance in lipid acylation/deacylation processes and cholesterol accumulation reported here could be considered as biomarkers of Fe-induced neurodegeneration and ferroptosis.

LI-P04-83

ARE PROSTAGLANDINS INVOLVED IN THE RESTITUTION OF AN OXALATE-DAMAGED EPITHELIUM?

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Renal collecting ducts, which are involved in the urine concentration mechanism, are immersed in an extracellular matrix with the highest body osmolarity. This hyperosmolarity is a key signal for renal cell differentiation and for the establishment of the urine concentration mechanism. However, hyperosmolarity can induce cell death when there is a great osmolarity change. Renal cells activate adaptive and protective mechanisms to survive in the hyperosmolar environment. One important cell mechanism is the expression of osmoprotective genes such as cyclooxygenase 2 (COX2). Moreover, renal ducts are exposed to wastes coming from blood filtration that include nephrotoxic drugs and kidney stones. Calcium oxalate stones are the most common type of kidney stone. Crystal aggregates are harmful for epithelial renal cells and tubular structures, and the damage could lead to renal kidney disease. Our prior results showed that oxalate modulates COX2 mRNA and protein expression in renal differentiated epithelial cells, but the role of this protein is still unknown. The aim of the present work is to evaluate whether prostaglandins, the COX2 products, are involved in the regeneration mechanism of differentiated renal epithelial cells damaged with oxalate. To do that, renal epithelial cells MDCK were grown in a hyperosmolar environment (512 mOsm/Kg H_2O) for 72 h to get a differentiated epithelium and then subjected to 1.5 mM oxalate (Ox) for 24, 48 and 72 h. To inhibit COX2, 10 µM NS398 was added 30 min before Ox treatment; and to restore the inhibition, PGE_2 (10^{-5}, 10^{-6} and 10^{-7} M) was added 30 min after Ox addition. After treatment, cells were harvested, counted and cell viability was determined. Cell morphology and COX2 expression was also evaluated. Cells treated with 24 h of Ox showed a spindle-shaped morphology characteristic of an epithelial mesenchymal transition (EMT) and NS398 addition before Ox treatment did not allow these EMT. After 48 h of Ox cells started to recover their typical epithelial morphology. Cell treated with NS398 before Ox showed a cobblestone morphology, but gaps in the monolayer were observed. Control conditions showed the typical epithelial cobblestone morphology after 24 and 48 h. PGE_2 addition to cells treated with NS398 and Ox did not allow the EMT at 24 and 48h. Moreover, PGE_2 treated cells showed a morphology characteristic of an epithelial cells (cobblestone). Ox decreased the number of cells at 24 h and 48 h compared to controls. The treatment with NS398 before Ox addition caused a slight decrease of cell numbers at 24 h but not at 48 h. PGE_2 addition did not affect cell number at 24 and 48 h. Cell viability did not change after all treatments. NS398 induced COX2 expression and the addition of PGE_2 slightly decreased it. The results showed that PGE_2 may be implicated in the restitution of the differentiated epithelia damaged with oxalate, but further experiments are needed to elucidate the molecular mechanisms involved.

LI-P05-89

XBP-1 REGULATION OF ARACHIDONIC ACID AND GLICEROLIPIDS METABOLISM IN RENAL EPITHELIAL CELLS UNDER OSMOTIC STRESS

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Hyperosmolarity is a key controversial signal for renal cells. Under physiological conditions, it induces renal cell differentiation and maturation of urine concentrating system. However, abrupt changes in environmental osmolarity may also

induce cell stress that can lead to death. To adapt and survive in such adverse conditions, renal cells implement different osmoprotective mechanisms that includes both the upregulation of cyclooxygenase-2 (COX-2) expression and prostaglandins (PGs) synthesis from arachidonic acid (AA), and a coordinated increase in phospholipids (PL) and triacylglycerides (TAG) biosynthesis. We previously shown that hyperosmolarity induces ER stress and activates the unfolded protein response (UPR) in Madyn Darby Canine Kidney Cells (MDCK) through IRE1α-XBP1s pathway, and that XBP1s modulates lipid synthesis regulating lipogenic enzymes expression. In the present work we evaluated how XBP1s modulates phospholipase A2 (PLA2)/COX-2/PGs pathway and its relationship with lipid synthesis induction under osmotic stress. MDCK cells were subjected to hyperosmolarity (298-512 mOsm/kg H2O) for different periods of time (0, 12, 24 and 48 h) and treated with different PLA2 (cPLA2, iPLA2 and sPLA2) and IRE1α inhibitors. RT-PCR studies showed that hyperosmolarity increased cPLA2 expression at 24 and 48 h but did not upregulate iPLA2 expression. Inhibition of cPLA2 but not iPLA2 nor sPLA2 prevented hyperosmolarity-induced lipid synthesis and lipid droplets accumulation. Furthermore, IRE1α RNase activity inhibition was accompanied by a decrease in cPLA2 and COX-2 but not in iPLA2 expression evaluated by RT-PCR. Instead, western blot analysis showed a significant increase in COX-2 protein levels when xbp1 (u) splicing was blocked by IRE1α inhibitor. Our findings suggest that the UPR modulates glycerolipids metabolism under osmotic stress by regulating cPLA2/COX-2/PGs axis.

LI-P06-103

GLYCOSPHINGOLIPIDS ARE ESSENTIAL FOR THE CORRECT SEGREGATION OF PHOSPHOINOSITIDES MEDIATED BY PTEN IN RENAL EPITHELIAL CELLS

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Phosphoinositides act as critical regulators of cell polarization. PtdIns(4,5)P2 is enriched in the apical membrane, whereas PtdIns(3,4,5)P3 is basolateral, and this segregation is regulated by PTEN. The apical localization of PTEN allows the local synthesis of PtdIns(4,5)P2 and the consequent apical recruitment of the apical protein complex required for lumen development. We previously demonstrated that sphingolipid synthesis is essential for the correct localization of PTEN during the differentiation of MDCK cells induced by hypertonicity, and that the inhibition of PTEN impairs MDCK cell differentiation. In this study we transfected cells with biosensors for PtdIns(4,5)P2 and PtdIns(3,4,5)P3 and cultured them under hypertonicity (inductor of cell differentiation) in the presence of D-PDMP (a glucosylceramide inhibitor), SF1670 (a PTEN inhibitor) or siRNA PTEN; followed by immunofluorescence staining for gp135, an apical marker. MDCK cells transient transfected with PLCδ1-PH – GFP, a PtdIns(4,5)P2 biosensor, showed a differentiated phenotype with PtdIns(4,5)P2 distributed at cell periphery, but accumulated at apical membrane in colocalization with gp135. Cells treated with SF1670 showed atypical gp135-containing lateral lumens with positive staining for PtdIns(4,5)P2. Similar results were observed in cells treated with siRNA PTEN, suggesting that the expression and the activity of PTEN are necessary for the correct localization of PtdIns(4,5)P2 and regulate the targeting of gp135 to induce the MDCK cell differentiation. To study the distribution of PtdIns(3,4,5)P3 we developed an MDCK cell line stably expressing the PH domain of Akt coupled to GFP (GFP-Akt-PH). Transfected cells cultured under hypertonicity 48 h post - confluence developed a differentiated phenotype with apical accumulation of gp135, as wild type cells. GFP-Akt-PH was mainly associated with lateral membranes. After treatment with D-PDMP, cells showed altered morphology with GFP-Akt-PH partially redistributed into apical membrane. The results shows that glycosphingolipid synthesis is necessary for the correct segregation of phospoinositides mediated by PTEN, suggesting an interplay between glycosphingolipids and phospoinositides that is essential for MDCK differentiation.

LI-P07-115

MENADIONE AND IRON OVERLOAD TRIGGER NEUTRAL LIPID REMODELLING IN ADIPOCYTES AND ADIPOSE TISSUE

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A growing body of evidence indicates that oxidative stress (OS) can cause an increase in preadipocyte proliferation, and adipocyte differentiation. However, the biochemical mechanisms by which OS alters adipocyte neutral lipid metabolism are not completely elucidated. Menadione and iron (Fe) overload are well known OS enhancers through the induction of Fenton and Haber-Weiss reactions. We have previously demonstrated that menadione exposure (20 and 50 µM) in differentiated 3T3-L1 adipocytes results in increased cell oxidant levels and downregulation of adipogenic genes and transcription factors. In this work, our goal was to investigate the effect of menadione and Fe overload in neutral lipid metabolism both in adipocyte cell culture and by using an *in vivo* model. For this purpose, we worked with differentiated adipocytes challenged with menadione and C57BL/6 mice exposed to Fe overload. Differentiated 3T3-L1 adipocytes were exposed to menadione for 24 h, and then neutral lipid profile was analyzed. For the *in vivo* OS model, Fe-saccharate (800 or 1332 mg/kg) or vehicle were administered by intraperitoneal injection (four-dose scheme in 16 days). Plasma, visceral and gonadal adipose tissue obtained from Fe-treated animals and controls were used to determine OS markers and neutral lipids. In mice, lipid peroxide levels and conjugated dienes derived from fatty acid oxidation were increased in visceral and gonadal adipose tissue after Fe treatment.