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Preliminary report

Corilagin is a potent inhibitor of NF-kappaB activity and downregulates TNF-alpha induced expression of IL-8 gene in cystic fibrosis IB3-1 cells

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ABSTRACT

Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose), a gallotannin identified in several plants, including *Phyllanthus urinaria*, has been shown to exhibit versatile medicinal activities. As far as possible anti-inflammatory effects of corilagin, only few reports are available, and the potential use of corilagin as possible therapeutic molecule for cystic fibrosis has not been evaluated. In the present paper we report experiments aimed at determining the activity of corilagin on nuclear factor kappaB (NF-kappaB) binding to DNA target and on the expression of the major pro-inflammatory gene involved in cystic fibrosis, interleukin-8 (IL-8). Both IL-8 mRNA content and IL-8 protein secretion were analyzed in cystic fibrosis bronchial IB3-1 cells stimulated by tumor necrosis factor-alpha (TNF-alpha), one of the most potent pro-inflammatory agents. The data obtained demonstrate that corilagin binds to NF-kappaB, inhibits NF-kappaB/DNA interactions and affects IL-8 gene expression in TNF-alpha treated IB3-1 cells. In addition, corilagin inhibits TNF-alpha induced secretion of MCP-1 and RANTES, exhibiting low or no effect on the release of G-CSF, IL-6 and VEGF. Therefore, corilagin might be of interest for experimental anti-inflammatory therapy of cystic fibrosis.

1. Introduction

The analysis of the effects of extracts from medicinal plants is one of the most interesting approaches for the identification of bioactive compounds to be used in the treatment of human pathologies [1,2]. In this respect *Phyllanthus urinaria* has been the object of several investigations [3–5]. One of the most relevant effects of *P. urinaria* is a modulation of inflammatory processes [4]. Lai et al. reported that *P. urinaria* extracts inhibit *Helicobacter pylori*-induced inflammation, by interfering with *H. pylori*-induced nuclear factor-kappaB (NF-kappaB) activation and subsequent release of interleukin-8 (IL-8) [4]. This effect might be of interest in the field of the development of anti-inflammatory agents in cystic fibrosis (CF) [6–11].

CF is a severe genetic disease due to defects of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene [12–15]. CF affects several organs with the chronic pulmonary disease being the major cause of reduction of the quality and expectancy of life [12]. Hallmark of CF lung disease is in most cases the chronic infection sustained by the gram negative bacterium *Pseudomonas aeruginosa* and the excessive lung inflammation with a huge infiltrate of neutrophils in the bronchial lumen, mainly due to the release of the chemokine interleukin (IL)-8 [7,8,16–18]. Accordingly, the identification of novel drugs able to reduce the excessive lung inflammation in CF is considered one of the key therapeutic targets to circumvent progressive lung tissue deterioration [19–21].

Starting from crude methanolic extracts of the aerial parts of *P. urinaria* and employing a sequence of three conventional steps including liquid–liquid distribution, gel permeation over Sephadex LH-20 and repeated reversed-phase HPLC chromatography, a very high yield of corilagin was obtained [22,23]. Corilagin (beta-1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose) (Fig. 1A) is a gallotannin demonstrated to exhibit versatile medicinal activities, for example hepatoprotective effects on Male Sprague–Dawley rats induced with galactosamine and

Abbreviations: CF, cystic fibrosis; TFD, transcription factor decoy; EMSA, electrophoretic mobility shift assay; TNF-alpha, tumor necrosis factor alpha; NF-kappaB, nuclear factor kappaB; IL-8, interleukin-8; IL-6, interleukin-6; G-CSF, granulocyte colony-stimulating factor; MCP-1, monocyte chemotactic protein-1; RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted; VEGF, vascular endothelial growth factor; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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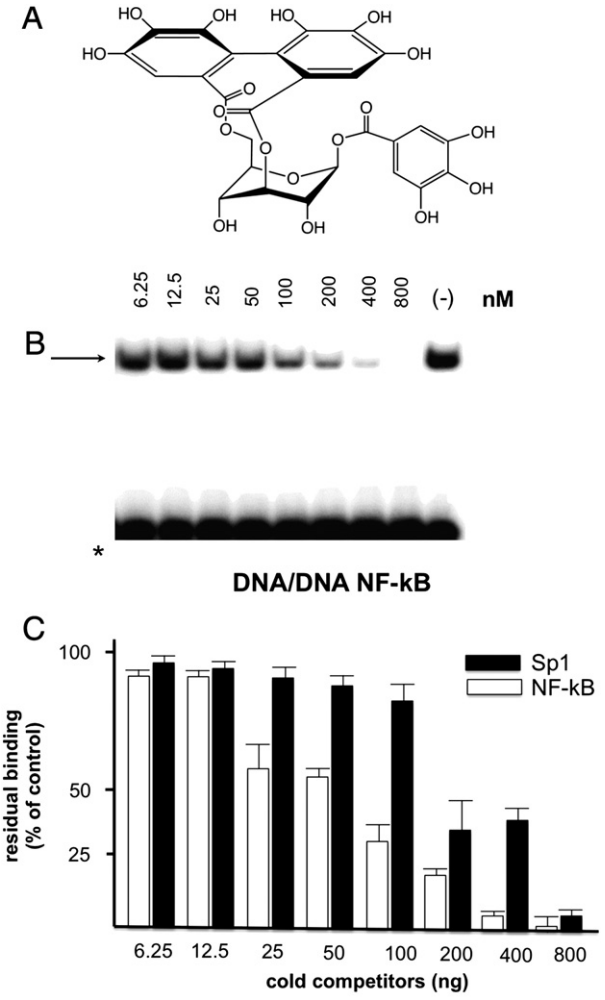

Fig. 1. A. Structure of corilagin. B. Effects of corilagin on NF-kappaB/DNA interactions. Purified p50 NF-kappaB was incubated for 20 min with the indicated concentrations of corilagin and then ^{32}P-labeled DNA/DNA, carrying the target sites for the transcription factors NF-kappaB, was added. After 40 min incubation, protein/DNA complexes were separated by polyacrylamide gel electrophoresis, and autoradiography was performed. Arrow indicates complexes between proteins and target molecules, asterisk indicates the free ^{32}P-labeled probe. C. Comparison of the effects of corilagin on NF-kappaB/DNA (white boxes) and Sp1/DNA (black boxes) interactions. Purified p50 NF-kappaB and Sp1 were incubated for 20 min with the indicated concentrations of corilagin and then for 40 min with ^{32}P-labeled DNA/DNA, carrying the target sites for the transcription factors NF-kappaB (white boxes) and Sp1/DNA (black boxes). Samples were then analyzed as described in (B).

lipopolysaccharide [1]. Recently, it was also reported that corilagin inhibits radiation-induced microglia activation through suppression of the NF-kappaB pathway, suggesting that this compound is a potential agent for the treatment of radiation-induced brain injury [24].

The possible effects on NF-kappaB are relevant in the context of the search of novel compounds for CF therapy, since we have previously reported that NF-kappaB inhibitors, including transcription factor decoy molecules (TFD) targeting NF-kappaB, are potent inhibitors of the expression of the IL-8 gene and of the release of IL-8 in cystic fibrosis cells infected with *P. aeruginosa* [6,25]. In addition to DNA-based NF-kappaB inhibitors, we have reported natural products as sources for the discovery of new drugs on the basis of their ability to interfere with NF-kappaB activity [2,26,27]. For instance, we found that the whole extract of *Aegle marmelos* exhibits strong inhibitory effect on the *P. aeruginosa*-dependent IL-8 induction in human CF-derived bronchial IB3-1 cells without affecting cell proliferation [27]. By separating the major components contained in *A. marmelos* extracts by gas chromatography and identifying their structures by mass spectrometry, we found that three components, namely 5,6-dimethoxy-1-indanone, 2-hydroxy-cinnamic acid and 5-methoxy psoralen (5-MOP), reproduce the inhibitory effects observed with the whole extract from *A. marmelos* [27].

Despite the fact that a relationship between NF-kappaB activity, IL-8 expression and inflammation in CF has been firmly established and possible anti-inflammatory effects of corilagin have been presented, only few reports are available on the effects of this compound on pathologies caused by inflammation. A preliminary exploration of anti-inflammatory mechanisms of corilagin has been reported using lipopolysaccharide activated murine macrophage cell line RAW 264.7 cells [23]. Although versatile medicinal use of corilagin has been demonstrated, the possible use of corilagin as a potential therapeutic molecule for cystic fibrosis has not been evaluated.

In the present paper we report experiments aimed at determining the activity of corilagin on NF-kappaB binding to target DNA and on the expression of the major pro-inflammatory genes activated in cystic fibrosis bronchial IB3-1 cells stimulated with TNF-alpha. We have elsewhere reported that TNF-alpha is in this cell line a potent inducer of pro-inflammatory genes, including IL-8 and IL-6 [28–30].

2. Materials and methods

2.1. Chemicals and reagents

Corilagin was obtained by the China National Institute for the Control of Pharmaceutical and Biological Products. All the other chemicals were purchased from Sigma-Aldrich.

2.2. High performance liquid chromatography analysis

The purity of corilagin was investigated by HPLC. Agilent 1100 series HPLC and Symmetry C18 (5 μm, 4.6 mm × 250 mm) column were used and detected with DAD detection using wavelength of 270 nm. Mobile phase consisted of (A) 0.1% acetonitrile and (B) trifluoroacetic acid. Column temperature was kept at 30 °C. Flow rate was adjusted to 0.8 ml per minute and injection volume was 10 μl. The final purity of corilagin was calculated from two concentrations of corilagin (25.93 mg/l and 259.3 mg/l).

2.3. Cell cultures

IB3-1 cells [31], derived from a CF patient with a DF508/W1282X mutant genotype and immortalized with adeno12/SV40, were grown in LHC-8 basal medium, supplemented with 5% FBS, at 37 °C/5% CO_2. The effects of corilagin on cell growth were analyzed as elsewhere described [32,33].

2.4. MTT assay

The MTT assay was carried out to estimate the viability of IB3-1 cells after incubation with corilagin (up to 2 mM final concentration) following a method described by Mosmann et al. [34]. Cells were plated onto a 96-well plate at a density of 6×10^4 cells/well in 100 μl medium, and were treated with a series of corilagin concentrations for 24 h. After 24 h, 20 μl of MTT solution (5 mg/ml) was added to each well and the plate was incubated for 4 h at 37 °C. Then the medium was carefully removed, 200 μl of DMSO was added to each well and the plate was shaken at room temperature for 15 min. Finally the plate was read at a wavelength of 570 nm. Cell viability was considered to be 100% in control untreated IB3-1 cells.

2.5. Computational studies

The 3D structures of the complexes NF-kappaB-DNA were retrieved from the Protein Data Bank (PDB codes: 1NFK and 1LE9)