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**FOOD DERIVED PEPTIDES WITH VASODILATOR EFFECT. STRUCTURE-ACTIVITY RELATIONSHIP**

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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 amino acids 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 μM), and cyclooxygenase (indomethacin, 10 μM) 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.

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

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**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).

**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*.

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.

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