Biomedicine & Pharmacotherapy 102 (2018) 699–710

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Oncosis-like cell death is induced by berberine through ERK1/2-mediated impairment of mitochondrial aerobic respiration in gliomas

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ARTICLE INFO

Keywords:
Oncosis
ATP
ERK1/2
Berberine
Glioma

ABSTRACT

Gliomas, the most common primary malignant brain tumor, exhibit high metabolic activity. The targeting of metabolism alterations, particularly in mitochondria, is emerging as an efficient approach for curing cancers. Here, we showed that berberine, a natural compound that is used as an antibacterial agent, could reduce cellular viability and induce oncosis-like death, characterized by cell swelling, cytoplasmic vacuoles and plasma membrane blebbing, in gliomas, and that these effects were correlated with intracellular adenosine triphosphate (ATP) depletion. We also found that berberine induced autophagy as a protective effect and decreased the oxygen consumption rate (OCR), which could inhibit mitochondrial aerobic respiration by repressing phosphorylated extracellular regulated protein kinases (p-ERK1/2). Furthermore, the down-regulation of mitochondrial p-ERK1/2 by berberine inhibited aerobic respiration and led to glycolysis, an inefficient energy production pathway. In addition, berberine reduced tumor growth and inhibited Ki-67 and p-ERK1/2 expression in vivo. The results demonstrate that berberine, which represses aerobic oxidation in mitochondria and decreases their energy production efficiency, decreases metabolic activity by reducing ERK1/2 activity.

1. Introduction

Gliomas account for approximately 70% of malignant primary brain tumors in adults, and these patients have short survival times [1]. Although patients receive postoperative chemotherapy and radiotherapy, gliomas with high metabolic activity survive due to their resistance to apoptosis [2]; thus, an alternative strategy decreases metabolic activity besides apoptosis is needed to treat gliomas.

Oncosis is a non-canonical form of programmed cell death that is related to a rapid decrease in intracellular adenosine triphosphate (ATP) and mitochondrial dysfunction [3–6]. Distinct from apoptosis, oncosis is emerging as a novel strategy for killing cancer cells [4]. Moreover, the induction of oncosis by natural or synthetic chemicals, such as artemisinin, solamargine and fluopsin C, has been demonstrated to be effective for treating cancer cells that are resistant to the currently available treatments [7–9].

Berberine (BBR, Fig. 1A), an alkaloid isolated from *Rhizoma coptidis*, has long been used in China as an antibacterial agent with anti-cancer effects [10–13]. Moreover, BBR can reduce the blood glucose levels and regulate lipid metabolism in a normal human being [14–19]. Moreover, glucose and fatty acid oxidation play an important role in supplying ATP, which is required for aerobic respiration in malignant glioma cells, a process that occurs in mitochondria [20,21].

It remains unclear whether the anti-cancer effects of BBR are related to oncosis-like death in gliomas through mitochondrial dysfunction. In this study, we found that BBR leads to oncosis by repressing mitochondrial aerobic respiration and thereby decreasing ATP production. Additionally, we further investigated the underlying mechanism, and the findings might shed new light on the use of BBR as a potential medicine for glioma treatment.

2. Materials and methods

2.1. Reagents and materials

BBR (CAS Number: 633-65-8, purity > 98%), which was dissolved in dimethyl sulfoxide, was purchased from Chroma Biotechnology Company (Chengdu, China). The ERK inhibitor U0126 (CAS Number:

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https://doi.org/10.1016/j.biopha.2018.03.132
Received 4 December 2017; Received in revised form 21 March 2018; Accepted 22 March 2018

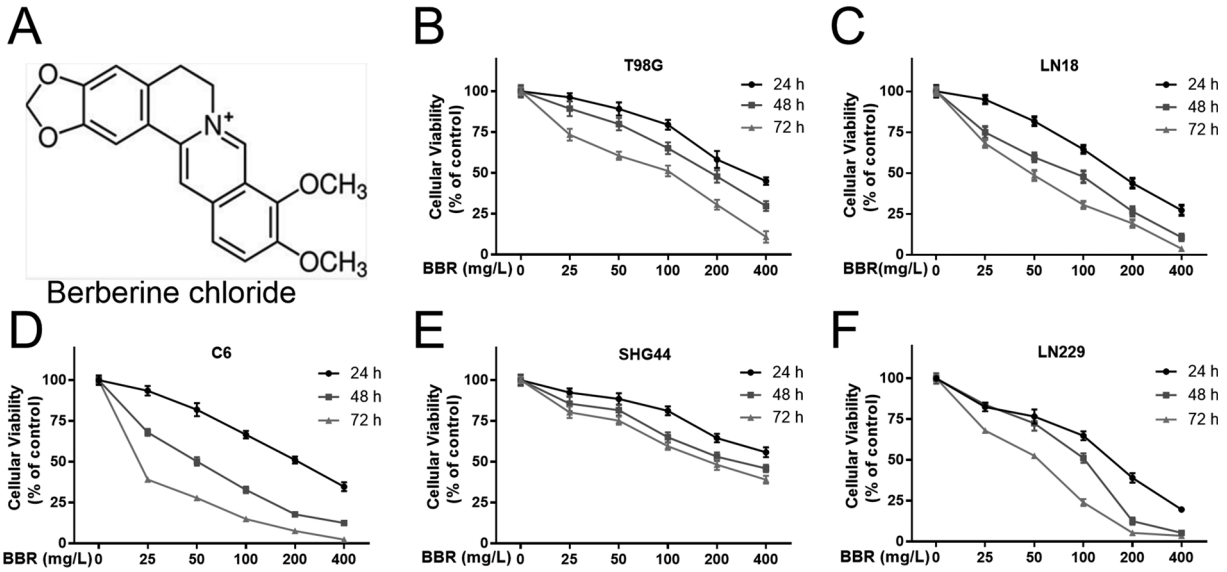

Fig. 1. Berberine reduced the viability of glioma cells.
(A) Structural formula of berberine hydrochloride. (B–F) MTT assay showing the viability of T98G, LN18, C6, SHG44 and LN229 glioma cells treated with increasing doses of BBR for increasing treatment times.

1173097-76-1) the autophagy inhibitor 3-methyladenine (3-MA, CAS Number: 5142-23-4) and the caspase inhibitor Z-VAD-FMK (Z-VAD, CAS Number: 187389-52-2) were purchased from Selleck Chemicals. Indirubin-3′-monoxime (IND, CAS Number: 160807-49-8), which is a glycogen synthase kinase-3β (GSK-3β) inhibitor, was obtained from MCE. Anti-PARP-1, anti-caspase-3, and Ki-67 antibodies, as well as goat anti-rabbit IgG and goat anti-mouse IgG, were purchased from Abcam (Cambridge, MA, USA). Anti-VDAC, anti-β-actin anti-ERK1/2 and anti-phosphor-ERK1/2 (p-ERK1/2) antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). The XF Mito Fuel Flex Test Kit was obtained from Seahorse Bioscience Company (Billerica, MA, USA). All other drugs, kits and adenovirus were obtained from Beyotime Biotechnology Company (Shanghai, China).

2.2. Cell lines and culture

Glioma cell lines were obtained from the cell bank of the Shanghai Biological Institute, Chinese Academy of Science (Shanghai, China), and were cultured in DMEM (Gibco, USA) containing 4 mM glutamine and supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL). The cells were cultured under a 5% CO_2 humidified atmosphere at 37 °C.

2.3. Cell viability analysis

T98G (5×10^3 cells/well), LN18 (1×10^4 cells/well), SHG44 (5×10^3 cells/well), C6 (5×10^3 cells/well) and LN229 (5×10^3 cells/well) glioma cells were seeded onto 96-well microplates, cultured for 24 h and then treated with the selected drug at the indicated concentrations for the indicated times. The cellular viability was assessed using an MTT assay and is expressed as a ratio relative to the absorbance at 570 nm of the control cells.

2.4. Time-lapse microscopy, lentivirus infection and transmission electron microscopy analysis

T98G and LN18 cells grown on 60 mm glass-bottom dishes were treated with the indicated concentration of BBR. The cells were observed with a live cell digital imaging system, and at least five bright-field photographs of live cells were obtained after 36, 42, and 48 h of treatment using the live cell digital imaging system.

One group of T98G cells was infected with adenovirus (20 MOI per cell) carrying mCherry-GFP-LC3B for 24 h following exposure to BBR; after the treatment, images were obtained using a confocal microscope.

Another group of cells was treated with the indicated drug, harvested, fixed with 2.5% glutaraldehyde for 24 h, rinsed with PBS, post-fixed in 1% osmium tetroxide containing 0.1% potassium ferricyanide, dehydrated with varying concentrations (30–100%) of alcohol and embedded in Epon 812. Ultrathin sections (50 nm) were cut using a Leica EM UC7 instrument, stained with 1% uranyl acetate and 0.1% lead citrate, and examined with an FEI Tecnai Spirit transmission electron microscope (JEOL, Pleasanton, CA, USA).

2.5. Analysis of cell death by flow cytometry

T98G, LN18 and C6 glioma cells were treated with the indicated BBR concentration for 48 h and collected. The collected cells were then subjected to Annexin V/propidium iodide double-staining using an Annexin V-FITC Apoptosis Detection kit according to the manufacturer's instructions (Becton Dickinson) and then analyzed using a flow cytometer (Becton Dickinson, San Diego, CA, USA).

2.6. Western blot analysis

T98G, LN18 and C6 cells were treated with the indicated concentrations of BBR for 48 h, and other groups of T98G cells were treated with a control agent, BBR (100 mg/L), or U0126 (10 μM) for 24 h. The mitochondrial and cytosolic proteins were extracted using a mitochondrial protein extraction kit according to the manufacturer's instructions. The protein samples were then separated on an 8–12% Tris-SDS gel and transferred to a PVDF membrane. The membranes were blocked with 5% bovine serum albumin and incubated with the corresponding antibody solutions overnight at 4 °C. After three 10-min washes with TBS-T (pH 7.40, 0.1% Tween 20), the membranes were incubated with horseradish peroxidase-conjugated IgG at room temperature for 1 h and then washed with TBS-T. The signals were detected by enhanced chemiluminescence, and a loading control was prepared with anti-β-actin or anti-VDAC antibody.