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Phloridzin derivatives inhibiting pro-inflammatory cytokine expression in human cystic fibrosis IB3-1 cells

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ABSTRACT

Cystic Fibrosis (CF) is the most diffuse autosomal recessive genetic disease affecting Caucasians. A persistent recruitment of neutrophils in the bronchi of CF patients contributes to exacerbate the airway tissue damage, suggesting that modulation of chemokine expression may be an important target for the patient's well being thus the identification of innovative anti-inflammatory drugs is considered a long-term goal to prevent progressive tissue deterioration. Phloridzin, isolated from *Malus domestica* by a selective molecular imprinting extraction, and its structural analogues, Phloridzin heptapropionate (F1) and Phloridzin tetrapropionate (F2), were initially investigated because of their ability to reduce IL-6 and IL-8 expression in human CF bronchial epithelial cells (IB3-1) stimulated with TNF-α. Release of these cytokines by CF cells was shown to be controlled by the Transcription Factor (TF) NF-kB.

The results of the present investigation show that of all the derivatives tested, Phloridzin tetrapropionate (F2) is the most interesting and has greatest potential as it demonstrates inhibitory effects on the expression and production of different cytokines involved in CF inflammation processes, including RANTES, VEGF, GM-CSF, IL-12, G-CSF, MIP-1b, IL-17, IL-10 and IP-10, without any correlated anti-proliferative and pro-apoptotic effects.

1. Introduction

Polyphenols are among the most abundant antioxidants present in the human diet and are primarily found in plant-derived products including fruits, vegetables, beverages, herbs and spices. In addition to their antioxidant activity, polyphenols display anti-inflammatory properties (Yoon and Beak, 2005; Gonzalez-Gallego et al., 2010) and thus may be exploited for the development of complementary approaches to be used in conjunction with conventional therapeutic protocols (i.e. non-steroidal anti-inflammatory drugs), for the management of inflammatory diseases. Accordingly, it is important to better define the molecular mechanisms and bio-molecular targets of polyphenols during the inflammatory process, which is regulated by cytokines as well as by a number of other classical mediators of inflammation.

Several investigators have reported that plant derivatives, including phenolic compounds and flavonoids, exhibit anti-inflammatory activity by modulating the expression levels of a number of cytokines including IL-1, IL-6, IL-8, IL-10, TNF-α and a variety of enzymes (i.e. inducible nitric oxide synthase and cyclooxygenase). Moreover, many plant-derived extracts and chemicals exhibit pharmacologic effects and clinical benefits. For instance, Phloridzin (also called Phlorizin), phloretin-2-β-D-glucose (Fig. 1), belongs to the chalcone class of flavonoids and is widely present in the bark, leaves and fruit of apple trees (Ehrenkranz et al., 2005). Several lines of evidence suggest that apples and apple products, such as apple juice and extracts, possess a wide range of biological activities, which may contribute to beneficial health effects against cardiovascular diseases, asthma and pulmonary dysfunction, diabetes, obesity, cancer and inflammation (Boyer and Liu, 2004). In this context, apple extracts and their constituents have been demonstrated to influence multiple mechanisms relevant for cancer prevention in *in vitro* studies. These include anti-mutagenic activity, modulation of carcinogen metabolism, antioxidant activity, anti-inflammatory mechanisms, modulation of signal transduction pathways, anti-proliferative and apoptosis-inducing activity, as well as novel mechanisms which are the basis of epigenetic events and innate immunity (Gerhäuser, 2008). In addition, some polyphenols are known to

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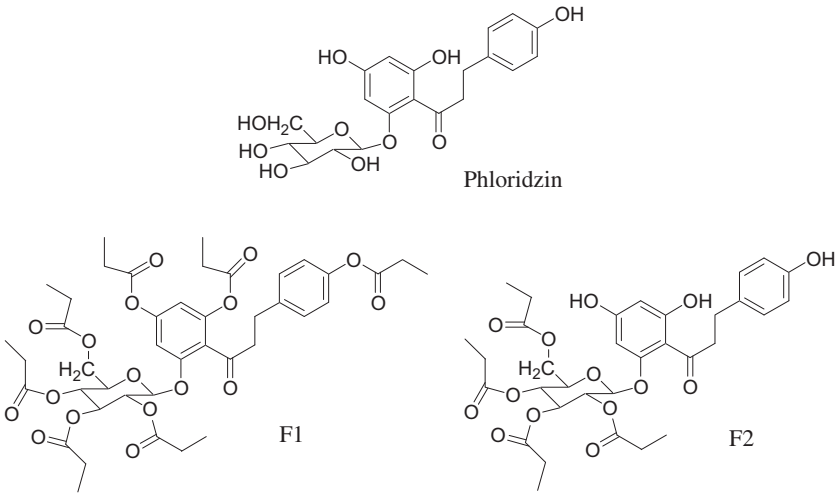

Fig. 1. Phloridzin and its semi-synthetic derivatives F1 and F2.

possess anti-inflammatory activity *in vitro* and *in vivo*, which have been attributed to the modification of signal transduction pathways (Santangelo et al., 2007). Polyphenols are capable of acting on the cytokine system by affecting the balance between pro- and anti-inflammatory cytokine expression (Lyu and Park, 2005). Quercetin and some catechins, for instance, inhibit TNF-α and IL-1β expression due to the enhancement of the release of IL-10 (Visioli and Galli, 1998).

Phloridzin has many biological functions, including antioxidase activity, regulation of glucose transporters, and the ability to induce apoptosis in tumor cell lines (Gonzalez-Gallego et al., 2010; Duge de Bernonville et al., 2010; Vasantha Rupasinghe and Yasmin, 2010). In addition, treatment with dihydrochalcone aglycone phloretin can inhibit the expression of IL-8, CXCL10, and TNF-α mRNAs in LPS-stimulated human acute monocytic leukemia-derived cell line (MonoMac 6) (Jung et al., 2009). One of the most important anti-inflammatory activities of phenolic compounds is their ability to modulate the activation of Nuclear Factor-kB (NF-kB). Because of this and since the anti-inflammatory effects of Phloridzin have not been studied in depth, we thought it would be important to investigate in greater detail the mechanism regulating the anti-inflammatory properties of Phloridzin in normal conditions as well in a defined pathogenic setting such as in Cystic Fibrosis (CF) (Bezzerri et al., 2008; DiMango et al., 1998; Joseph et al., 2005; Saadane et al., 2007; Sadikot et al., 2006; Tchilibon et al., 2005). CF is a severe genetic disease due to mutations in the CF Transmembrane Conductance Regulator (CFTR) gene and affecting several organs with chronic pulmonary disease being the major cause of reduction of the quality and expectancy of life (Boucher, 2004). It is well established that an important hallmark of CF lung disease is the chronic infection sustained by the gram negative bacterium *Pseudomonas aeruginosa* and the excessive lung inflammation characterized by a huge inflammatory infiltrate of neutrophils in the bronchial lumen, mainly due to the release of the chemokine interleukin (IL)-8 (Nicolis et al., 2009; Bonfield et al., 1995; Khan et al., 1995; Puchelle et al., 2001; Belcher and Vij, 2010). Accordingly, the identification of novel drugs able to reduce the excessive lung inflammation is considered one of the key therapeutic targets to circumvent progressive lung tissue deterioration (Ross et al., 2009; Zemanick et al., 2010; Fuchs and Milbradt, 1993).

The development of modern therapies that try to counteract the inflammation in CF patients is aimed at finding new potential anti-inflammatory drugs with different modes of action that may replace the use of several drugs, such corticosteroids that posses, in addition to great benefits, many important side effects. It should be underlined that the Transcriptional Factor (TF) NF-kB plays a crucial role in regulating the inflammatory processes affecting patients with CF. Accordingly, several research groups have focused their research activity in finding novel active principles capable of inhibiting the biological activity of NF-kB (Seeram et al., 2001; Sasaki et al., 2007; Lampronti et al., 2013) and showing inhibitory activity on IL-6 and IL-8 transcription in bronchial epithelial cells exposed to *P. aeruginosa* or TNF-α (Tumor Necrosis Factor-alpha). In order to identify novel molecules, we have focused our studies on natural plants extracts as a source of potential regulators of proinflammatory genes (Vertuani et al., 2011). In particular, we have made great efforts aimed at designing semisynthetic analogues of natural phenylpropanoids with increased biological activity and stability (Baldisserotto et al., 2012, 2015).

In this study we focused our interest on the natural molecule Phloridzin and its semisynthetic derivatives F1 and F2 (Fig. 1) previously synthesized by our group (Baldisserotto et al., 2012) in order to verify potential anti-inflammatory properties of interest in the development of therapeutic protocols for CF.

2. Material and methods

2.1. Extraction of Phloridzin from Malus domestica: analysis of major components

Phloridzin was extracted from fruit extracts of *Malus domestica* using the molecular imprinting technique described previously (Lampronti et al., 2013). The extracts were analyzed using high performance liquid chromatography (HPLC) followed by mass spectrometry (MS), in order to recognize and quantify the presence of active principle(s) (Fig. 1).

The molecular weights of the compounds were determined by ESI (Micromass ZMD 2000) and the values expressed as $[MH]^+$. ^1H NMR spectroscopy was obtained using a Bruker AC-200, a Varian VXR-200 or a Mercury Plus 400 spectrometer.

2.2. HPLC analysis

HPLC analysis was performed using an Agilent 1100 Series HPLC System equipped with a G1315A DAD and with a Hydro RP18 Sinergi 80A column (4.6 × 150 mm, 4 μm) from Phenomenex. The mobile phase consisted of water (0.01 M H_3PO_4) (solvent A) and acetonitrile (0.01 M H_3PO_4) (solvent B). The determination