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ABSTRACT

predictor of PWV in healthy subjects points to the relevance of HDL function in vascular physiology and arterial stiffness prevention along life.

DEVELOPMENT OF NEW RAC1 INHIBITORS AS POTENTIAL PHARMACOLOGICAL AGENTS FOR THE TREATMENT OF ATHEROSCLEROSIS

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Experimental and clinical observations have documented the role of the small GTPase Rac1 protein and cardiovascular diseases opening to the development of new potential pharmacological intervention. In this study we characterized a new class of selective small molecule Rac inhibitors with the 3-aryl-1H-pyrazole-5-carboxamide nucleus. Starting from our previous identification of Rac inhibitors (1), through a computational approach, 57 chemical entities were identified and their Rac inhibitory efficacy evaluated by G-LISA assay. 23 compounds were found to reduce, Rac-GTP levels in cultured cells by more than 24.8%. A comparative analysis at 25 µM was then performed and compounds 1, 2, 3, 11 and 21 resulted the most potent. Compounds 1, 2, 3 and 21 did not affect the activation of RhoA protein, while compound 11 partially reduced RhoA-GTP levels. The IC50s for Rac inhibition of these compounds were between 2.9 and 29.1 µM and similar potencies were observed in a cell adhesion assay, a Rac1-dependent cellular event. From these analysis it was selected compound 2 as a most promising inhibitor to test in in-vitro and in-vivo models of atherosclerosis. Compound 2 profoundly affected cytoskeleton organization of cultured human SMCs and inhibited SMC migration in response to PDGF-BB in a concentration dependent manner with an IC50 value of 5.8 µM. More interestingly, incubation of human monocytic cell line THP-1 with Compound 2 (10 µM) completely abrogated their adhesion to cultured human umbilical endothelial cells (HUVEC) indicating a potent anti-inflammatory activity.
Taken together, in the present study we identified a new selective small molecule Rac inhibitor capable to interfere with SMC migration and monocyte-endothelial cell adhesion, two pivotal features of atherogenesis. Further analysis will be carried out to test the effect of compound 2 on spontaneous atherosclerotic plaque development in apoE-/- mice.

Reference

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EVALUATION OF EFFICACY OF A NEW NUTRICEUTICAL PRODUCT CONTAINING PLANT STEROLS IN BAG (STEROLIP ESI) IN REDUCING CHOLESTEROL LEVELS IN PATIENTS WITH POLYGENIC HYPERCHOLESTEROLEMIA

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Primary objective was to establish how lipid profile is modified in patients affected by polygenic hypercholesterolemia after three and six weeks of therapy with Sterolip (a new nutriceutical product in bag containing 1.6 g of plant sterol) with respect to a placebo; secondary objectives were to verify the tolerability of the product, to test the long term efficacy after 12 weeks of treatment, to evaluate the response to the therapy in relation with three genetic variants involved in sterol absorption.
Methods. We enrolled 60 patients over 18 years of age, affected by polygenic hypercholesterolemia in primary prevention with cardiovascular risk <20% in the next ten years. 30 patients were double-blind randomized with placebo and 30 patients with Sterolip. The double blind treatment lasted six weeks at the end of which all patients were treated with Sterolip in open-label for other six weeks. Lipid profile was determined before treatment and every three weeks. We genotyped all patients for APOE, NPC1L1 c. 816C>G (L272L) and ABCG8 D19H polymorphisms.
Results. The parameters that in the treatment group were significantly reduced compared to the placebo group were total cholesterol (TC) and LDL Cholesterol (LDL-C) at three weeks of treatment ($p<0.05$). The product was good tolerated. Long term therapy is efficacious only in a few cases with a large interindividual variability. Carriers of G allele of NPC1L1 polimorphism have demonstrated a significant more consistent reduction of TC after three and six weeks of treatment in comparison to C allele carriers ($p<0.05$).
Conclusions. In according with data published in literature this study demonstrated a clinically significant action of plant sterols after three weeks of treatment. In genetically predisposed individuals, the therapy may be considered for a more time.

INCIDENCE AND SEVERITY OF NONALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH IMPAIRED LIPID AND GLUCOSE METABOLISM ARE PREDICTED BY SREBF POLYMORPHISM

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Background. Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease, encompasses a histological spectrum, ranging from simple steatosis (SS) to steatosis plus necroinflammation (nonalcoholic steatohepatitis, NASH), which can be differentiated only by liver biopsy. While SS has a benign hepatological course, NASH can progress to end-stage liver disease. Sterol regulatory element-binding factor (SREBF) genes code for key

nuclear transcription factors regulating lipid homeostasis: sterol regulatory element binding protein (SREBP)-1c regulates hepatic de novo lipogenesis and insulin sensitivity, while sterol regulatory element-binding factor-2 (SREBF-2) codes for SREBP-2, a master transcriptional regulator of genes involved in cellular cholesterol biosynthesis, uptake, and export. Therefore, SREBF-1c and SREBF-2 may modulate the genetic susceptibility to NAFLD and NASH.
Objective. Factors predisposing to non-alcoholic fatty liver disease (NAFLD) and associated cardio-metabolic disorders are unknown. Sterol regulatory element-binding protein (SREBP)-1c and SREBP-2, transcription factors regulating lipogenesis and cholesterol metabolism have been experimentally connected to NAFLD, but no human data exist. In population-based studies, single nucleotide polymorphisms (SNPs) in SREBF-1 gene have been connected to obesity, insulin resistance and T2DM, and the functional SNP rs133291 C/T in the SREBF2 gene has been linked to serum LDL-cholesterol, but there are no human data on the impact of these SNPs on the risk of developing NAFLD and associated metabolic abnormalities. We hypothesized SREBF-1c/2 SNPs may not only predispose not only to NAFLD/NASH, but also affect NAFLD-associated cardio-metabolic risk. We aimed at:
1) prospectively assessing the role of SREBF-1c/2 SNPs in promoting NAFLD development in apparently healthy subjects;
2) elucidating mechanisms connecting SREBF-1c/2 to liver injury, glucose and lipid homeostasis in established NAFLD.

Methods. We followed-up 165 non-obese nondiabetic participants in a population-based study, without NAFLD/metabolic syndrome, characterized for 2 common SREBF-1c and SREBF-2 polymorphisms, dietary habits, physical activity, adipokines, CRP, and endothelial dysfunction markers. NAFLD developers underwent an OGTT with Minimal Model analysis of glucose homeostasis parameters, and an oral fat tolerance test with measurement of plasma lipoproteins, adipocytes, and hepatocyte apoptosis marker cytokeratin-18 fragments.
Results. After 7 years, 29% subjects developed NAFLD and 5% developed diabetes. SREBF-1c and SREBF-2 predicted incident NAFLD; SREBF-2 predicted non-alcoholic steatohepatitis (NASH), diabetes, CRP and endothelial dysfunction markers. In NAFLD, while SREBF-1c predicted histological steatosis and hepatic insulin resistance, SREBF-2 predicted progressive liver histology, hepatic/muscle/adipose insulin resistance, pancreatic β-cell dysfunction, and an altered fat tolerance: higher postprandial lipemia, cholesterol enrichment of triglyceride-rich lipoproteins and ox-LDLs, HDL-C reduction, adipokine imbalance (lower adiponectin and higher resistin), and higher cytokeratin-18 fragments.
Conclusions. SREBF-2 predisposes to NASH and cardio-metabolic disorders by affecting glucose homeostasis and dietary fat tolerance. We showed polymorphisms in genes coding for nuclear transcription factor SREBP-2 predict incidence and severity of NAFLD and of associated abnormalities in glucose and lipid metabolism and in adipokine response to dietary fat. Our findings may have relevant implications: from a clinical standpoint, SREBF-1/2 SNPs may be used to select NAFLD patients at higher risk of developing progressive liver disease and cardio-metabolic complications for tight monitoring and experimental treatments. Furthermore, future research should verify whether a genetically-mediated maladaptive response to a chronic, daily, repetitive stress like fat ingestion may link chronic overfeeding to obesity and its complications and to assess whether SREBP-2 pathway modulation may prevent cholesterol lipotoxicity in different tissues more effectively than currently available strategies, which selectively target hepatic cholesterol synthesis or intestinal absorption.

SMALL DENSE LDL PROFILES IN FAMILIAL COMBINED HYPERLIPIDEMIA AND IN FAMILIAL HYPERCOLESTEROLEMIA

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Introduction. Differences between small dense LDL (sd-LDL) profiles were evaluated in 108 probands with Familial Combined Hyperlipidemia (FCHL), 117 probands with Familial Hypercolesterolemia (FH) and 146 normolipidemic, normotensive, normoglicaemic healthy subjects among those consecutively admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples.
Methods. LDL particle separation was performed by Lipoprint System: 7 LDL subfractions (LDL 1 to LDL 7) were obtained, mean LDL particle size and LDL score (% of sd-LDL) were calculated.
Results. LDL score was significantly ($p<0.001$) higher in FCHL patients as compared to FH patients (21.6±1.3 vs 15.3±1.0 mg/dL) and controls (3.3±5.0 mg/dL); mean LDL size was significantly lower ($p<0.001$) in FCHL patients than FH patients (262.8±0.5 vs 264. ±0.4 Å) and controls (271.1±2.8 mg/dL). In a subsequent analysis we compared the amount of cholesterol in each LDL subfractions of the two groups of patients and controls. LDL I and LDL II cholesterol were significantly ($p<0.001$) higher in FH patients as compared to FCHL patients (69.0±1.9 vs 44.4±1.9 mg/dL for LDL I) and controls (41.7±11.5 mg/dL); (69.5±2.0 vs 42.8±1.5 vs 19.1±8.6 mg/dL for LDL II) (FH vs FCHL vs controls).
Conclusions. These findings indicate that FCHL patients have LDL score significantly higher and mean LDL size significantly lower than FH patients and healthy subjects. Small, dense LDL particles have been associated with an increased risk of coronary artery disease. More studies are envisaged to investigate the specific contribution of LDL subfractions to cardiovascular risk in FH patient.

THE IMPROVED BIOCHEMICAL DIAGNOSTICS OF THE LIPID PROFILES IN THE FRAMEWORK OF REGIONAL NETWORK FOR INHERITED LIPID DISORDERS: FIRST REPORT

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Familial combined hyperlipidemia (FCHL) is a severe inherited hyperlipidemia with a high cardiovascular mortality. Affected individuals have elevated cholesterol or trygliceride concentrations or both. Such a lipid profile is frequently associated with an unfavourable decrease in high density lipoprotein concentration, an elevated apolipoprotein B and an increased prevalence of atherogenic, small, dense low-density lipoprotein (sd-LDL) subfractions. Family studies are necessary to establish the diagnosis of FCHL in each patient.
Since it is not always possible to get some biochemical data from