Ημερίδα Ελληνικής Εταιρείας Βασικής και Κλινικής Φαρμακολογίας

07-03-2015
Hotel Titania
Athens

ΠΡΟΓΡΑΜΜΑ/PROGRAM

9.00-9.30 Registration

9.30-10.30 Short Oral presentations
Chairs: Profs Papadimitriou E, Marselos M

9.30-9.45 Determination of a transmembrane amino acid of the CRF1 receptor that is critical for receptor's activation. Spyridaki et al. University of Crete
9.45-10.00 Toxicity assessment of novel anti-amyloidogenic substances. Chalatsa et al., University of Athens
10.00-10.15 Deficiency in apolipoprotein A-I ablates the pharmacological effects of metformin on plasma glucose homeostasis and hepatic lipid deposition. Karavia et al., University of Patras
10.15-10.30 Oxygen-glucose deprivation (OGD) modulates the unfolded protein response (UPR) and inflicts autophagy in a PC12 hypoxia cell line model. Vavilis et al, Aristotle University of Thessaloniki

Chairs: Prof Gravanis A, Karakioulakis G
10.30-11.30 Old Hormone, Old Drug: New Applications for Treatment of Mental Disorders "The Oxytocin Story"
Alexis Bailey, University of Surrey

11.30-12.00 *Coffee break*

Chairs: Profs Papaioannidou P, Kypreos K
12.00-12.30 Towards immunization against prion diseases
Prof. Thodoros Sklaviadis, Aristotle University of Thessaloniki
12.30-13.00 The Corticotropin Releasing Factor System in the periphery or what can stress do to your body
Dr. Katerina Chatzaki, Democritus University of Thrace
13.00-13.15 The European Certified Pharmacologist Program
Prof. Vangelis Manolopoulos

13.15-14.30 *Lunch break*

Chairs: Prof Kouvelas D, Daifoti-Papadopoulou
14.30-15.00 "What is so special about advanced therapy (medicinal products)?"
Dr. Jan Mueller-Berghaus, Paul-Ehrlich-Institute, Germany and CHMP/EMA

15.00-16.20 Ρυθμιστικό πλαίσιο φαρμάκων/Update on drug regulation

Στρογγυλό τραπέζι που οργανώνεται από τον ΕΟΦ/Round table organized by the Greek National Organization for Medicines (ΕΟΦ)

15.00-15.20 ""Διαδικασίες έγκρισης, τροποποίησης και ανανέωσης άδειας κυκλοφορίας - Κεντρική Διαδικασία"
Δρ. Γιώργος Αϊσλάιτνερ, Προϊστάμενος Τμήματος Γραμματείας και Διοικητικού Ελέγχου

15.20-15.40 "Διαδικασίες έγκρισης φαρμακευτικών προϊόντων προηγμένης θεραπείας (AMTPs)"
Δρ. Αγγελική Ρομποτή, Προϊσταμένη Τμήματος Βιολογικών Προϊόντων

15.40-16.00 "Κανονιστικό πλαίσιο απλουστευμένων αιτήσεων έγκρισης φαρμακευτικών προϊόντων" Δρ. Ελευθερία Νικολαΐδη, Προϊσταμένη Τμήματος Γραμματείας ΕΣΕ

16.00-16.20 ""Επισκόπηση της νέας νομοθεσίας φαρμακοεπαγρύπνησης"
Δρ. Λεωνίδας Κληρονόμος, Προϊστάμενος Τμήματος Ανεπιθύμητων Ενεργειών

16.30 Απολογισμός απερχόμενου ΔΣ και επιλογή πόλης για τη διεξαγωγή του συνεδρίου 2016/Report from the Executive Committee-2016 Annual Conference

17.00 Εκλογές/Elections

1. DISTINCT ROLES OF APOLIPOPROTEIN A1 AND LECITHIN-CHOLESTEROL ACYLTRANSFERASE INLIPOPOLYSACCHARIDE-INDUCED INFLAMMATION

P.I. Petropoulou[1*], J.F.P. Berbée[1*], Aikaterini Hatziri[1],I. Karagiannides[1]and K.E. Kypreos[1]
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* Equal contribution to this work

AIM: Chronic or recurrent microbial infections enhance the risk of cardiovascular disease as the result of a heightened inflammatory state. HDL has great immunomodulatory properties, primarily mediated by the proteins associated to HDL. Here we investigated lipopolysaccharide (LPS)-induced inflammation in Apoa1-deficient mice that lack classical HDL and Lcat-deficient mice that lack mature HDL.

METHODS: RAW 264.7 macrophages, whole blood and peritoneal macrophages from C57Bl/6 (wild-type, WT), Lcat-deficient (*Lcat*$^{-/-}$) and Apoa1-deficient (*Apoa1*$^{-/-}$) mice were challenged with LPS from Escherichia coli (1-100 ng/mL; 4-18 h) and TNFα secretion was quantified by ELISA. WT, *Lcat*$^{-/-}$ and *Apoa1*$^{-/-}$ mice were challenged intravenously with LPS (100 μg/kg body weight) and several plasma cytokines/chemokines were monitored. Leukocytes from WT, *Lcat*$^{-/-}$ and *Apoa1*$^{-/-}$ micewere stained with anti-mouse Cd11b, Ly-6C, CD4 and CD8a antibodies for immuno-phenotyping using FACS.

RESULTS: Serum and HDL from *Lcat*$^{-/-}$ and *Apoa1*$^{-/-}$ miceshow a reduced capacity to attenuate the LPS-induced TNFα response, *in vitro*. Whole blood from *Lcat*$^{-/-}$ and *Apoa1*$^{-/-}$ mice show an increased TNFα response when stimulated with LPS *ex vivo,* an effect more pronounced for *Lcat*$^{-/-}$blood. *Lcat*$^{-/-}$ and *Apoa1*$^{-/-}$mice show a markedly enhanced and prolonged LPS-induced inflammatory response *in vivo*. At baseline, LCAT-deficiency is associated with leukocytosis, increasedCD11b+/Ly-6C^{med}monocyte levels and CD4+ lymphocyte levels, but low CD11b+/Ly-6C^{hi}monocyte levels and low CD11b expression in all types monocytes. Similarly, peritoneal macrophages from *Lcat*$^{-/-}$ mice show a markedly reduced LPS-induced TNF-alpha response. On the other hand, APOA1 deficiency is associated with decreased leukocyte and CD11b+/Ly-6C$^{lo/med/hi}$monocyte and CD4^{+}, CD8^{+} lymphocyteslevelsat baseline.

CONCLUSION: Though the systemic effect of APOA1 and LCAT deficiency appear to be similar in mice challenged with LPS, the underlined mechanism differs. LCAT deficiency renders leukocytesless responsive, but increases their number, while APOA1 deficiency renders leukocytesmore responsive, though it reduces their numbers in circulation.

2. BEHAVIORAL EFFECTS OF THE OLIVE COMPOUND OLEUROPEINE

Electra Papakonstadinou[1], Nikolaos Kokras[1], Antonios Tsarbopoulos[1], Alexios-Leandros Skaltsounis[2], Zeta Papadopoulou-Daifoti[1] and Christina Dalla[1]
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Olives and olive products, as part of the Mediterranean diet have been suggested to be beneficial against cognitive decline, dementia and Alzheimer's disease. Oleuropein (oleu) is a natural phenolic antioxidant, which is present in elevated concentration in olives, olive oil and olive tree leaves (Oleaeuropaea L.). In the current study we investigated the effects of the compound Oleuropein, on the behavioral phenotype of an Alzheimer disease mouse model (3xTg), which expresses mutated forms of human proteins Tau, APP and Presenilin1. This model has high validity because it exhibits the main pathological aspects of Alzheimer's disease. Male and female wild type (wt) and transgenic (3xTg) mice were used at the age of 9-11 months old. The behavioral screening included activity, anxiety, depression and memory/learning tests, such as the open field, light/dark, Y maze, novel object recognition and tail suspension tests. The 3xTg mice exhibit reduced locomotor activity, which was not reversed by oleuropein treatment. However, oleuropein treatment resulted in the improvement of the reduced exploratory activity of 3xTg mice towards a novel environment. Sex differences were only observed in stereotypic behavior of 3xTg mice. Anxiety levels and "depressive-like" behavior were not affected by genotype, sex or treatment. In novel object recognition task 3xTg mice exhibited mild cognitive deficits, which were reversed by oleuropein treatment. Conclusively, it seems that oleuropein exerts a mild beneficial effect in cognition.However, more studies are required, in order to elucidate its role of in Alzheimer's disease.

Acknowledgements: This work has been supported by a "Large Scale Cooperative Project" (TreatAD, 09SYN-21-1003) co-financed by the European Social Fund (ESF) and the General Secretariat for Research and Technology in Greece.

3. EFFECTS OF THE NITRIC OXIDE (NO) DONORS MOLSIDOMINE AND SODIUM NITROPRUSSIDE (SNP) ON ANXIETY-LIKE BEHAVIOUR IN RATS

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BACKGROUND & OBJECTIVES: There is poor experimental evidence concerning the role of nitric oxide (NO) on anxiety. The present study investigated the effects of intraperitoneal (i.p.) administration of the NO donors molsidomine(1, 2 and 4 mg/kg) and sodium nitroprusside (SNP) (0.1, 0.3 and 1 mg/kg) in an animal model of anxiety in rats.
METHODS: For this purpose, the light/dark (LD)test was used.
RESULTS: Molsidomine (1 and 2 mg/kg) and SNP (0.3 and 1 mg/kg) significantly increased, with respect to the control animals, the amount of time spent by rats in the illuminated compartment of the LD box. Interestingly, both compounds did not affect the total number of transitions between the lit and the dark chamber of the apparatus.
CONCLUSIONS: The present results indicate that either molsidomine or SNP produced an anxiolytic-like effect that cannot be attributed to changes in motor activity. Further, the current findings suggest a possible implication of NO donors in anxiety.

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4. PHARMACOLOGICAL EVALUATION OF NOVEL ADENINE-HYDROGEN SULFIDE SLOW RELEASE HYBRIDS DESIGNED AS MULTI-TARGET CARDIOPROTECTIVE AGENTS

N. Lougiakis[1], A. Papapetropoulos[1], S. Toumpas[1], A. Zoga[2], Z. Zhou[1], N. Pouli[1], P. Marakos[1], E. Gikas[1], EK. Iliodromitis[2], I. Andreadou[1]
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Adenosine receptors have a major role in triggering the intracellular signal transduction pathways involved in the prevention of reperfusion (rep) injury. Exogenous administration of hydrogen sulfide (H2S) triggers cardioprotection in animal models of ischemia (isc)/rep injury. However, the exposure of biological systems to inorganic H2S "donors", such as NaHS, causes a burst of H2S release that does not recapitulate the low level of continuous production of H2S that occurs in vivo.
PURPOSE: We aimed to evaluate the cardioprotective effect of novel hybrid compounds that simultaneously contain two pharmacophore groups, an adenine and a H2S releasing moiety.
METHODS: The slow releasing sulfur containing agent 4-hydroxythiobenzamide (4-OH-TBZ) was coupled either with 9-(4-hydroxybutyl) adenine providing the product S1, or with adenosine providing the product S6. The capability of the new hybrids to release H2S was assessed in vitro. Anesthetized rabbits were subjected to 30 min isc and 3 hours rep and were divided into 8 groups: 1) Control, no further intervention; 2) PostC, with 8 cycles of 30 sec isc/rep; 3) S1, treated with the compound S1 at a dose of 1.79 μmol.kg-1 at the 20th min of isc; 4) S6, treated with the compound S6 at the same mode as S1; 5) S1+SPT, treated as S1 with the addition of the adenosine receptor blocker SPT; 6) S6+SPT treated as S6 with the addition of SPT; 7) 9-(4-hydroxybutyl) adenine at a dose of 1.79 μmol kg-1; 8) NaHS, treated with NaHS at a dose of 1.79 μmol kg-1 bolus, followed by infusion of 17.9 μmol kg-1 for the next 120 min. Doses of S1 and S6 were estimated at 20-fold lower dose compared to NaHS. The infarcted (I) to risk (R) areas were estimated as % I/R. The circulating plasma levels of thiosulfates were assessed at rep by a developed and validated UPLC-UV methodology using a derivatization reaction with the CMQT reagent.
RESULTS: The S1 and S6 compounds reduced the infarct size (17.4±0.7% and 20.1±0.9% respectively vs 47.9±0.7% in Control, $p<0.05$), as NaHS (16.7±2. 1%) and PostC (24.3±0.5%). SPT did not abrogate the benefit of S1 (16.9±1.8%) and S6 (18.3±0.7%). Compound S1, closely resembling to adenosine, was more potent ($p<0.05$) than 9-(4-hydroxybutyl) adenine (28.5±1.1%) indicating that the H2S releasing moiety is critical. S1 and NaHS increased circulating thiosulfates, with S1 exhibiting only 1/10 of the levels observed in the NaHS group.
CONCLUSIONS: The novel adenine-H2S hybrids reduce the infarct size independently of the adenosine receptors, providing the basis for further development of slow releasing H2S donors in myocardial isc.

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5. DEFICIENCY IN APOLIPOPROTEIN A-I ABLATES THE PHARMACOLOGICAL EFFECTS OF METFORMIN ON PLASMA GLUCOSE HOMEOSTASIS AND HEPATIC LIPID DEPOSITION

Eleni A. Karavia[a,$], Aikaterini Hatziri[a,$], Christina Kalogeropoulou[a,$], Nikolaos Papachristou[b], Eva Xepapadaki[a], Anastasios Natsos[a], Caterina Constantinou[a], Peristera-Ioanna Petropoulou[a], Shlomo Sasson[c], Dionysios J Papachristou[b], and Kyriakos E. Kypreos[a*]

ABSTRACT
Recently we showed that deficiency in apolipoprotein A-I (apoA-I), the main protein component of HDL, sensitizes mice to diet-induced obesity, glucose intolerance and NAFLD development. Based on this knowledge, in the present study we investigated the potential involvement of apoA-I in the pharmacological effects of metformin on

glucose intolerance and NAFLD development. Groups of apoA-I-deficient (apoA-$I^{-/-}$) and wild-type C57BL/6 mice fed western-type diet were either treated with a daily dose of 300 mg/kg metformin for 18 weeks or left untreated for the same period. Then, histological and biochemical analyses were performed. Metformin treatment led to a comparable reduction in plasma insulin levels in both C57BL/6 and apoA-$I^{-/-}$ mice following intraperitoneal challenge with glucose. However, only metformin-treated C57BL/6 mice maintain sufficient peripheral insulin sensitivity to effectively clear glucose following the challenge, as shown by a [^{3}H]-2-deoxy-D-glucose uptake assay in isolated soleus muscle. Similarly, deficiency in apoA-I ablated the pharmacological effect of metformin on hepatic lipid deposition and NAFLD development. Interestingly, metformin treatment resulted in a comparable hepatic AMPK activation in apoA-$I^{-/-}$ and C57BL/6 mice suggesting that apoA-I may impact subsequent steps in AMPK signaling involved in maintenance of normal glucose homeostasis. Gene expression analysis of key lipogenic genes FASN, DGAT-1, MTTP and PPARγ suggested that the effects of apoA-I on metformin action may be independent of *de novo* lipogenesis. Our data show that the recently identified role of apoA-I in diabetes extends even further to the modulation of the pharmacological actions of metformin, a common insulin sensitizer for the treatment of type 2 diabetes.

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6. *ALDH1A7* EXPRESSION IN RR AND rr RATS: STRUCTURAL ALTERATIONS OF THE PROMOTER AND THE ROLE OF NUCLEAR RECEPTORS

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BACKGROUND & OBJECTIVES: Two populations of Wistar rats had been selected and regenerated by inbreeding, based on the differential induction of *ALDH1A* subfamily genes, especially after administration of phenobarbital (PB). Our previous studies of the enzymatic, mRNA and protein levels of ALDH1As have shown a remarkable difference of *ALDH1A7* gene expression between *RR* (responsive) and *rr* (non-responsive) rats. This project is supplemented by experiments examining the structural alterations of the *RR* and *rr* promoter sequences and the potential role of nuclear receptors CAR and PXR.

METHODS: *ALDH1A7* promoters from *RR* and *rr* strains were cloned and sequenced. Promoter deletion constructed were cloned upstream of pGL3-Basic luciferase reporter plasmid by standard molecular biology methods. Co-transfections with empty and CAR or PXR expression vectors, *RR* and *rr* reporters, and control PBREM and XREM plasmids in hepatoma cells. Luciferase and beta-galactosidase activities were measured as before (Küblbeck et al. 2010).

RESULTS: Remarkably high levels of reporter gene were observed by the *RR* proximal promoter from *RR* rats, whereas the respective fragment from the *rr* substrain showed low basal activity. The upstream *RR* promoter appeared to contain negative elements, depressing the activity.

CONCLUSIONS: Structural alterations in ALDH1A7 promoter sequence in rr rats lead to low expression levels in this substrain. Only minor, if any, activation by CAR and PXR was observed in co-transfection assays.

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7. OXYGEN-GLUCOSE DEPRIVATION (OGD) MODULATES THE UNFOLDED PROTEIN RESPONSE (UPR) AND INFLICTS AUTOPHAGY IN A PC12 HYPOXIA CELL LINE MODEL.

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Laboratories of Physiology[1], Histology, Embryology and Anthropology[3], Pharmacology[5], and 1st Department of Obstetrics and Gynaecology[6], School of Medicine, Faculty of Health Sciences, Laboratory of Physiology, School of Biology, Faculty of Sciences[2], Aristotle University of Thessaloniki, and Department of Pathology[4], General Hospital "G. Papanikolaou", Thessaloniki, Greece

BACKGROUND & OBJECTIVES: Hypoxiaimposes severe stress upon cells. It is a major feature of many pathological conditions like, traumatic brain injury, cerebral haemorrhage, perinatal asphyxia and can lead to cell death due to energy depletion and increased free radical generation. This study investigates the effect of hypoxia on the unfolded protein response (UPR), utilizing a 16 hour oxygen glucose deprivation protocol (OGD) in a PC12 cell line model. Expression of glucose regulated protein 78 (GRP78) and glucose regulated protein 94 (GRP94), key players of the UPR, was studied along with the expression of glucose regulated protein 75 (GRP75), heat shock cognate 70 (HSC70) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), all with respect to the cell death mechanism(s).

METHODS: PC12 cells were cultivated under OGD and normoxic conditions. Protein and mRNA were isolated and GRP78, GRP94, GRP75, HSC70 and GAPDH levels were

assessed at translational and transcriptional level using western blotting and real-time PCR. Cell death mechanism was elucidated using TUNEL assay, DNA electrophoresis and LC3I to LC3II conversion. Electron microscopy and PAP stain of the samples was carried out.
RESULTS: Cells subjected to OGD displayed upregulation of GRP78 and GRP94 and concurrent downregulation of GRP75. These findings were accompanied with minimal apoptotic cell death and induction of autophagy.
CONCLUSIONS: The above observation warrants further investigation to elucidate whether autophagy acts as a prosurvival mechanism that upon severe and prolonged hypoxia acts as a concerted cell response leading to cell death. In our OGD model hypoxia modulates UPR and induces autophagy.

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8. THE EFFECT OF HYDROGEN SULFIDE DONORS ON TNF-A INDUCED EXPRESSION OF NADPH SUBUNITS IN ENDOTHELIAL CELLS

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3. Medical School, University of Exeter, Exeter, UK

BACKGROUND & OBJECTIVES: Hydrogen sulfide (H_2S) appears to confer cytoprotection via multiple mechanisms including anti-oxidant and anti-inflammatory effects. Recent reports demonstrated that H_2S ameliorates cellular oxidative stress and also acts as an endogenous scavenger for reactive oxygen species (ROS). The NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) is a membrane-bound enzyme complex, responsible for ROS production in cells. The present study aimed to investigate the effect of pretreatment with two H_2S donors, GYY4137 and thioglycine, on the expression of two central components of this enzyme complex (subunits gp91phox and p47phox) in endothelial cells.
METHODS: EAhy926 cells were grown to confluence in 6-well plates. Cells were serum-starved for 12h and then incubated with 500μM of GYY4137 or thioglycine for 1h. After H_2S donor treatment, cells were incubated with TNF-α (10ng/ml) for 6h. Protein expression of NADPH oxidase subunits was assessed by Western blot analysis of whole cell lysates. The levels of subunit protein expression were quantified by measuring band intensities and expressed as a ratio to β-actin expression.
RESULTS: The expression of cytoplasmic p47phox subunit was increased after TNF-α stimulation while the expression of gp91phox plasma-membrane seems to be unchanged. In preliminary experiments, pre-treatment of EAhy926 with GYY4137 (500μM) or thioglycine (500μM) for 1h before TNF-α activation seems to affect expression levels of both subunits.
CONCLUSION: H_2S may have the ability to attenuate and prevent the known deleterious effects of elevated ROS through modification of the NADPH oxidase-dependent redox signaling pathway.

Acknowledgements: This research has been co-financed by the European Union (European Social Fund –ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales: "Hydrogen sulfide a new endogenous regulator of angiogenesis: signaling, physiology/pathophysiology and development of pharmacological inhibitors" (MIS 380259).

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9. EFFECTS OF THE NOVEL DEHYDROEPIANDROSTERONE (DHEA) ANALOGUEBNN27 ON DIFFERENT MEMORY COMPONENTS AND ON SCOPOLAMINE-INDUCED AMNESIA IN A RECOGNITION MEMORY TASK IN RATS

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BACKGROUND & OBJECTIVES: Consistent experimental evidence suggests the involvement of neurosteroiddehydroepiandrosterone (DHEA) in cognition. BNN27 is a novel DHEA analogue, which devoid of steroidogenic activity and its neurotrophic effect has been observed. Its role, however, in learning and memory has not yet established. Thus, the present study was designed to investigate the effects of BNN27 (1, 3 and 10 mg/kg) on rats'recognition memory.
METHODS: For this purpose, the novel object recognition task (NORT) was used.
RESULTS: Intraperitoneal (i.p.) administration of BNN27 (3 and10 mg/kg)antagonized delay-dependent deficits in the NORT in the normal rat, suggesting that this DHEA analogue affected acquisition, storage and retrieval of information. In addition, BNN27 (3 and 10 mg/kg, i.p.) counteracted the scopolamine [0.2 mg/kg, subcutaneously (s.c.)]-induced performance deficits in this recognition memory paradigm.
CONCLUSIONS: The present results indicate that BNN27 may modulates different aspects of recognition memory and suggest that its interaction with the cholinergic system is relevant to cognition.

10. EFFECT OF DIETARY CROCUS SATIVUS L. ON THE ACTIVITY OF CYP1A2 IN HEALTHY SUBJECTS

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INTRODUCTION : Crocus sativus L. is a plant extensively grown in regions around the Mediterranean, Iran, India, Tibet and regions in China. Saffron, the dried red stigmas of C. sativus, is one of the most expensive spices used as food flavouring and colouring substance and consists of a complex mixture of volatile and non-volatile compounds that contribute to its aroma and flavour. The major components of dried stigmas are picrocrocin, safranal, crocin and its aglycone crocetin. C. sativus has been used as a folk medicine for treating diseases for ages and it has been recently shown as a potential agent for cancer prevention. Cytochrome CYP1A2 has been shown to participate in the metabolic bioactivation of several environmental and dietary procarcinogens to active intermediates which can trigger carcinogenesis. Moreover, CYP1A2 activity can be induced and inhibited by several environmental and endogenous compounds.

OBJECTIVES: The purpose of the present study was to examine the effect of saffron water extract on the in vivo CYP1A2 activity.

MATERIALS AND METHODS: Healthy, non-smoking, volunteers (n=19; 15 women, 4 men; mean age 41.4±8.7 y, range: 23-55 y) participated in the study. Volunteers followed a carotenoid-free diet for 13 days. During this period, volunteers abstained also from any foods and drugs known to induce or inhibit CYP1A2 activity. On day 7 of the protocol, subjects commenced daily consumption of 150 ml water extract of commercially available saffron at the physiologically relevant dose of 300 mg. On days 7 and 13 of the study protocol, and following a 12-hour abstinence from caffeine-containing food and beverages, volunteers consumed 200 mg of caffeine and provided, 6 hours later, a saliva sample. Caffeine metabolites were assessed in saliva samples by HPLC. CYP1A2 activity was determined by the use of the caffeine metabolite ratio Paraxanthine/Caffeine (PX/CAF).

RESULTS: PX/CAF ratio values (mean±S.D.) determined in 19 volunteers prior to (0.47±0.23) and at the end of (0.40±0.13) saffron consumption were statistically different (p=0.042, paired samples t-test). Mean difference was 13.58% (0.58-26.57, 95% C.I.). The reduction of PX/CAF ratio values was not uniform across the study sample. Specifically, it was reduced in 13 volunteers, increased in 5 volunteers and remained unchanged in one subject.

CONCLUSION: Daily consumption of water saffron extract reduces CYP1A2 activity. This reduction may contribute towards cancer prevention through the reduced metabolic activation of environmental procarcinogens by CYP1A2.

11. DEFICIENCY IN SCAVENGER RECEPTOR CLASS B TYPE I RESULTS IN PLASMA ACCUMULATION OF APOLIPOPROTEIN E-CONTAINING LIPOPROTEINS AND RESISTANCE IN THE DEVELOPMENT OF DIET-INDUCED OBESITY, GLUCOSE INTOLERANCE AND HEPATIC LIPID DEPOSITION.

Eleni A. Karavia[1], Nikolaos J. Papachristou[2], Eva Xepapadaki[1], Caterina Constantinou[1], Peristera-Ioanna Petropoulou[1], Eleni Papamichail[1], Dionysios J. Papachristou[2], and Kyriakos E. Kypreos[1]

ABSTRACT

Scavenger receptor class B type I (SR-BI) is primarily responsible for the uptake of cholesteryl esters (CE) of HDL by the liver and other tissues, playing important role in reverse cholesterol transport. Recently, we reported that apolipoprotein A-I and lecithin-cholesterol acyltransferase, another two very important protein components of HDL metabolic pathway, have important role in diet-induced obesity, hepatic lipid deposition and glucose intolerance. Here, we investigated the potential role of SR-BI in the development of these conditions. SR-BI deficient ($SR\text{-}BI^{-/-}$) and C57BL/6 mice were fed western type diet for 24 weeks and biochemical and histological analyses were performed. Though $SR\text{-}BI^{-/-}$ mice consumed more food they were resistant to diet-induced obesity and hepatic lipid deposition, and displayed physiological tolerance to intraperitoneal glucose administration, suggesting that SR-BI-mediated uptake of cholesteryl esters is crucial for development of NAFLD and related metabolic perturbations. Kinetic and gene expression analyses indicated that the reduced hepatic lipid deposition in $SR\text{-}BI^{-/-}$ mice is also associated with reduced de novo fatty acid synthesis and AMPK activation. Apolipoprotein analysis of lipoproteins showed that deficiency in SR-BI results in a significant increase in large apoE-containing HDL particles, as well as triglyceride-rich lipoproteins, and inhibition of plasma lipoprotein lipase (LpL). Our data suggest that $SR\text{-}BI^{-/-}$ mice fed western-type diet for 24 weeks, accumulate CE-rich HDL of abnormal apoprotein composition and size. The gradually increasing HDL-apoE ultimately inhibits plasma LpL activity, inhibiting the clearance of all triglyceride-rich lipoproteins and preventing the shuttling of dietary lipids to the liver.

12. A RAPID HPLC METHOD FOR DETERMINATION OF DRUG INDUCED CHANGES IN NEUROTRANSMITTERS RELEASE RATES IN RAT HYPOTHALAMUS, USING NBD AS A DERIVATIVE

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BACKGROUND AND OBJECTIVES: Changes in the release rates of the neurotransmitters, induced from drug administration, can be monitored by the push–pull superfusion technique. We present a rapid and simple method for the determination of the release rates of glutamate, aspartate, serine, taurine and γ-aminobutyric acid in the superfusate of rat hypothalamus, using High Performance Liquid Chromatography (HPLC) and fluorimetric detection.

METHODS: Several conditions which influenced derivatization and separation, such as pH, temperature, acetonitrile percentage mobile phase and flow rate, were optimized to obtain a suitable protocol for amino acids quantification in samples. The separation of the five amino acid neurotransmitters was performed with pre-column derivatization with 4-fluoro-7-nitrobenzofurazan (NBD) and a borax buffer solution. The detection was performed using a column with 142 bar pressure and a mobile phase consisting of phosphate buffer and methanol (90/10 % v/v) at a flow rate of 1.0 mL/min. The wavelengths were 470/540nm (Ext/Em.).

RESULTS: The five neurotransmitter derivatives were completely separated within 20 min. The linear relation was good in the range from 25 to 300 (nmol L^{-1}), and the correlation coefficients were ≥0.999. Intra-day precision was between 1.8 and 3.2%, and inter-day precision was between 2.4 and 4.7%. The established method was used to determine amino acid neurotransmitters with satisfactory recoveries varying from 92.3to 102.9%.

CONCLUSIONS: In this study, an optimized HPLC fluorescence method for determination of aspartate, glutamate, serine, taurine and GABA in rat hypothalamus was developed. The advantages of this method are simple derivatization and rapid analysis.

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13. ENDOTHELIAL NITRIC OXIDE SYNTHASE ACTIVATION AS A NOVEL MECHANISM FOR NITROGLYCERIN-INDUCED CARDIOPROTECTION

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PURPOSE: Cardioprotection by NO donors at the end of ischemia (isc) has not been studied in detail. We sought to evaluate the contribution of the endogenous NO pathway to the cardioprotective action of nitroglycerin (NTG) and to compare this with ischemic postconditioning (PostC).

METHODS AND RESULTS: Anesthetized rabbits were subjected to 30-min myocardial isc and 3-hour reperfusion (rep) and randomized into 15 groups: 1) Control, no further intervention, 2) PostC with 8 cycles of 30-sec isc/rep, 3) NTG treated (2 μg/kg-1/min-1) for a total time of 65 min starting 10 min prior to rep, 4) NTG plus the NOS inhibitor L-NAME, 5) NTG plus the selective nNOS inhibitor 7-NI, 6) NTG plus the selective iNOS inhibitor 1400W, 7) NTG plus the PI3K inhibitor Wortmannin (Wor), 8) NTG plus the adenosine receptor inhibitor SPT, and 9) NTG plus the PKG inhibitor DT-2. In six additional groups, L-NAME, 7-NI, 1400W, Wor, SPT and DT-2 were administered alone at the same time and dose as previously. The infarcted (I) to risk (R) areas were estimated in % I/R. In a second series of experiments animals of the groups Control, PostC, NTG and NTG+L-NAME were subjected to the above interventions up to 10th min of rep when tissue samples were collected for determination of eNOS and Akt and of myocardial ROS by DHE fluorescence. Co-administration of L-NAME or Wor along with NTG eliminated the effect of NTG on %I/R (37.9±2.0%, and 38.3±2.6% respectively vs 23.0±3.2%, $p<0.05$). Inhibition of adenosine and PKG did not affect the protection afforded by NTG (16.0±1.8% and 18.5±3.4% respectively, p=NS vs NTG). Inhibition of nNOS increased NTG protection (12.3±1.0%, $p<0.05$) whilst 7-NI itself exerted protection (27.05±1.6% vs 48.05±2.0% in Control, $p<0.05$). Inhibition of iNOS did not affect the benefit of NTG on %I/R (14.4±1.3%). eNOS and Akt phosphorylation was higher in PostC and NTG groups, while ROS formation was reduced compared to Control and NTG+L-NAME groups. To further investigate the role of eNOS on NTG-mediated protection, wild type and eNOS(-/-) mice underwent isc-rep with NTG administration. NTG had no effect on %I/R in eNOS(-/-) mice compared to wild type. In order to evaluate the effect of mPTP, wild type and cyclophiline D (CypD)(-/-) mice underwent isc-rep with NTG administration. CypD(-/-) hearts had smaller %I/R than wild type and NTG failed to confer additional protection, indicating that NTG protecs through a CypD-dependent mPTP function.

CONCLUSION: NTG induces protection through activation of the PI3K-Akt pathway; this effect is mainly mediated by eNOS activation which further causes inhibition of mPTP.

14. TOXICITY ASSESSMENTOF NOVEL ANTI-AMYLOIDOGENIC SUBSTANCES

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INTRODUCTION: Natural products have played a dominant role in the discovery of leads for the development of drugs aimed at the treatment of human diseases.Aiming to identify novel potentially anti-amyloidogenic agents for the treatment of AD, we performed toxicity screening for 21 promising natural and synthetic bioactive compounds.

METHODS: To assess the effects of the selected substances on cell viability, the human neuroblastoma wild-type SH-SY5Y cells were selected and differentiated to neuron-like cells. The effect of the selected substances on differentiated SH-SY5Y cells was assessed by performing the WST-1 cell viability assay.A broad range of incubation times and compound concentrations were screened.

RESULTS: The successful differentiation to neuron-like cells was confirmed by western blotting and immunofluorescence using antibodies against the neuron-specific markers MAP2, TuJ1 and NeuN. Cells were exposed to concentrations 0.1 μM – 1 mM of each compound and 0.038μg/ml – 380 μg/ml of total extracts for 24h and 72h. Of the 21 substances screened , 11 promoted cell viability at certain concentrations,6 substancesdid not affect cell viability and 4led to increased cell death at most concentrations tested.

CONCLUSIONS: Our findings demonstrate that*trans*-crocin 4, *trans*-crocetin, oleuropein, *Cichoriumspinosum*and *Sideritisscardica*H_2O extractsappear to be the safest, or even beneficial for neuronal cell survival at the in vitro level, and are now being assessed for their effects on the amyloid beta peptide, as well as, the hyper-phosphorylated tau aggregation pathways.

15. THE LOW DENSITY LIPOPROTEIN RECEPTOR MODULATES THE EFFECTS OF TESTOSTERONE ON WHITE ADIPOSE TISSUE METABOLIC ACTIVATION THROUGH ESTROGEN-INDEPENDENT MECHANISMS

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AIM: Epidemiological studies as well as studies in prostate cancer patients undergoing androgen deprivation therapy established that testosterone deficiency (TD) plays a causative role in the development of metabolic syndrome and cardiovascular disease. The aim of the study was the delineation of the role of estrogens in the functional cross-talk between LDL receptor (LDLR) and testosterone (T). **METHODS:** Mice deficient in LDLR (*Ldlr*$^{-/-}$) were used in the study. Male mice were castrated and female mice were ovariectomized. Indirect calorimetry studies were performed using the Calocage small animal colorimetry system (*TSE Systems*). Following surgery, metabolic and biochemical analyses were performed. **RESULTS**: Though sham operated male *Ldlr*$^{-/-}$ mice fed western-type diet for 12 weeks became obese, their castrated littermates were resistant to diet-induced obesity. A T replacement study confirmed that TD was the critical parameter in the resistance in diet-induced body weight gain of castrated *Ldlr*$^{-/-}$ mice. Sham operated *Ldlr*$^{-/-}$ mice treated with exemestane, a third generation irreversible aromatase inhibitor, showed a similar body weight gain to placebo-treated animals, providing a first indication that the resistance of castrated *Ldlr*$^{-/-}$ mice towards diet-induced obesity is directly due to TD and not to estrogen deficiency. Ovariectomized female *Ldlr*$^{-/-}$ mice (do not synthesize either estrogens or androgens) confirmed that the resistance of castrated *Ldlr*$^{-/-}$ mice towards diet-induced obesity is directly due to TD. Surprisingly, these experiments further dissected the metabolic effects of testosterone and estradiol showing that estradiol counteracts the functions of testosterone in the regulation of plasma triglyceride levels in mice lacking a functional LDLR. Indirect calorimetry analysis indicated that hypogonadism in male *Ldlr*$^{-/-}$ mice, was associated with increased metabolism while treatment of castrated *Ldlr*$^{-/-}$ mice with T reverted the observed phenotype. The elevated metabolic activity of castrated *Ldlr*$^{-/-}$ mice was confirmed by the markedly increased mitochondrial cytochrome c and UCP1 protein levels in white adipose tissue (WAT) of castrated male mice but not in brown adipose tissue (BAT), providing strong evidence of metabolic activation of WAT (WAT browning). **CONCLUSIONS**: Our data indicate that LDLR modulates the effects of testosterone on white adipose tissue metabolic activation through estrogen independent mechanisms.

16. THE ROLE OF RECEPTOR PROTEIN TYROSINE PHOSPHATASE BETA/ZETA AND $\alpha_v\beta_3$ INTEGRIN IN C6 CELL MIGRATION

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BACKGROUND: Receptor protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) binds various extracellular matrix and cell adhesion molecules, as well as soluble ligands such as pleiotrophin (PTN) and vascular endothelial growth factor A (VEGF). We have previously shown that in order for PTN to induce cell migration, presence of $\alpha_v\beta_3$ integrin, which forms a functional complex with RPTPβ/ζ, is required, while in cells that do not express $\alpha_v\beta_3$, PTN inhibits cell migration. In the present work, we studied the role of RPTPβ/ζ in the effect of PTN and VEGF on the migration of cells that do not express $\alpha_v\beta_3$, as well as whether $\alpha_v\beta_3$ affects the interaction of PTN or VEGF with RPTPβ/ζ.
METHODS: Rat glioma C6 cells that express α_v but not β_3 were used. Down-regulation of RPTPβ/ζ was performed by siRNA and over-expression of β_3 by a pCDNA3.1 vector containing full length β_3 cDNA. Cell migration was studied by using 24-well microchemotaxis chambers. The interaction of PTN or VEGF with RPTPβ/ζ was studied by a combination of immunoprecipitation/Western blot assays.
RESULTS: Down-regulation of RPTPβ/ζ in C6 cells reversed the inhibitory effect of PTN on cell migration and PTN caused a significant increase of C6 cell migration. VEGF stimulated C6 cell migration and its effect was independent of RPTPβ/ζ expression. Interestingly, over-expression of β_3 subunit in C6 cells resulted in decreased growth and cell migration compared with untrasfected or cells transfected with a plasmid control vector. Moreover, over-expression of β_3 decreased the interaction of both PTN and VEGF with RPTPβ/ζ.
CONCLUSION: Our data suggest that RPTPβ/ζ may have a significant role on migration of glioma cells that do not express $\alpha_v\beta_3$ and verify that $\alpha_v\beta_3$ is a key molecule in RPTPβ/ζ signaling.

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17. DETERMINATION OF A TRANSMEMBRANE AMINO ACID OF THE CRF1 RECEPTOR THAT IS CRITICAL FOR RECEPTOR'S ACTIVATION

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BACKGROUND & OBJECTIVES: The type 1 receptor (CRF_1R) for the corticotropin releasing factor (CRF) plays a key role in the response of our body to stressful stimuli and the maintenance of homeostasis.Malfunctioning of CRF/CRF_1R-neuronal circuits is closely associated with the appearance of anxiety and depression. The CRF_1R is a family B G-protein coupled receptor (GPCR), which contains seven transmembrane domains (TMs). This study aims to functionally and structurally characterize the third TM (TM3) of CRF_1R.
METHODS: To determine the role of TM3 residues in the function of CRF_1R we used a site-mutagenesis approach along with radioligand binding studies and signaling experiments.
RESULTS: Among the TM3 residues of CRF_1R, tested in this study Gly210 hasbeen shown to play an important functional role. In specific, we found that mutations of Gly210 that strengthened its interaction with TM5 residues stabilized the inactive state of CRF_1R, thus impeding receptor's activation.
CONCLUSIONS: The Gly210 in TM3 plays an important role in CRF_1R activation, by participating in functionally important inter-TM interactions. Similarly, receptor inactivation by the non-peptide CRF_1R-selective antagonists could be partly due to the reinforcement of the TM3-TM5 interface. Non-peptide antagonistshave been previously shown to interact with Asn283in TM5and the TM3 residues, Met206 and Phe203. These results are of high significance because they shed light on the molecular mechanisms underlying CRF_1R activation, thus further advancing receptor-based rational design of CRF_1Rselective non-peptide antagonists.Small non-peptide CRF_1R-selective antagonists have been shown to display antidepressant and anxiolytic properties.

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18. AN ASSOCIATION STUDY OF SERUM AMYLOID A WITH ESSENTIAL HYPERTENSION AND THE *TNF* -850C>T AND *ABCB1* 2677G>T/A, 3435C>T GENE POLYMORPHISMS

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BACKGROUND-OBJECTIVES: Serum Amyloid A (SAA) is marker of both acute and chronic inflammation, associated with obesity, metabolic syndrome, insulin resistance and diabetes mellitus. SAA functions as a lipoprotein, and possibly an adipokine, carried predominantly on high density lipoprotein (HDL). In this study we have examined the distribution of SAA among normotensive and newly-diagnosed hypertensive subjects as well as its association with common *TNF* and *ABCB1* polymorphisms.
METHODS: One hundred never-treated, hypertensive patients and 60 normotensive volunteers, of similar age and sex distribution, participated in this study. Genotyping was accomplished with standard PCR-RLFP methods. The association of the polymorphisms with SAA levels was tested with univariate and multivariate analyses, using a general linear model with type III sum of square statistics (ANCOVA). Age, sex, smoking and BMI were used as co-variates.
RESULTS: We have observed, 1) a statistically significant increase of SAA levels in hypertensives, compared to normotensives, 2) a statistically significant association of *TNF* -850C>T with SAA albeit not independent from BMI, and, 3) a statistically significant, BMI-independent, association of both *ABCB1* 2677G>T/A and 3435C>T with SAA in normotensives.
CONCLUSIONS: SAA's association with hypertension may reflect its correlation with BMI. The association with *TNF* -850C>T could be related to the regulation of SAA's expression by TNF, while the association with *ABCB1* 2677G>T/A, 3435C>T is in line with previous reports linking inflammation with P-glycoprotein expression.

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19. HYDROGEN SULFIDE REGULATES THE REDOX STATUS OF SOLUBLE GUANYLYL CYCLASE

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INTRODUCTION: Soluble guanylate cyclase (sGC) is a "receptor" for the endogenously produced gasotransmitter nitric oxide (NO). sGC exists as a heterodimer of an α and a β subunit, that carries a heme prosthetic group. Binding of NO to ferrous (Fe^{2+}) heme in the N-terminus of sGC β1 induces structural changes that are transmitted to the C-terminus of the protein increasing its catalytic activity and leading to the production of the second messenger cyclic guanosine-3',5'-monophosphate (cGMP) from guanosine 5`-triphosphate (GTP). About a decade ago, NO-independent activators and stimulators were discovered as promising agents for the treatment of cardiovascular and pulmonary diseases. These agents can activate sGC in a heme-dependent manner (sGC stimulators) or heme-independent manner (activators). Hydrogen sulfide (H_2S) is a new gasotransmitter with pleiotropic actions in mammalian cells. H_2S exerts both direct (ROS scavenging) and indirect (up-regulation of redox-sensitive genes and mechanisms) anti-oxidant actions.
PURPOSE: In the present study we aimed to determine whether H_2S could regulate sGC redox state and affect its responsiveness to NO-releasing agents and sGC activators.
MATERIALS AND METHODS: The ability of H_2S to alter responsiveness to a NO donor and BAY 58-2667 was tested using purified recombinant sGC, cultured rat aortic smooth muscle cells and pre-constricted aortic rings in vitro.
RESULTS: Supplementation of ferric (Fe^{3+}) recombinant sGC with NaHS led to heme reduction and to increased enzyme responsiveness to the NO donor sodium nitroprusside, while the same treatment caused a decrease in the responsiveness to BAY 58-2667. Using cultured cells, we observed that treatment with rotenone, that increases endogenous ROS production elevating ferric sGC, augmented cGMP accumulation in response to BAY58-2667; this effect was reversed by NaHS. In contrast, treatment of cells with rotenone reduced DEANO-induced cGMP accumulation in a NaHS-reversible manner.
In experiments with phenylephrine-constricted aortic rings, treatment with rotenone caused a rightward shift of DEANO concentration-response curve, an effect partially restored by incubation with NaHS. When rings were pre-treated with NaHS presumably leading to higher content of ferrous sGC heme, the concentration-response curve to BAY 58-2667 was shifted to the right.

CONCLUSION: These results suggest that H_2S can facilitate the reduction of sGC heme Fe from ferric to ferrous and maintain sGC in a NO-responsive state. This also limits the action of sGC to activators (BAY 58-2667). The described effect of H_2S on sGC provides an additional mechanism of cross-talk between the NO and H_2S pathways.

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