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Research article

North American ginseng influences adipocyte–macrophage crosstalk regulation of inflammatory gene expression

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ABSTRACT

Background: Adipocyte–macrophage communication plays a critical role regulating white adipose tissue (WAT) inflammatory gene expression. Because WAT inflammation contributes to the development of metabolic diseases, there is significant interest in understanding how exogenous compounds regulate the adipocyte–macrophage crosstalk. An aqueous (AQ) extract of North American (NA) ginseng (*Panax quinquefolius*) was previously shown to have strong inflammo-regulatory properties in adipocytes. This study examined whether different ginseng extracts influence adipocyte–macrophage crosstalk, as well as WAT inflammatory gene expression.

Methods: The effects of AQ and ethanol (EtOH) ginseng extracts (5 μg/mL) on adipocyte and macrophage inflammatory gene expression were studied in 3T3-L1 and RAW264.7 cells, respectively, using real-time reverse transcription polymerase chain reaction. Adipose tissue organ culture was also used to examine the effects of ginseng extracts on epididymal WAT (EWAT) and inguinal subcutaneous WAT (SWAT) inflammatory gene expression.

Results: The AQ extract caused significant increases in the expression of common inflammatory genes (e.g., *Mcp1*, *Ccl5*, *Tnf-α*, *Nos2*) in both cell types. Culturing adipocytes in media from macrophages treated with the AQ extract, and vice versa, also induced inflammatory gene expression. Adipocyte *Ppar-γ* expression was reduced with the AQ extract. The AQ extract strongly induced inflammatory gene expression in EWAT, but not in SWAT. The EtOH extract had no effect on inflammatory gene expression in either both cell types or WAT.

Conclusion: These findings provide important new insights into the inflammo-regulatory role of NA ginseng in WAT.

1. Introduction

White adipose tissue (WAT) is a metabolically active endocrine organ that plays a central role in whole-body energy homeostasis [1]. The secretion of a wide range of proteins from WAT, collectively termed "adipokines," is important for communication between adipocytes and other cell types. Of particular relevance is the paracrine dialog that exists between adipocytes and macrophages. Hypertrophic adipocytes secrete specific chemokines [e.g., monocyte chemoattractant protein 1 (MCP1)] that promote macrophage recruitment and activation [2]. These macrophages secrete a myriad of cytokines [e.g., tumor necrosis factor-alpha (TNF-α)] that activate adipocyte inflammatory signaling pathways, leading to recruitment of additional immune cells. Together, this cyclical dialog induces a chronic, low-grade inflammatory state in WAT. Because WAT inflammation is a major contributor to the development of metabolic diseases, there is significant interest in understanding how exogenous compounds can regulate adipocyte–macrophage crosstalk.

Increasing evidence demonstrates that many natural health products (NHPs) are able to regulate WAT inflammation [3]. This is particularly relevant given recent reports highlighting the

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widespread use of NHPs in the US population [4]. Ginseng, which is the fifth most highly consumed NHP in the United States [4], has been shown to regulate immune function, inflammatory processes, glucose metabolism, and response to stress and fatigue [5–8]. Ginseng constitutes a family of herbs in which the primary species are *Panax ginseng* (Korean ginseng) and *Panax quinquefolius* [North American (NA) ginseng]. Although similar in genus, these species have distinct profiles of bioactive compounds, such as ginsenosides and polysaccharides. Compared with *P. ginseng*, relatively little is known about the bioactivity of *P. quinquefolius*.

We have previously reported that different NA ginseng extracts (which vary in ginsenoside and polysaccharide contents) had differential effects on inflammatory signaling pathways in differentiated 3T3-L1 adipocytes [5]. Specifically, an aqueous (AQ) ginseng extract induced adipocyte inflammatory signaling through the Toll-like receptor-4 (TLR4) pathway, whereas an ethanol (EtOH) extract had no effect. Azike et al [8] showed that these same NA ginseng extracts had different effects on cytokine production in macrophages. In their study, the AQ extract showed immune-stimulating effects and the EtOH extract showed immunoinhibitory effects [8]. Although the bioactivity of these extracts has been studied in cultured adipocytes and macrophages individually, it is unknown whether ginseng regulates the paracrine dialog between them. The goal of this study was to investigate how AQ and EtOH ginseng extracts influence the crosstalk between adipocytes and macrophages. We also examined whether the effects of these extracts were observed in different mouse WAT depots. Together, this research provides new insights into the molecular mechanisms by which NA ginseng extracts mediate cellular communication in adipose tissue.

2. Materials and methods

2.1. Ginseng acquisition and extraction

The Ontario Ginseng Growers Association provided 4-yr-old NA ginseng roots. Ginseng roots were pooled from five different Ontario farms and prepared at Naturex (South Hackensack, NJ, USA) for The Ontario Ginseng Research and Innovation Consortium [8]. In brief, dried ginseng root samples were soaked three times during 5 h at 40°C in either 16 L of water or an EtOH/water (75/25, v/v) solution to obtain the AQ and EtOH extracts, respectively [8]. Following extraction, solutions were filtered, solvent removed using a rotary evaporator, and concentrates were lyophilized using a freeze dryer. The contents of ginsenoside and polysaccharide were measured using high-performance liquid chromatography and size exclusion chromatography, as described elsewhere [8,9]. The EtOH extract contained more than two times the total ginsenoside content than the AQ extract: 28.25% versus 13.87% dry weight of the extract. Rb1 and Re were the two most predominant ginsenosides in both extracts, however, the Rb1/Re ratio was higher in the EtOH extract: 1.8 versus 1.1 [8]. For all cell culture studies, ginseng extracts were dissolved in Hank's balanced salt solution (HBSS) and filtered using 0.2-μm filters (Fisher Scientific, ON, Canada) prior to their use. The concentration of extracts used in cell culture studies was selected following a dose–response pilot study to maximize the biological effects of the extracts while preventing cell toxicity. Toxicity was assessed using the Promega cytotoxicity assay (Madison, WI, USA).

2.2. Cell culture reagents

Murine 3T3-L1 preadipocytes were purchased from American Type Culture Collection (ATCC; Rockville, MD, USA). Murine RAW264.7 macrophages (ATCC) were kindly provided by Dr. Lindsay Robinson (University of Guelph, Guelph, ON, Canada). Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin, and 0.25% trypsin–EDTA were purchased from HyClone Laboratories (Logan, UT, USA). Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), human insulin, fetal bovine serum (FBS), and HBSS were obtained from Sigma Aldrich (Oakville, ON, Canada).

2.3. Adipocyte cell culture studies

Four separate studies were conducted with murine 3T3-L1 preadipocytes, with each performed in triplicate using different passage numbers (Table 1, Fig. S1 in the supplementary material online). Each study used the same basic protocol for adipocyte differentiation, as previously described [5]. In brief, preadipocytes were seeded at a density of 6.0×10^4 and cultured in DMEM containing 5% FBS and 1% penicillin–streptomycin (i.e., basic media). Differentiation was induced 2 d postconfluence (i.e., Day 0) using a standard differentiation cocktail consisting of basic media supplemented with 1μM DEX, 1mM IBMX, and 5 μg/mL human insulin (i.e., differentiation media). After 2 d (i.e., Day 2), the differentiation media was removed and replaced with the basic media containing only 5 μg/mL human insulin (i.e., maintenance media). Maintenance media was changed every 2 d during the differentiation protocol. On Day 7, FBS was removed and the remainder of the experiments were conducted in serum-free conditions. Treatment

Table 1
Overview of various adipocyte and macrophage studies

Study No.	Study description
Adipocyte studies	
A1	Differentiated adipocytes were treated for 48 h with 5 μg/mL of the EtOH and AQ ginseng extracts. The CTRL corresponded to serum-free media. After 48 h, total RNA was extracted and ACMs were collected and used for Study M3.
A2	Differentiated adipocytes were treated for 24 h with 5 μg/mL of the EtOH and AQ ginseng extracts. The CTRL corresponded to serum-free media. After 24 h, media containing ginseng extracts were removed and replaced with ginseng-free, serum-free media for an additional 24 h. ACMs from this second 24-h period were collected and used for Study M4.
A3	Differentiated adipocytes were treated for 48 h with MCMs collected from Study M1 (see below).
A4	Differentiated adipocytes were treated for 48 h with MCMs collected from Study M2 (see below).
Macrophage studies	
M1	Macrophages were treated with 5 μg/mL of the EtOH and AQ ginseng extracts for 24 h. The CTRL corresponded to serum-free media. After 24 h, total RNA was extracted and MCMs were collected and used for Study A3.
M2	Macrophages were treated with 5 μg/mL of the EtOH and AQ ginseng extracts for 12 h. The CTRL corresponded to serum-free media. After the 12-h treatment period, media containing ginseng extracts were removed and replaced with ginseng-free, serum-free media for an additional 12 h. MCMs from this second 12-h period were collected and used for Study A4.
M3	Macrophages were treated for 24 h with ACM collected from Study A1 (see above).
M4	Macrophages were treated for 24 h with ACM collected from Study A2 (see above).

ACMs, adipocyte-conditioned media; AQ, aqueous; CTRL, control; EtOH, ethanol; MCMs, macrophage-conditioned media