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Review

Ixeris dentata-induced regulation of amylase synthesis and secretion in glucose-treated human salivary gland cells

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ABSTRACT

The endoplasmic reticulum (ER) is an organelle which controls synthesis of secretory and membrane proteins. Alterations in protein folding capacity, leading to ER stress, can be observed in patients with diabetes and related diseases such as xerostomia. The objectives of this study were to investigate the effects of *Ixeris dentata* (IXD) extract, which has been used for diabetes treatment, and compounds purified from IXD, 8-epidesacylcynaropicrin-3-O-beta-glucopyranoside (ID-57D), on amylase synthesis and secretion in human salivary gland (HSG) cells exposed to a high concentration of glucose. A high concentration of glucose in the experimental medium of cultured cells can model diabetes *in vitro*. IXD extracts and ID-57D increased oxidative folding-associated protein expression, including p-IRE-1α, PDI and ERO-1α, with the enhanced oxidative folding pattern seen in HSG cells transiently exposed to a high concentration of glucose. Moreover, the treatments reduced the ER stress response, such as the expression of GRP78, maintaining amylase synthesis and secretion in chronically glucose-exposed HSG cells. This study suggests the potential therapeutic value of IXD extract for the treatment of diabetes or its complications such as xerostomia.

Contents

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1. Introduction

Salivary α-amylase, one of the most important enzymes in saliva (Kandra and Gyemant, 2000), consists of two families of isoenzymes, of which one is glycosylated while the other contains no carbohydrate. Salivary α-amylase accounts for 40–50% of total salivary gland-produced proteins. Among the enzymatic proteins in saliva, amylase is synthesized by and secreted from salivary gland cells through an intra-endoplasmic reticulum protein-folding processes (Astor et al., 1999).

The endoplasmic reticulum (ER) is an organelle that controls protein folding and secretion (Chevet et al., 2001). Furthermore, it plays an important role in the biosynthesis of secretory proteins, including amylase (Malo et al., 2013). Any imbalance between the physiological load of secretory protein translocation into the ER and its folding capacity can lead to ER stress (Kaufman, 2002; Mori, 2000). Deprivation of essential nutrients or energy sources, or exposure to toxins may also disturb normal protein folding and secretion (Yu et al., 2007).

The accumulation of incorrectly-folded proteins triggers an ER stress response that has at least two distinct components. The first, known as the unfolded-protein response (UPR), consists of the transcriptional induction of genes that encode ER resident proteins such as GRP78 and protein disulfide isomerase (PDI). These proteins are believed to promote the folding of newly synthesized peptides in the ER lumen (Gething and Sambrook, 1992; Kozutsumi et al., 1988; Lai et al., 2007). The second component consists of a profound and rapid repression of protein synthesis. Both responses can be rationalized in terms of the need to relieve ER stress: the first response increases the capacity of the ER to actively fold proteins, and the second decreases the demands made on the organelle by attenuating protein synthesis rates.

The ER provides an environment that is highly optimized for oxidative protein folding. Endoplasmic reticulum oxidation 1 (ERO1α) transfers disulfides to PDI and is essential for oxidative protein folding even in simple eukaryotes such as yeast and worms (Tu and Weissman, 2004; Zito et al., 2010). PDI and ERO-1α, key components of the oxidative folding machinery, have been studied as enhancers of protein secretion (Sevier and Kaiser, 2008). General ER stress signaling, including expression of GRP78, has also been explained as an alteration in secretory processes (Kaufman, 2002; Mori, 2000). Modifying the ER protein folding environment may enhance the efficiency of protein folding, but this process not has been examined with respect to its application to secretory protein-associated pathological conditions, including diabetes or its associated complications, such as dry mouth.

Ixeris dentata (IXD, Asteraceae), a typical medicinal herb, has been used for the treatment of indigestion, pneumonia, hepatitis, contusions and tumors (Yi et al., 2002). IXD is known to contain aliphatic compounds, triterpenoids and sesquiterpene glycosides (Seto et al., 1986). The methanolic extract of IXD has been postulated to contain one or more hypoglycemic and hypolipidemic constituents that significantly reduced triglyceride and total cholesterol levels in streptozotocin-diabetic rats (Choi et al., 1990), suggesting an effect of IXD on controlling metabolic alteration in diabetes or its associated disease conditions. ER stress has been investigated in cases of metabolism-associated glucose disturbance, such as diabetes (Bánhegyi et al., 2007). If ER stress is modified, or some oxidative folding process prior to general ER stress is enhanced, some pathological conditions in which secretory protein folding is altered could be relieved. For example, the potential effect of IXD treatment on ER stress and secretion in diabetes serves as a hypothesis for this study.

The effect of IXD extracts on secretory protein maturation steps, such as amylase folding and the secretion process, were examined in a human submandibular salivary gland (HSG) cell line. The HSG cell line was chosen for this study because primary salivary gland cells, which secrete high levels of amylase, have limited capacity for regeneration, are highly differentiated and are difficult to expand *in vitro*. This study suggests that IXD treatment may contribute to relieving the symptoms of dry mouth through controlling protein folding processes.

2. Materials and methods

2.1. Materials

IXD extracts was purchased from the Korea Research Institute of Bioscience & Biotechnology (Daejeon, South Korea). Thapsigargin, tunicamycin, and 4-phenylbutyrate (4-PBA) were obtained from Sigma Chemical Company (St. Louis, MO). Luteolin-7-o-glucoside was purchased from EXTRASYNTHESE (Genay Cedex France). 8-epidesacylcynaropicrin-3-O-beta-glucopyranoside and *Ixeris F* were obtained from Yonsei International Campus (Songdo, South Korea).

2.2. Plant material and preparation

IXD was purchased from PULGREEN (Seoul, Korea). The powdered IXD was weighed and extracted with methanol for 20 min at 50 °C, using an ASE300 accelerated solvent extractor (DIONEX Corporation). After filtering, the IXD extracts were dried for 24 h at 40 °C in a MODULSPIN 40 (Biotron Corporation) and kept at −4 °C.

2.3. Quantitation of pure compound using HPLC–DAD

The content of the main active constituent of IXD in each extract was determined by HPLC–DAD as described in the Chinese Pharmacopoeia (Li et al., 2008). The HPLC system consisted of a Waters Acquity UPLC H-Class System, and the output signal was recorded using Empower™ Software. In brief, the chromatographic separation was carried out using a linear gradient elution of acetonitrile (A) and 0.1% formic acid (B) (0 min, 5% A; 15 min, 27.5% A; 15.5 min, 100% A; 21 min, 100% A; 21.5 min, 5% A; 30 min, 5% A) on an Innopia Inno C18 column (4.6 × 150 mm, 5 μm) at a flow rate of 1.0 mL/min. The wavelength for quantification of 8-epidesacylcynaropicrin-3-O-beta-glucopyranoside and *Ixeris F* was set at 210 nm.

Stock standard solution of 8-epidesacylcynaropicrin-3-O-beta-glucopyranoside and *Ixeris F* was prepared in methanol at a concentration of 1.0 mg/mL. The appropriate amount of the standard solution was mixed and diluted with methanol as indicated. The dried extracts of *IXD* were weighed and dissolved by methanol. These sample solutions were filtered through a 0.45 mm membrane filter (Millipore, Nylon, 170 μm) and analyzed with HPLC–DAD.

2.4. Cell culture

Human salivary gland cells, HSG cells (Sato et al., 1987) were cultured in RPMI 1640 (sigma R1363, 2 mM L-glutamine, without glucose & $NaHCO_3$) supplemented with 10% fetal bovine serum (FBS, Gibco), 5 mM glucose (Sigma, G-8270), 100 U/mL