METHODS AND FINDINGS

IN EXPERIMENTAL AND CLINICAL PHARMACOLOGY

VOLUME 31 SUPPLEMENT A SEPTEMBER 2009

XXXI CONGRESS OF THE SPANISH SOCIETY OF PHARMACOLOGY

HEALTHCARE & SCIENCE

THOMSON REUTERS

P-025 FOOD DERIVED PEPTIDES WITH VASODILATOR EFFECT. STRUCTURE-ACTIVITY RELATIONSHIP

A.B. García-Redondo,[1] F.R. Roque,[1] M.S. Avendaño,[1] M.J. Alonso,[3] R. López-Fandiño,[2] M. Miguel,[1,2] M. Salaices[1]

[1]Depto. de Farmacología y Terapéutica, Universidad Autónoma de Madrid, Madrid, Spain; [2]Instituto de Fermentaciones Industriales, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [3]Depto. de Ciencias de la Salud III, Universidad Rey Juan Carlos, Alcorcón, Spain

Biologically active peptide fragments are formed during proteolysis of food proteins, and have been shown to possess multiple physiological properties, including properties related to cardiovascular health such as blood pressure lowering effect. Most of food-derived peptides with antihypertensive activity have been also characterized as in vitro angiotensin converting enzyme (ACE) inhibitory agents, but, only a few studies have shown in vivo ACE-inhibitory activity of these peptides. This suggests that other mechanisms of action could be implicated in their antihypertensive effect. The aim of this study was to analyze, in resistance arteries, the vasodilator activity of several peptide sequences obtained from food protein hydrolysates and to establish whether there is a relationship between the aminoacids present in peptide sequences and the vasodilator effect. For this, third order branch of the mesenteric artery from 6 months old male Wistar Kyoto rats were used. The vasodilator response of arterial segments with or without endothelium to several peptides (0.1 mM) was analyzed in an isometric myograph. Moreover, the effect of NO synthase (L-NAME, 100 microM), and ciclooxygenase (indomethacin, 10 microM) inhibitors on the vasodilator response was tested. Peptides RADHPFL, RADHPF, RADHP, YRGGLEPINF, RDILNQ and VPP showed an endothelium-dependent vasorelaxation, whereas the vasodilator effect of FRADHPFL was only partially dependent of endothelium. The maximum relaxation (~75%), belongs to YRGGLEPINF peptide. In addition, the relaxation induced by the peptides RADHPFL, RADHPF, RADHP, RDILNQ and VPP is mainly mediated by NO, since the response was inhibited only by L-NAME, while both L-NAME and indomethacin inhibited the vasodilator response induced by FRADHPFL and YRGGLEPINF. It seems that the presence of arginine or tyrosine in the N-terminal extreme could be related with the vasodilator activity of these compounds. In conclusion, these results suggest that endothelium-dependent relaxation could be also a mechanism involved in the antihypertensive effect of food derived peptides.

Supported by RD06/0014/0011, SAF 2006-02376 and FMM.

P-026 8-EPIGROSHEIMIN, FROM *CREPIS DIOSCORIDIS*, REDUCES INOS AND COX-2 EXPRESSION IN RAW 264.7 MACROPHAGES VIA INHIBITION OF NF-KB

R.M. Giner,[1] M. Tsoukalas,[1,2] E. Skaltsa,[2] M.C. Recio,[1] J.L. Ríos[1]

[1]Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Spain; [2]Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Greece

Introduction. Sesquiterpene lactones are a large group of active constituents found in medicinal plants from the Asteraceae family. They are considered to be important chemotaxonomic markers and particularly effective inhibitors of nuclear factor-kB (NF-kB).(1)

Objective. The aim of this work is to isolate and identify a sesquiterpene lactone from *Crepis dioscoridis* (Asteraceae) and to evaluate its ability to inhibit the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) along with NF-kB activation in RAW 264.7 murine macrophages stimulated with lipopolysaccharide (LPS).

Methodology. The sesquiterpenoid lactone has been isolated by applying different chromatographic techniques. Its effect on NO production in culture supernatant has been spectrophotometrically evaluated through measurement of nitrite. The iNOS and COX-2 expressions have been determined with the aid of Western blot analysis; an electrophoretic mobility shift assay (EMSA) was performed to detect the nuclear translocation of NF-kB. **Results.** A costus lactone-type guaianolide has been isolated from the cyclohexane/diethyl ether/methanol (1:1:1) extract of the aerial parts of *C. dioscoridis*. It has been characterized with the aid of 1D & 2D NMR spectroscopy and identified as 8-epigrosheimin, the occurrence of which has been previously reported in two other *Crepis* species, *C. capillaris* and *C. mollis*.(2) After confirming the absence of any cytotoxicity for the lactone (up to 25 µM) by means of the MTT assay, the exposure to LPS (1 µg/ml) of macrophages pretreated with 8-epigrosheimin (1-25 µM) resulted in a reduction of the expression of COX-2 and iNOS in a concentration dependent manner. The inhibitory effect on iNOS was accompanied by a decrease in nitrite accumulation in the culture medium of the treated cells (IC_{50} = 6.6 µM, r^2 = 0.9674). The lactone also reduced the DNA binding of NF-kB in nuclear extracts of the LPS-stimulated macrophages. **Conclusions.** These results indicate that 8-epigrosheimin is able to reduce iNOS and COX-2 expression in part through inhibition of the NF-kB transcriptional activity.

Suported by GVPRE/2008/155 (Generalitat Valenciana) and SAF2006-06726 (Spanish Government through the MIC and FEDER).

1. Siedle B, García-Piñeres AJ, Murillo R et al. Quantitative structure-activity relationship of sesquiterpene lactones as inhibitors of the transcription factor NF-kB. *J Med Chem* 2004; 47: 6042-54. / 2. Zidorn C. Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Phytochemistry* 2008; 69: 2270-96.

P-120 ACTIVATION OF p38 AND ERK1/2 MAPK BY SUPEROXIDE ANION PARTICIPATES IN ANGIOTENSIN II-INDUCED COX-2 EXPRESSION IN SMOOTH MUSCLE CELLS FROM RESISTANCE ARTERIES

R. Hernanz,[1] A. Beltran,[2] J.V. Pérez-Girón,[1] A. Martín,[1] A. Briones,[2] R. Palacios,[1] M. Salaices,[2] M.J. Alonso[1]

[1]Depto. Ciencias de la Salud III, Universidad Rey Juan Carlos, Alcorcón, Spain; [2]Depto. Farmacología, Universidad Autónoma de Madrid, Madrid, Spain

Introduction: Angiotensin II (Ang II) regulates vascular smooth muscle cell (VSMC) function by activating signalling cascades that promote vasoconstriction, growth and inflammation. The mechanisms implicated in Ang II-induced pro-inflammatory actions include activation of several mitogen-activated protein kinases (MAPKs), reactive oxygen species generation and the modulation of prostaglandins production by regulating cyclooxygenase-2 (COX-2) expression.

Aim: To investigate the effect of Ang II on COX-2 expression in VSMC derived from small resistance arteries and the mechanisms involved.

Methods: VSMC derived from rat mesenteric resistance arteries were used. Protein expression was determined by Western Blot, mRNA levels by Q-RT-PCR and superoxide anion (O_2-) production by dihydroethidine fluorescence.

Results: Ang II (0.1 μM) time-dependently increased COX-2 protein expression (30 min - 8 h) and mRNA levels (15 min - 4 h), while COX-1 expression remained unmodified. In addition, Ang II did not modify AT_1 receptor expression. The AT_1 antagonist losartan (10 μM), but not the AT_2 antagonist PD 122319 (10 μM), abolished the increase in COX-2 expression induced by Ang II (2 h). COX-2 expression was also reduced by the respective NADPHox and xanthine oxidase inhibitors, apocynin (30 mM) and allopurinol (10 mM). Furthermore, Ang II (3-30 min) increased O_2- production; this effect was reduced by losartan, allopurinol and apocynin but not by PD 123319. Ang II (2-30 min) induced the phosphorylation of p38 and ERK1/2 MAPK; this effect was reduced by losartan, allopurinol and apocynin. In addition, the respective inhibitors of p38 and ERK1/2, SB 203580 (10 mM) and PD 98059 (25 mM), reduced the Ang II-induced COX-2 expression.

Conclusions: The present results provide evidences that angiotensin II increases COX-2 expression in VSMC from resistance arteries, at least in part, through mechanisms that include O_2- production and the subsequent activation of p38 and ERK1/2 MAPK. Supported by Red RECAVA (RD06/0014/0011), DGICYT (SAF2006-02376) and Fundación Mutua Madrileña.

P-121 SIRT1 INHIBITION INDUCES AORTIC ENDOTHELIAL DYSFUNCTION

R. López-Sepúlveda, R. Jiménez, M. Sánchez, M. Romero, M. Gómez-Guzmán, P. Galindo, M.J. Zarzuelo, A. Quintela, J. Duarte

Departamento de Farmacología, Facultad de Farmacia, Universidad de Granada, Granada, Spain

Introduction: Sirtuins are a family of redox-sensitive NAD+-dependent deacetylases. It has been previously described that mammalian homolog SIRT1 deacetylates eNOS and thus increases eNOS catalytic activity. Hypothesizing that the beneficial effects of Resveratrol (one of the main polyphenols in red wine) on vascular function is mediated, in part, by its role as a SIRT1 activator, we investigated if the inhibition of SIRT1 turns into endothelial-dysfunction and so if Resveratrol is able to prevent that.

Material and methods: Experiments were conducted in aortic rings from male Wistar rats. Rings were incubated in Krebs solution for 5 h in a cell culture incubator in the absence or presence of Nicotinamide (NAM) (1 mM) or Sirtinol (30μM), both of them SIRT1 inhibitors, and in the presence of Resveratrol (0.1 mM). After equilibration, arteries were stimulated with phenylephrine (1 μM) and a concentration-response curve was constructed by cumulative addition of acetylcholine (ACh). In the case of NAM after stimulation with phenylephrine (1 μM) a concentration-response curve was also constructed by cumulative addition of calcium ionophore (A23187). The protocol using ACh was repeated adding SOD (100 U/ml) to the organ chamber 30 min before the addition of phenylephrine.

Results: Incubation of the aortic rings for 5 h in the absence of NAM or Sirtinol produced no significant changes in the contractile response to phenylephrine or in the relaxant response to Ach or A23187. Incubating NAM or Sirtinol for 5 h led to the development of endothelial dysfunction as indicated by the reduction in the maximal relaxant effect of Ach or A23187, while coincubation with Resveratrol (0,1 mM) prevented endothelial dysfunction induced by NAM or Sirtinol. The impaired relaxant response to ACh induced by NAM was completely restored by SOD and partially restored in the case of Sirtinol, showing the implication of reactive oxygen species, like superoxide anion (O2-).

Conclusion: Taken together these results indicate that SIRT1 inhibition induced endothelial dysfunction, which was associated to increased O2- production and was prevented by Resveratrol.