Topic 6 – Diabetes, lipids, metabolism – B

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0260

Imidazoline I1 receptor ligands activate hepatic adiponectin pathways and thus improve insulin sensitivity

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Metabolic syndrome is defined as a cluster of cardiovascular and metabolic disorders. Previous studies in rat models of metabolic syndrome have demonstrated that ligands selective for I1 imidazoline receptor (LNPs) increase insulin sensitivity through central sympathoinhibition and an additional peripheral effect attributable to adiponectin, a major insulin-sensitizer adipokine. The objective of this study was to explore possible direct actions on hepatocytes, one of the target cells of insulin and adiponectin.

Experiments were carried out in HepG2 cells, a cell line of hepatocytes. In order to evaluate the effect of LNPs on insulin sensitivity, the activation (i.e. phosphorylation) of a key actor of insulin pathways, AKT, was evaluated by measuring the ratio pAKT/AKT by Western Blot. Similarly, the effect of LNPs on adiponectin signaling was evaluated by measuring the rate of phosphorylation of the central kinase involved in adiponectin pathways, AMPK, by Western Blot. Insulin (10 μM) induced the phosphorylation of AKT (pAKT/AKT=0.50±0.05) compared to control without insulin (pAKT/AKT=0.11±0.03; p≤0.05) whereas LNPs (1μM) alone did not. Interestingly, pretreatment by LNPs (1 μM) during 60 min could potentiate the insulin-induced activation of AKT: LNP509: pAKT/AKT=1.50±0.06 (p≤0.05 vs insulin alone); LNP599: pAKT/AKT=0.41±0.16 (p=0.045 vs insulin alone).

Concerning adiponectin signaling pathways, LNPs alone (from 10⁻⁸ M to 10⁻⁴ M) increased AMPK phosphorylation in a concentration- and time-dependent manner. The maximal effect was obtained after 10 min exposure of LNPs (10 μM) to untreated cells: pAMPK/AMPK=0.18±0.04; LNP 509 pAMPK/AMPK=0.38±0.05 p≤0.05; LNP599 pAMPK/AMPK=0.46±0.17). These data suggest that LNPs on hepatic cells activate adiponectin pathways and potentiate insulin action. These two direct effects on insulin sensitive cells could account for the ameliorated insulin sensitivity observed in vivo.

0418

Sodium-glucose cotransporters (SGLT) in the heart. Contribution of SGLT-type of transport in hyperglycemia-induced signaling pathway in adult cardiomyocytes

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Background: Exposure to hyperglycemic conditions increases reactive oxygen species (ROS) production in adult cardiomyocytes, inducing glucotoxicity. This is due to an NADPH oxidase activation and more particularly the NOX2 isoform. Our group has demonstrated that hyperglycemia-induced toxic effect does not required glucose metabolism but results from glucose transport through a SGLT type of transport. SGLT acts as glucose sensor. Seven SGLT isoforms have been described (SGLT1 to 6 and SMT1) but their expression in the heart remains to be elucidated. The aim of this work is to study SGLT isoforms expression in the heart and identify the isoform responsible for glucotoxicity.

Methods: SGLT isoforms expression has been performed in heart extracts (from rats, mice and humans) and in isolated cardiomyocytes (from rats and mice) using PCR and westernblotting. The study of the contribution of each isoforms to glucotoxicity is based on the substrates specificity of all these SGLT isoforms.

Results: SGLT1, SGLT3 and SMT1 are expressed in the heart and in cardiomyocytes from rats and mice as well as in human heart. In human heart, SGLT3 expression is marginal. SGLT4 is only expressed in rat heart. In presence of 5 mM glucose, rat cardiomyocytes exposure to high concentration of galactose (16 mM, transported through SGLT1) does not activate NOX2. By contrast, myo-inositol (16 mM, transported through SMT1) completely reproduces hyperglycemic effects. Indeed, it favors p47phox translocation inducing NOX2 activation and stimulates ROS production. This ROS production is blocked by a NOX2 specific inhibitor (gp91dstat). Similar observation was performed in mice cardiomyocytes.

Conclusion: SGLT1 and SMT1 are expressed in rats, mice and human cardiomyocytes. Increased transport through SMT1 activates NOX2.

0347

Leucine, a potent inhibitor of cardiac glucose uptake

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Background: It was demonstrated that branched-chain amino acids like leucine induce insulin resistance in muscle and adipose tissues. The mechanism proposed to explain leucine action involves mTOR/p70S6K signaling. This pathway can be activated by leucine and is implicated in the stimulation of an insulin negative feedback loop. Knowing that insulin-resistance participates in diabetic cardiomyopathy, we were interested in studying leucine effect in cardiomyocytes.

Methods: Primary cultured adult rat cardiomyocytes were pretreated with different concentrations of leucine (from 1 to 10 mM) during different periods of time (up to 20h) before being exposed to insulin (3x10⁻⁹ M, 30 min).

Results: In absence of leucine, insulin induced a 6-fold increase in glucose uptake (0.31±0.04 vs. 0.05±0.01 μmoles/mg.h). This correlated with the increase in phosphorylation state of PKB and AS160, both known to regulate glucose transport downstream of insulin. Pre-incubation with leucine for 1 h stimulated mTOR/p70S6K pathway resulting in the inhibiting phosphorylation of IRS-1 located in the proximal insulin signaling pathway. This is accompanied by a significant decrease in PKB and AS160 phosphorylation but, surprisingly, insulin-stimulated glucose uptake was preserved (0.31±0.04 vs. 0.05 μmoles/mg.h). On the other hand, a longer incubation (14h) with leucine induced a drastic decrease in glucose transport (0.05±0.01 μmoles/mg.h). The mTOR/p70S6K inhibitor rapamycin did not prevent this inhibition. Moreover, the non-metabolized leucine analog BCh was able to stimulate mTOR/p70S6K pathway but had no effect on the insulin-mediated stimulation of glucose uptake. By contrast, intermediates of leucine catabolism, alpha-ketoisocaproate, acetoacetate and beta-hydroxybutyrate, inhibited glucose uptake similarly to leucine.

Conclusion: Leucine catabolism reduces insulin-dependent glucose transport independently of insulin signaling.

0447

Elevated plasma PCSK9 is equally detrimental for non-familial hypercholesterolemic (non-FH) and heterozygous FH patients, irrespective of their LDL receptor defects

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Objectives: Do elevated PCSK9 levels constitute an even greater risk for people who already have reduced LDL receptor (LDLR) levels, such as heterozygous familial hypercholesterolemic (HeFH) patients?

Methods: Circulating PCSK9 was measured by ELISA in non-treated HeFH patients carrying either a D209E (n=237), V48M (n=117), or D154N (n=38) LDLR missense mutation and in normolipidemic controls (n=152). Skin fibroblasts and lymphocytes were isolated from a subset of patients and grown in 0.5% serum and mevastatin with increasing amounts of recombinant PCSK9. LDLR abundance at the cell surface was determined by flow cytometry.

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Results: PCSK9 dose-dependently reduced LDLr expression in control and FH fibroblasts to similar extents, by up to 77±8% and 82±7%. Likewise, PCSK9 reduced LDLr abundance by 39±8% in non-FH and by 45±10% in HeFH fibroblasts, irrespective of their LDLr mutation status. We found positive correlations of the same magnitude between PCSK9 and LDLr-C in controls (b=0.22, p=0.0003), D206E (b=0.20, p=0.0002), V408M (b=0.24, p=0.0002), and D154N (b=0.25, p=0.0484) HeFH patients. The strengths of these associations were all similar.

Conclusion: Elevated PCSK9 levels are equally detrimental for HeFH and non-FH patients: a 100mg/mL increase in PCSK9 will lead to an increase in LDL-C of 0.20-0.25mmol/L, in controls and HeFH alike, irrespective of their LDLr mutation. This explains why non-FH and HeFH patients respond equally well to monoclonal antibodies targeting PCSK9.

0169

Paraoxonase 1 activity, in the fructose-fed rats, in the presence and in the absence of an antioxidant treatment with alpha-lipoic acid

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Introduction: Paraoxonase 1 (PON1) is an esterase synthesized by the liver and secreted into the plasma, where it is associated with high density lipoproteins (HDL). Its role is to protect LDL and HDL from oxidation, thus preventing atherosclerosis. A decreased level of plasma PON1 activities has been found in diabetes mellitus, cardiovascular disorders and chronic liver diseases; but, it can also be influenced by diet and lifestyle. The purpose of this study was to assess the PON1 activities in the insulin-resistant rats fed with a fructose-enriched diet, in the presence and in the absence of an antioxidant treatment with alpha-lipoic acid (AL).

Methods: 48 male Sprague-Dawley rats were randomized into two series: rats fed for 3 months with standard chow (Control) or with standard chow supplemented with fructose (60%). In each series, a group of rats was treated intraperitoneally during 14 days/month with NaCl 0.9% and another group with 50 mg/kg/day AL. At the end of the 3 months, we assessed: 1) peripheral tissue resistance to insulin (HOMA-IR) and plasma lipid profile, 2) paraoxonase, arylesterase and lactonase activities of PON1, 3) plasma homocysteine (Hcy) level and 4) hepatic transaminase activities: aspartate-aminotransferase and alanine-aminotransferase.

Results: The fructose intake increased peripheral tissue resistance to insulin (HOMA-IR) and plasma lipid profile, less the HDL. Also, transaminase and PON1 activities, asparatate-aminotransferase, alanine-aminotransferase, arylesterase and lactonase activities, and the plasma homocysteine (Hcy) level were significantly (p>0.05) enhanced in the fructose group. The AL discontinuous treatment associated with the fructose-enriched diet improved the tissue sensitivity to insulin and decreased the plasma lipid profile levels. Moreover, the AL treatment restored PON1 and transaminase activities, without influencing the Hcy concentration. A decrease in plasma transaminase activities was noted even when AL was associated with standard diet.

Conclusions: In our experimental conditions, the fructose intake induced an increase in plasma transaminase and PON1 activities in association with a Hyperhomocysteinemia. The AL treatment restored the enzymes’ activities and had a hepatoprotective effect, but without influence on Hcy level.

0261

Hao Ling pu-erh tea attenuates lipid accumulation in primary culture rat hepatocytes.

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Aim: Tea is one of the most consumed beverages in the world and its health-promoting effects have been widely investigated. Lipid-lowering effects of pu-erh tea have attracted growing interest. The importance of liver in lipid metabolism prompted us to investigate the lipid-lowering properties of pu-erh tea in rat primary culture hepatocytes. We tested the effect of a Hao Ling pu-erh tea extract (HLPT) and one of the major components of tea as Epigallocatechin-3-gallate (EGCG) which is largely recognized as a hypolipidemic molecule.

Methods: HLPT: an infusion of Hao Ling pu-erh tea was lyophilized and quantified for its composition in catechins by LC-MS. 24h after seeding on collagen, rat hepatocytes in primary culture were treated for 24h with various concentrations of HLPT (100, 200, 400, 600 μg/ml) and EGCG (30, 100 μM) and compared to CyclosporinA (hyperlipidemic reference) and Clofibrate (hypolipidemic drug used in human) (n=3 in triplicate). Lipid droplet accumulation was measured by LipidTox staining and evaluated by fluorescence microscopy on an ArrayScanXTI high Content Analysis Reader (Cellomics Inc.).

Results: We found that HLPT significantly prevented hepatocyte lipid accumulation (-56%) and in the same proportion to Clofibrate. EGCG also significantly attenuated lipid accumulation (-19%) but less than HLPT.

Conclusion: The main result of this study was to point out the major implication of liver cells in the hypolipidemic effects of HLPT. Moreover, we have shown here that this effect was partly due to the EGCG, well known for its antioxidant effects. However as we reported here that HLPT has a higher hypolipidemic effect than EGCG alone, which means that EGCG acts in synergy with other HLPT components such as theaflavins but this hypothesis has to be confirmed in further experiments. In future, the variation of expression of genes involved in lipid metabolism (THRHSP.LXRP.PPARα) by qPCR induced by HLPT will allow us to improve the understanding of the effect induced by HLPT.

0211

Prevention of cardiovascular, renal and metabolic abnormalities by soluble epoxide hydrolase inhibition in a murine model of type 2 diabetes

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Objective: Epoxycosatrienoic acids (EETs) are synthesized from arachidonic acid, notably in endothelial cells, and display attractive metabolic, vasodilatory and anti-inflammatory properties. We demonstrated previously that inhibiting EET degradation mediated by soluble epoxide hydrolase (sEH) reduces hypertension and heart failure, and others reported that it improves glucose homeostasis in type 2 diabetes. However, the impact of such strategy on target organ damage in diabetes remains to be clarified.

Materials and methods: The pharmacological sEH inhibitor r-AUCB (10 mg/l in drinking water) was administered for 8 weeks in mice subjected to a high fat diet (HFD; 60% fat) for 16 weeks. Mice on control chow diet (10% fat) served as controls.

Results: Glibenclamide and r-AUCB similarly prevented the increased fasting glycemia in HFD mice (Control: 5.4±0.2; HFD: 8.0±0.8; HFD+G: 5.1±0.3; HFD+r-AUCB: 5.6±0.2 mmol/L; p<0.05). However, only r-AUCB improved glucose tolerance and decreased gluconeogenesis. In parallel, r-AUCB prevented adipose tissue activation and dyslipidemia. Moreover, r-AUCB prevented coronary artery thrombosis and sEH reduced hypertension and heart failure, and others reported that it improves glucose homeostasis in type 2 diabetes. However, the impact of such strategy on target organ damage in diabetes remains to be clarified.

Discussion: These results demonstrate that beyond its glucose-lowering effects sEH inhibition improves coronary endothelial function, diabetic dysfunctions and prevents early kidney damage in a murine model of type 2 diabetes. This positive impact on target organ damage and metabolic homeostasis prompts sEH inhibition as a promising therapeutic perspective in type 2 diabetes.