Therapeutic potential of resveratrol for the treatment of type III Gaucher disease

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Abstract

Gaucher disease is the most common lysosomal storage disorder. Resveratrol is a natural polyphenol that possesses a wide range of beneficial effects, including anti-inflammatory, anti-oxidant, and neuroprotective activities. The aim of this study was to determine if resveratrol has a therapeutic effect on primary fibroblast cells derived from a patient with type III Gaucher disease. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed to determine the effect of resveratrol on cell survival. The expression levels of apoptosis-inducing factor (AIF), Bcl-2-associated X protein (Bax), caspase-3, acetyl-coenzyme A acetyltransferase 1 (ACAT1), E3-binding protein (E3BP), and citrate synthase (CS) were evaluated by western blotting to characterize the effect of resveratrol treatment on Gaucher disease cells. Thin-layer chromatography (TLC) was carried out to measure changes in glucosylceramide levels in resveratrol-treated patient cells. Resveratrol increased the viability of patient cells compared to that of untreated control cells. Resveratrol treatment dose-dependently decreased AIF, Bax, and cleaved caspase-3 levels, whereas ACAT1, E3BP, and CS expression dose-dependently increased. TLC analysis showed reduced levels of glucosylceramides in resveratrol-treated patient cells. These findings demonstrate that resveratrol can relieve cellular stress due to glucosylceramide accumulation, and suggest that it should be studied further as a new therapeutic approach for the treatment of Gaucher disease.

INTRODUCTION

Gaucher disease (GD) is an autosomal recessive inherited disease caused by a deficiency in the lysosomal enzyme glucocerebrosidase (GCase). GD is the most prevalent sphingolipid storage disease and is characterized by intracellular accumulation of glucosylceramide (GluCer) in many tissues, resulting in a number of pathological conditions, including hepatosplenomegaly, bone lesions, blood disorders, including anemia, leukopenia, and thrombocytopenia, and involvement of other organs, such as the brain and lungs in some GD variants. GD has been divided into 3 subtypes based on the clinical symptoms present and the extent of central nervous system involvement. Type I GD is a chronic non-neuronopathic disease. Type II GD, acute neuronopathic form, is an early onset subtype with rapid neurodegeneration and death usually occurs within the first 2 years of life. Type III GD is called the juvenile from, although it can also occur in adults, and is a subacute neuronopathic form of intermediate severity, with patients frequently surviving into their teens or adulthood. Part of the pathology of GD is due to the frequent accumulation of defective GCase in the endoplasmic reticulum (ER) in an unfolded state. The accumulation and impaired trafficking of GCase from the ER to lysosomes can induce ER stress, causing the activation of apoptotic enzymes, including Bcl-2 family members and caspases, resulting in cell death. Metabolic abnormalities, such as insulin resistance and abnormal lipid metabolism, are also reported in GD. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenolic phytoalexin and is produced naturally by several plants, such as grapes and mulberries. It is known to have beneficial properties, including anti-inflammatory, anti-oxidant, anti-tumor, and neuroprotective activities. Moreover, resveratrol regulates carbohydrate and lipid metabolism. Current GD therapies have a limited ability to reduce disease symptoms, in particular neurological symptoms of type II and type III GD, because of the inability to cross the blood–brain barrier or limited efficacy per se. There is a significant need to develop novel GD therapeutics that are more effective. We
investigated the therapeutic potential of resveratrol on cells derived from a patient with type III GD by evaluating cell survival, biomarkers involved in metabolic and apoptotic pathways, and changes in GluCer levels.

METHODS

Cell line and cell culture
Type III GD fibroblasts were obtained from the Coriell Institute for Medical Research (GM# 20272; homozygous for the L444P mutation, Coriell, Camden, NJ, USA). Normal fibroblasts were obtained from the Korean Cell Line Bank (KCLB# 21947, CCD-986sk; Seoul, South Korea). Cells were grown in 100 mm dishes in Dulbecco’s modified Eagle’s medium (DMEM; Mediatech Inc., Herndon, VA, USA) containing 20% fetal bovine serum (Thermo Scientific, South Logan, UT, USA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Invitrogen, Carlsbad, CA, USA). Cultures were maintained at 37°C in a 5% CO₂ humidified incubator.

Cell viability assay
The GD fibroblasts and normal fibroblasts were seeded in 96-well plates at a density of 10⁴ cells/200 μL. Cells were then cultured in DMEM containing 0.1 μM, 1 μM, or 10 μM resveratrol (prepared in dimethyl sulfoxide (DMSO); Sigma, Saint Louis, MO, USA) for 24 h at 37°C in a 5% CO₂ incubator. Both type III GD fibroblasts and normal fibroblasts were treated with 0.1% DMSO (vehicle control). Ten microliters of EZ-cytox 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay solution (Daellab service, Seoul, South Korea) was added per well and incubated for 2 h at 37°C. After incubation, MTT reduction was determined by measuring the absorbance at 450 nm using a microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). Cell viability was expressed as a percentage of the cell viability of the control group.

Western blot procedure
Approximately 1 x 10⁶ GD and normal fibroblasts were cultured in 100 mm dishes in culture medium containing resveratrol (0.1 μM, 1 μM, or 10 μM) at 37°C. GD fibroblasts and normal fibroblasts were also cultured in 0.1% DMSO (vehicle control). After incubation for 24 h, cells were washed with cold phosphate buffered saline and lysed in whole cell lysis buffer (20 mM HEPES pH 7.5, 0.5 mM EDTA, 0.1 mM EGTA, 350 mM NaCl, 10 mM NaF, 1 mM MgCl₂, and 1% Triton X-100 [Sigma]) with a protease inhibitor cocktail (Roche, Penzberg, Germany). Protein concentration was determined with the Coomassie Plus protein assay reagent (Pierce, Rockford, IL, USA). Thirty micrograms of protein were collected from each sample, run on electrophoresis gels, and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Membranes were incubated with antibodies against apoptosis-inducing factor (AIF; Abcam, Cambridge, MA, USA), Bcl-2-associated X protein (Bax; Cell Signaling, Beverly, USA), cleaved caspase-3 (Cell Signaling), E3-binding protein (E3BP; Abcam), citrate synthase (CS; Abcam), acetyl-coenzyme A acetyltransferase 1 (ACAT1; Abcam), and -actin (Sigma). Membranes were then incubated with anti-mouse (Abcam) and anti-rabbit (Abcam) secondary antibodies. Bands were visualized with an enhanced chemiluminescence system (SuperSignal West Pico Luminol/Enhancer Solution, Pierce). Densitometric analysis was performed to quantify protein levels with TINA 2.0 software (Raytest Isotopenmessgeräte GmbH, Straubenhardt, Germany).

Lipid extraction and measurement of GluCer concentration
Quantitative changes in GluCer levels in cells treated with resveratrol or DMSO (vehicle control) were evaluated by thin-layer chromatography (TLC). Lipid extraction and GluCer separation were carried out as described in a previous article.¹⁰ Densitometric analysis was performed to examine GluCer levels with TINA 2.0 software (Raytest Isotopenmessgeräte GmbH).

Statistical analysis
Each experiment was performed independently for a minimum of three times up to six times. Student’s t-test was performed to confirm the presence of significant differences. P values less than 0.05 were considered statistically significant.

RESULTS

Cell viability after resveratrol treatment
We treated type III GD fibroblasts with increasing concentrations of resveratrol (0.1 μM, 1 μM, and 10 μM), and analyzed its effect on cell viability
by MTT assay. Resveratrol treatment at 0.1 μM, 1 μM, and 10 μM dose-dependently increased the viability of GD fibroblasts 17–46%, compared to DMSO-treated GD fibroblasts (\(P < 0.05\), Fig. 1). To characterize the molecular effects of resveratrol, the expression levels of AIF, Bax, and caspase-3, which are associated with apoptosis, were evaluated. Resveratrol treatment at 0.1 μM, 1 μM, and 10 μM resulted in dose-dependent reductions in the expression of AIF (34–63%; \(P < 0.05\)), Bax (23–46%; \(P < 0.05\)), and cleaved caspase-3 (35–70%; \(P < 0.05\)), compared to DMSO-treated GD fibroblasts (Figure 2).

Western blot analysis of metabolic markers

Because metabolic imbalances, such as insulin resistance and abnormal lipid metabolism, have been described in GD5, and resveratrol has been shown to regulate glucose and lipid metabolism8,9, we evaluated resveratrol’s effects on factors associated with metabolic pathways in GD fibroblasts. After 24 h treatment with different concentrations of resveratrol, the expression level of ACAT1 and E3BP dose-dependently increased (23–58% and 19–67%, respectively), compared to the vehicle control (\(P < 0.05\)). In addition, the expression level of CS increased by 10–54% in a dose-dependent manner (\(P < 0.05\), Fig. 3) in response to resveratrol treatment.

Measurement of changes in intracellular GluCer levels

Quantitative changes in GluCer concentrations in type III GD cells were determined by TLC. Increased levels of GluCer were observed in GD cells treated with DMSO, compared to those in the normal cells. Treating GD cells with increasing concentrations of resveratrol dose-dependently decreased GluCer accumulation in the treated cells (21–38% reduction by relative density scan, \(P < 0.05\), Figure 4).
Figure 3. Western blot and densitometric analyses of metabolic markers. (A) Resveratrol dose-dependently increased expression levels of acetyl-coenzyme A acetyltransferase 1 (ACAT1), E3-binding protein (E3BP), and citrate synthase (CS) in type III GD cells. (B) Densitometric analysis. β-actin served as a loading control. Vehicle represents type III GD cells treated with DMSO, which was defined as 100% relative density. Normal-vehicle, normal cells treated with DMSO; GD III, type III GD cells. *, P value of <0.05 by Student’s t-test.

DISCUSSION

Previous studies have reported beneficial activities of resveratrol in several pathological conditions, but not in GD. The ER is the site of protein production, processing, and maturation. Defective processing of mutant GCase results in the accumulation of this protein in the ER lumen, which can cause ER stress. Prolonged ER stress can activate the intrinsic apoptotic pathway, which ultimately activates effector caspases, such as caspase-3, leading to cell death. The results of the present study demonstrated the novel finding that resveratrol dose-dependently decreases the expression level of AIF, Bax, and cleaved caspase-3 in type III GD cells with increased cell viability. AIF mediates a caspase-independent apoptotic pathway. Therefore, this finding indicates that resveratrol has a wide spectrum of mechanisms of action affecting both caspase-dependent and caspase-independent cell death pathways. In addition, because insulin resistance and impaired lipid metabolism were reported in GD, and resveratrol was documented to improve carbohydrate and lipid metabolism, we also evaluated resveratrol’s effect on factors associated with these metabolic pathways in type III GD cells. Our results showed that GD cells treated with resveratrol had a dose-dependent decrease in the concentration of GluCer, and resveratrol reduced the concentration of GluCer in GD cells. Vehicle represents type III GD cells treated with DMSO, which was defined as 100% relative density. Normal-vehicle, normal cells treated with DMSO; GD III, type III GD cells. *, P value of <0.05 by Student’s t-test.
stress by reducing GluCer accumulation in GD cells. Cells can then recuperate from this pathological condition by increasing the activity of metabolic pathways with increased acetyl-CoA requirements (Figure 5). The enzyme ACAT1 catalyzes the formation of acetacetyl-CoA from two molecules of acetyl-CoA, which serves as the basis for the synthesis of bioactive materials required for cellular processes, such as growth and differentiation, by the mevalonate pathway. Thus, the upregulation of ACAT1 in GD cells in response to resveratrol treatment indicates that acetyl-CoA, the byproduct of GluCer degradation, is converted into acetacetyl-CoA, which can be used to produce the molecules required for cell growth, proliferation, and differentiation by the mevalonate pathway. In this study, resveratrol did not enhance GCase activity (data not shown). Therefore, the observed decrease in GluCer levels in patient cells may be due to the degradation of the substrate, which yields glucose and acetyl-CoA that can contribute to the upregulation of glucose and lipid metabolism.

In conclusion, this study examined the effects of resveratrol on type III GD cells in vitro. We observed that resveratrol decreased intracellular GluCer accumulation and apoptotic factors, and increased the expression of enzymes associated with glucose and lipid metabolism in type III GD cells. Resveratrol can cross the blood–brain barrier, and it has potential protective activities in neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease. These findings support the further investigation of resveratrol as a new therapeutic agent, because of its potential merit in the treatment of currently intractable neurological symptoms of GD.

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DISCLOSURE

Conflicts of interest: None

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