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The Inhibitory Effect of *Weissella koreensis* 521 Isolated from Kimchi on 3T3-L1 Adipocyte Differentiation

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***Abstract*—**Abnormal adipocyte growth, in terms of increased cell numbers and increased cell differentiation, is considered to be a major pathological feature of obesity. Thus, the inhibition of preadipocyte mitogenesis and differentiation could help prevent and suppress obesity. The aim of this study was to assess whether extracts from *Weissella koreensis* 521 cells isolated from kimchi could exert anti-adipogenic effects in 3T3-L1 cells (fat cells). Differentiating 3T3-L1 cells were treated with *W. koreensis* 521 cell extracts (*W. koreensis* 521_CE), and cell viability was assessed by MTT assays. At concentrations below 0.2 mg/ml, *W. koreensis* 521_CE did not exert any cytotoxic effect in 3T3-L1 cells. However, treatment with *W. koreensis* 521_CE significantly inhibited adipocyte differentiation, as assessed by morphological analysis and Oil Red O staining of fat. *W. koreensis* 521_CE treatment (0.2 mg/ml) also reduced lipid accumulation by 24% in fully differentiated 3T3-L1 adipocytes. These findings collectively indicate that *Weissella koreensis* 521 may help prevent obesity.

Keywords*—Weissella koreensis* 521, 3T3-L1 cells, adipocyte differentiation, obesity.

I. Introduction

OBESITY, which occurs when excessive fat accumulates due to an average of energy intake compared to consumption, is associated with various health consequences, such as cardiovascular disease, diabetes mellitus, and other chronic disorders [1], [2]. Obesity is widely recognized as a worldwide problem due to its negative health consequences and huge cost to society. Thus, we critically need safe and effective anti-obesity measures. Despite this urgent need and the potential size of the market for anti-obesity drugs, however, the developed drugs have proven unsatisfactory to date, due to their deleterious side effects. Thus, some researchers are turning to edible natural products that have 'historically' been used as dietary supplements for body-weight management and control in many countries [3].

Obesity primarily arises via increased cytoplasmic triglyceride deposition, which leads to adipocyte enlargement, and elevated adipogenesis, which results in the formation of new adipocytes from precursor cells [4]. Adipose tissue has been shown to secrete a variety of bioactive peptides (i.e., adipokines) that can potentially affect glucose and lipid metabolism [1]. As adipocyte differentiation and the extent of subsequent fat accumulation are closely related to the occurrence and advancement of various diseases, inhibiting the proliferation and differentiation of fat cells is considered to be an important strategy for the potential treatment of obesity [3], [5].

Probiotics are defined as live microbial feed supplements that can benefit human health via the gastrointestinal tract [6]. According to a recent study, live probiotics, dead probiotic cells, and even probiotic cell components can exert significant biological effects outside the gastrointestinal tract. Thus, probiotics are believed to be an important part of an overall dietary strategy for maintaining health. Some probiotics have been found to be effective in regulating adipose tissue in overweight adults and animal models of obesity. In particular, administration of *L. plantarum* to mice led to reductions in adipose tissue weights [7], [8].

Kimchi is a fermented cabbage product that is traditional in the Republic of Korea [9]. Among the lactic acid bacteria (LAB; a subset of probiotic organisms), isolated from kimchi, *W. koreensis* is a psychrophilic bacterium that is found as the dominant species in kimchi produced at -1^{o}C [10]. *W. koreensis* raises interest on anti-obesity LAB. However, there is little information available on the anti-obesity effects of *W. koreensis* LAB. Here, we investigated the effects of *W. koreensis* cell extracts on the cell viability and intracellular lipid accumulation in cultured and differentiating 3T3-L1 (adipocyte) cells.

II. Material and Methods

A. Isolation of Weissella koreensis 521 and Preparation of the Cell Extract

W. koreensis 521 was isolated from kimchi and confirmed by DNA sequencing of 16S rRNA. For experiments, *W. koreensis* 521 was grown anaerobically in deMan-Rogosa-Sharpe (MRS) medium at 37^{o}C for 18hr, and then the cells were collected by centrifugation and washed with phosphate-buffered saline (PBS). The cells were counted and suspended in PBS at 10^{10} colony-forming units (CFU)/ml, and then subjected to sonication and centrifugation. Supernatants containing the *W. koreensis* 521 _CE' were filter-sterilized (pore size, 0.45μm), lyophilized, and kept at –20^{o}C until use.

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B. Cell Culture and Differentiation

The 3T3-L1 preadipocyte cell line was obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in 1% penicillin-streptomycin (PS)/DMEM containing 10% FBS (Gibco-BRL) [Lonza, Walkersville, MD, USA] at 37°C in a 5% CO_2 incubator. Differentiation was induced by incubating confluent cells for 6 days in differentiation medium (DM) containing 1% PS/DMEM, 10% FBS, 0.25 mM IBMX, 0.25μM dexamethasone and 1μg/ml insulin. The cells were then maintained in post-differentiation medium (DMEM containing 1% PS and 10% FBS), with replacement of the medium every 2 days. To examine the effects of the cell extract on the differentiation of preadipocytes to adipocytes, cells were cultured with MDI in the presence of various concentrations of cell extract [0–2.0mg/ml].

C. Quantification of Cell Viability via MTT Assay

The MTT assay is a standard colorimetric assay used to measure cellular proliferation (cell growth). Yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced to purple formazan in the mitochondria of living cells. The number of surviving cells is directly proportional to the level of the formazan product created; the amount of colored product is directly proportional to the number of viable cells, and can be read on a multi-well scanning spectrophotometer [11]. The 3T3-L1 preadipocytes were treated with 1 x 10^5 *W. koreensis* 521_CE/well. After 48 hr, the cells were incubated with MTT working solution [Promega] for 3hr. The fat was then removed from the plate, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan dye. The absorbance of the resulting colored solution was quantified (absorbance 570nm/reference 630nm) using an ELISA plate reader [Bio-TEK Power-Wave XS, VT, USA]

D. Oil-Red O Staining

The cellular lipid content was assessed by Oil Red O staining. After the induction of differentiation, cells were washed twice with PBS, fixed in 3.7% formaldehyde (Sigma-Aldrich) in PBS for 1hr, and stained with Oil Red O [Cayman, USA] for 1hr. The stained fat droplets were dissolved in isopropanol containing 4% Nonidet P-40, and quantified by measuring the absorbance at 520 nm. Pictures were taken using an Olympus microscope.

Lipid accumulation (%) = 100 – (A–B)/A x 100

A : $A_{520\,nm}$ [control]

B : $A_{520\,nm}$ [LAB sample]

E. Statistical Analysis

Statistical analyses were performed using Sigma Plot 10.0 [Systat software, USA]. Values are expressed as the means ± standard error (SE) from three independent experiments. Statistical significance was determined using the paired Student's *t* test.

III. Results

A. Effects of W. koreensis 521 on Cell Viability in 3T3-L1 Preadipocytes

To distinguish any inhibitory effect of *W. koreensis* 521_CE from a possible cytotoxic effect on 3T3-L1 preadipocytes, we treated cells with various concentrations of *W. koreensis* 521_CE and performed MTT assays. The extract failed to show any cytotoxic activity against 3T3-L1 cells up to 0.2 mg/ml (Fig. 1).

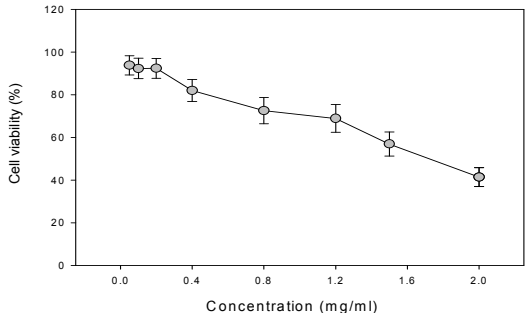

Fig. 1 Effect of *W. koreensis* 521 on the cell viability of post-confluence preadipocytes. The indicated concentrations of *W. koreensis* 521_CE were added to the differentiation medium at day 0. After 8 days of treatment, viability was determined by MTT assay. No cytotoxic effect was noted up to 0.2 mg/ml. Assays were performed in 3 replicates from 3 independent experiments. Values are means ± SEM (p<0.01)

B. Inhibition of Adipocyte Differentiation and Lipid Accumulation

To test whether *W. koreensis* 521_CE inhibited adipocyte differentiation, we used insulin, dexamethasone, and isobutylmethyl xanthine (differentiation medium, DM) to induce the differentiation of 3T3-L1 preadipocytes, treated the differentiating cells with [0.8 x 10^5 cells/well in a 6-well plate] *W. koreensis* 521_CE on day 0, and then changed the culture medium every 2 days for a total of 2 days. *W. koreensis* 521_CE were then switched to add to the 3T3-L1 cells at 2~8 days, and the adipocytes were stained with Oil Red O for visualization of fat droplets. The staining results showed that an 8-day treatment with various concentrations [0–0.8 mg/ml] of *W. koreensis* 521_CE during the differentiation period significantly and dose-dependently inhibited 3T3-L1 adipogenesis (Fig. 2, lower panels) in terms of both cell differentiation (Fig. 2) and lipid accumulation (Fig. 3), compared with control cells. Among the tested concentrations of *W. koreensis* 521_CE, 0.2 mg/ml was the most effective at reducing the lipid content in differentiated cells (by 24% compared with control cells) (Fig. 3). These results suggest that *W. koreensis* 521_CE inhibited the differentiation of 3T3-L1 preadipocytes by suppressing lipid accumulation.

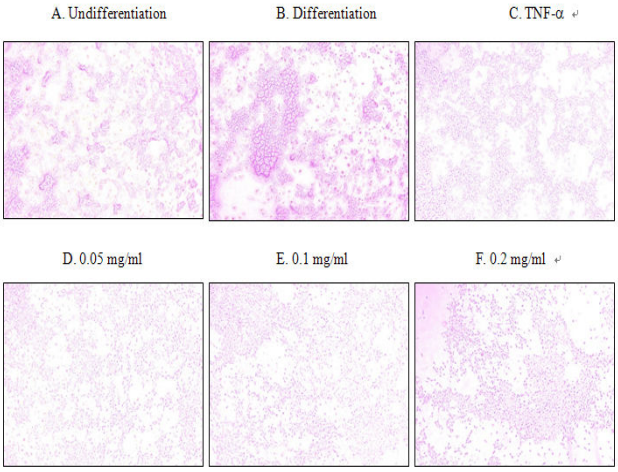

Fig. 2 Morphological examination of undifferentiated cells (A), control differentiated cells (B), tumor-necrosis factor (TNF)-α treated control cells (C), cells treated with various concentrations of *W. koreensis* 521_CE (D, 0.05 mg/ml; E, 0.1 mg/ml; F, 0.2 mg/ml). Confluent 3T3-L1 cells were treated with various concentrations of *W. koreensis* 521_CE for 8 days and lipid accumulation was measured by Oil Red O staining. A significant inhibitory effect [using red staining as a proxy for differentiation here and counting the red-stained cells] was noted

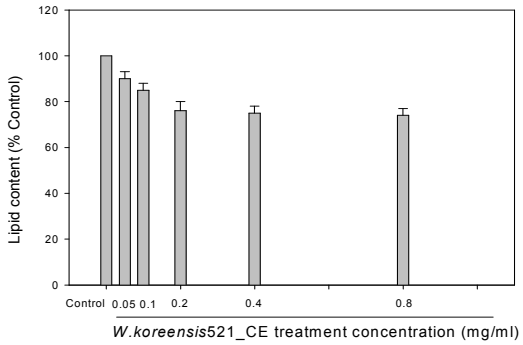

Fig. 3 Inhibitory effect of *W. koreensis* 521_CE on lipid accumulation in 3T3-L1 adipocytes. Confluent 3T3-L1 cells were treated with various concentrations of *W. koreensis* 521_CE for 8 days, and lipid accumulation was examined by Oil Red O staining. Assays were performed in 3 replicates from 3 independent experiments. Values are means ± SEM (p<0.01)

IV. Discussions

The recent noticeable increases in the worldwide numbers of overweight and obese people are due in part to diet and lifestyle changes [12]. Some natural products have been shown to protect against obesity and have beneficial health effects, and have attracted the attention of researchers because of their relatively good safety profiles [13]. Among these natural products, probiotic preparations comprising dead cells or their metabolites have been shown to exert biological responses [14], [15]. Among the various beneficial health effects of probiotics, their biological impact on obesity has generated considerable interest. For example, the probiotic species, *L. plantarum*, was shown to have specific biological effects (including anti-obesity effects) *in vitro* [16].

In this study, the potential anti-obesity effects of *W. koreensis* 521_CE were investigated through cell viability assays and Oil red O staining of 3T3-L1 cells treated with various concentrations of the extract either in maintenance culture or during differentiation. For increased adipose tissue to be created, the number or size of adipocytes must increase by either proliferation or differentiation [17]. Conversely, the reduction of adipose tissue mass involves the loss of lipids through lipolysis, the inhibition of preadipocyte proliferation, and/or the decreased differentiation of mature adipocytes. Treatment of 3T3-L1 cells with various concentrations [0–0.2 mg/ml] of *W. koreensis* 521_CE did not significantly alter cell viability, indicating that the inhibitory effect of *W. koreensis* 521_CE on lipid accumulation was due not to reduced cell viability. Such reduction was seen up to a 0.2 mg/ml. Instead, we observed decreased adipogenesis in *W. koreensis* 521_CE-treated cells compared to control cells (Fig. 2), indicating that the extract reduced the lipid accumulation in mature adipocytes. The loss of fat mass can be partly attributed to lipolysis, in which triglycerides are broken down into glycerol and fatty acids in adipocytes. Together, our results indicate that *W. koreensis* 521_CE may contain components that inhibit lipid accumulation in 3T3-L1 cells. The observed inhibitory effects were more significant in cells treated with a higher concentration of *W. koreensis* 521_CE versus a lower concentration of *W. koreensis* 521_CE (Fig. 3). Thus, our results confirm the potential anti-obesity effects of *W. koreensis* 521_CE and suggest that *W. koreensis* 521 may have similar effects. Further studies on *W. koreensis* 521 may provide additional insights into these potential effects and allow us to identify other relevant factors.

V. Conclusion

W. koreensis 521_CE exerted inhibitory effects on adipocyte differentiation and lipid accumulation, suggesting that *W. koreensis* 521 may have important anti-obesity effects and could thus possibly be developed as a therapeutic substance for preventing or treating obesity.

Acknowledgment

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