Am J Transl Res 2017;9(9):3977-3989
www.ajtr.org /ISSN:1943-8141/AJTR0058435

Original Article

Solasonine inhibits glioma growth through anti-inflammatory pathways

Xun Wang[1,2,3*], Shuang Zou[4#], Yu-Long Lan[1,2,3*#], Jin-Shan Xing[1], Xiao-Qiang Lan[1], Bo Zhang[1]

[1]Department of Neurosurgery, The Second Affiliated Hospital of Dalian Medical University, Dalian 116023, P. R. China; [2]Department of Neurosurgery, The Third People's Hospital of Dalian, Non-Directly Affiliated Hospital of Dalian Medical University, Dalian 116033, P. R. China; [3]Department of Pharmacy, Dalian Medical University, Dalian 116044, P. R. China; [4]Department of Neurology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, P. R. China. [*]Equal contributors. [#]Co-first authors.

Received May 29, 2016; Accepted August 9, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: The global burden of malignant glioma is expected to increase and new therapy approaches are urgently required. Solasonine is a natural glycoalkaloid compound that has been used in cancer treatment for many years; however the precise mechanisms are poorly understood. Here we seek to explore the potential roles of solasonine in glioma that could add to the development of newer therapeutic approaches for the treatment of malignant glioma. Cell proliferation of glioma cells was determined by MTT assay, and the biological functions of solasonine were investigated by migration, colony formation, apoptosis assays and cell cycle analysis in glioma cells. Western blotting and RT-qPCR were used to detect the protein and gene expression levels respectively. The nuclear localization of NF-κB p50/p65 was analyzed after treatment with solasonine. The roles of MAPKs in the anticancer effect of solasonine were then examined. The in vivo anti-tumor efficacy of dopamine was also analyzed in xenografts nude mice. We report that solasonine could inhibit cell proliferation, migration and colony formation of glioma cells. Treatment of solasonine induced apoptosis via modulating cytochrome c and caspase signaling. Besides, solasonine decreased the expression of proinflammatory mediators and nuclear translocalization of NF-κB p50/p65. Mechanistic investigation further revealed that solasonine may target anti-inflammatory signaling pathway, and more specifically p-p38 and p-JNK MAPKs. All these indicated that solasonine could inhibit glioma growth via inhibiting inflammatory signaling pathway.

Keywords: Solasonine, glioma, inflammation, therapy

Introduction

There are many difficulties in the treatment of cancer, but the most frequent are drug resistance, toxicity and low specificity [1]. Plant molecules and their semi-synthetic and synthetic derivates are important sources of antitumor drugs. According to Cragg and Newman [2], over 50 % of the drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them. *Solanum lycocarpum* A. St.-Hil (Solanaceae) is a plant found in Southeast and West-Central Brazil, on native Brazilian savanna [3]. The genus *Solanum* has anti-inflammatory [4, 5], antihepatotoxic [6] and hypotensive activities [7], besides inhibiting allergic reactions and histamine release [8]. Plants of the *Solanum* genus are known for their high alkaloid concentration and part of their toxicity may be due to these alkaloids [9, 10]. Solasonine (**Figure 1**) are the major glycoalkaloid found in at least 100 *Solanum* species [11] from which a 3b-acetoxipregno-5,16-dien-20-one is obtained [12]. Munari et al. [13] have investigated the antiproliferative activity of solasonine against cancer cell lines and demonstrated that solasonine may be good candidates for anticancer drug development.

Glioma is by far the most common primary brain tumor in adults [14]. The median survival of glioma patients is only 12-15 months, and this has not improved despite multimodal treatment [15]. Despite the advances in the management of malignant gliomas, they remain characterized by dismal prognoses [16]. Glioblastomas are the most common brain malignancies and the most challenging brain tumors to treat,

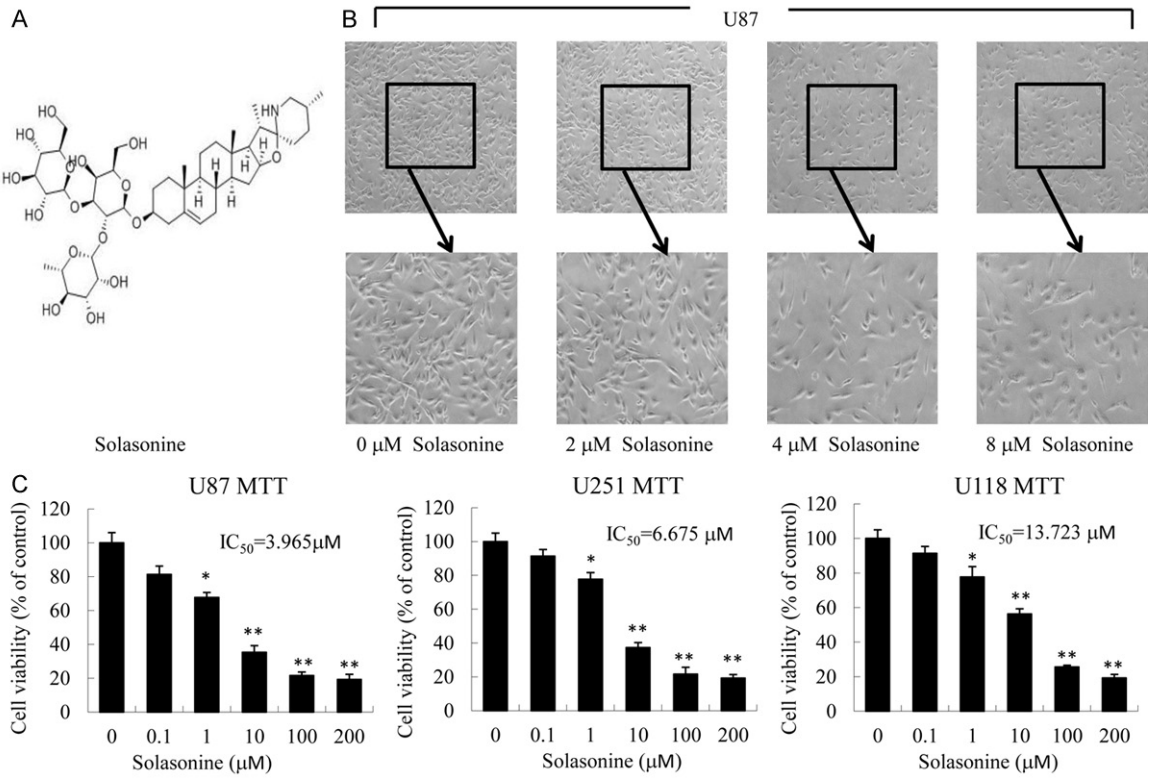

Figure 1. Solasonine inhibits the growth of human glioma cells. (A) The chemical structure of solasonine. (B, C) U87 MG, U251 and U118 MG cells were cultured with indicated concentrations of solasonine for indicated hours, and then, morphological observation (B) and MTT assay (C) were performed. Results represent the mean ± SD of three experiments done in triplicate. The inhibition rate of solasonine on U87 MG cells, U251 and U118 MG cells were detected by MTT after 24 h of solasonine treatments, IC_{50} value was calculated. *P < 0.05 and **P < 0.01 vs. DMSO-treated group.

accounting for 15.1% of the total central nervous system tumors [17]. Therefore, the development of new treatments is urgently needed.

Mounting evidence suggests an important role for inflammation in tumor development [18, 19]. The development of gliomas, in particular, is closely associated with inflammation status and immune response [20, 21]. It is therefore proposed that more complete studies are needed to fully assess the roles and status of systemic inflammation in malignant gliomas and inflammation-targeted methods could be potential for drug development.

NF-κB is a central regulator of the inflammatory process and plays a critical role in inflammation. This molecule regulates the expression of a group of proinflammatory mediators, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) [22]. NF-κB signaling is an optimal therapeutic target for the pathogenesis of inflammation. Besides, mitogen-activated protein kinases (MAPKs) signaling pathways such as those mediated by p38 MAPK (p38), c-Jun NH2-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK) are important for NF-κB transactivation or translocation [23]. Therefore, NF-κB nuclear translocation is an active inflammatory response, suggesting the possibility for its manipulation in anti-inflammatory drug development [24]. Therefore, the aim of the current study was to confirm the anti-inflammatory effects of solasonine and determine the role of NF-κB and its upper regulators to evaluate the potential of dopamine as an alternative drug in the treatment of glioma.

In the present study, we seek to explore whether solasonine was able to inhibit glioma growth *in vivo* and *in vitro*. These findings could provide strong evidence to support the anti-tumor activity of solasonine in glioma and indicated that

solasonine could be potential to be novel therapeutic strategy for human malignant glioma. This study could present a strong and mechanistic basis for pharmacokinetic studies and clinical trials to evaluate the efficacy of solasonine as a glioma therapy.

Materials and methods

Antibodies and other materials

The primary antibodies for p-p38, p-JNK, Bax, Bcl-2, cleaved caspase-3, cleaved caspase-9, NF-κB p65 and p-p65, β-actin and all the secondary antibodies were obtained from Cell Signaling Technology (Cell Signaling Technology, Inc, USA). The primary antibodies for NF-κB p50 and cytochrome C were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Trypsin, Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from HyClone Laboratories (HyClone Laboratories Inc.). Solasonine was purchased from Yuanye Biotech. (Jinan, China), and was dissolved in DMSO and kept at -20°C, as the stock solution. MBG was diluted in culture medium to obtain the desired concentration, which was stable in DMSO. The Phosphate Buffered Saline (PBS), protease inhibitor cocktail and 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co (St. Louis, MO). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Cell culture

Human U87 MG, U251 and U118 MG cell lines were obtained from ATCC (Manassas, VA). Cells were maintained in either DMEM medium supplemented with 10% fetal bovine serum. All cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cell viability assay

Cell viability was determined by a MTT assay (Roche Diagnosis, Indianapolis, IN). Briefly, lung cancer cell lines were seeded at 6 × 10^3 cells/well in 96-well plates. Cells were allowed to adhere for overnight, and then the cells were changed to fresh medium containing various concentrations of solasonine dissolved in DMSO (final concentration, 0.1%). After 48 h incubation, the growth of cells was measured. The effect on cell viability was assessed as the percent cell viability compared with untreated control group, which were arbitrarily assigned 100% viability. The solasonine concentration required to cause 50% cell growth inhibition (IC_{50}) was determined by interpolation from dose-response curves. The OD values were determined. All experiments were performed in triplicate.

In vitro migration assay

Scratch assay (wound healing assay) was performed to detect cell migration. The cells were grown to full confluence in six-well plates and wounded with a sterile 100 ul pipette tip after 6 h of serum starvation and then washed with starvation medium to remove detached cells from the plates. Cells were treated with indicated doses of solasonine in full medium and kept in a CO_2 incubator. After 48 h, medium was replaced with phosphate buffered saline (PBS) buffer, the wound gap was observed, and cells were photographed using a Leica DM 14000B microscope fitted with digital camera.

Colony formation assay

To analyze the cell sensitivity to solasonine, we used a colony formation assay in vitro. Briefly, U87 cells (0.8 × 10^3 per well) were seeded in six-well plate containing 2 ml growth medium with 10% FBS and cultured for 24 h. Then, removed the medium, and cells were exposed to various concentrations of solasonine. After 24 h, cells were washed with PBS and supplemented fresh medium containing 10% FBS. The cultures were maintained in a 37°C, 5% CO_2 incubator for 14 days, allowing viable cells to grow into macroscopic colonies. Removed the medium, and the colonies were counted after staining with 0.1% crystal violet.

Apoptosis assay

Apoptosis was measured by fluorescence-activated cell sorter (FACS) using the Annexin V-FITC Apoptosis Detection Kit (Nanjing KeyGEN Biotech. CO., LTD.). In brief, cells plated in 6-well plates were treated with solasonine. After treatment of 12 h, cells were collected and washed once with cold PBS, and subsequently stained simultaneously with FITC-labeled annexin V and PI. Stained cells were analyzed using FACS Accuri C6 (Genetimes Technology Inc.).

Cell cycle analysis by flow cytometry

U87 MG cells were incubated with different concentrations of solasonine for 24 h. Then, the cells were trypsinized into single cells and collected, washed with phosphate-buffered saline (PBS), and then suspended in a staining buffer (10 μg/mL propidium iodide, 0.5% Tween 20, 0.1% RNase in PBS). The stained cells were analyzed by flow cytometer with CellQuest acquisition and analysis software program (Becton Dickinson and Co., San Jose, CA).

Mitochondrial membrane potentials assay

JC-1 probe was employed to measure mitochondrial depolarization in glioma cells. Briefly, Cells cultured in six-well plates after indicated treatments were incubated with an equal volume of JC-1 staining solution (5 μg/ml) at 37°C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using an Olympus fluorescent microscope under Argon-ion 488 nm laser excitation. Mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio.

Western blotting analysis

Cell lysate proteins were separated by electrophoresis on a 7.5-12% sodium dodecyl sulfate-polyacrylamide minigels (SDS-PAGE) and then electrophoretically transferred to a PVDF membrane. Western blots were probed with the specific antibodies. The protein bands were detected by enhanced chemiluminescence. Similar experiments were performed at least three times. The total protein concentration was determined using a BCA protein assay kit.

Quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from U87 MG cells after treatment with solasonine for 48 h, using TRIzol reagent according to the kit protocol (TaKaRa Bio, Dalian, China). cDNA was reverse-transcribed using the PrimeScript RT Reagent Kit (TaKaRa Bio, Dalian, China) according to the manufacturer's instructions. The Q-PCR reaction was performed following the kit protocol (TaKaRa Bio, Dalian, China), and amplification was performed using the Mx3005P Real-Time PCR System (Agilent, CA, USA). The relative mRNA expression of each gene was normalized to GAPDH RNA levels and analyzed using the $2^{-\Delta\Delta CT}$ method. The primers were synthesized by Invitrogen (Shanghai, China).

Confocal immunofluorescence

Immunofluorescence staining was done in cells cultured in chamber slides. After solasonine treatment, the cells were washed in phosphate-buffered saline (PBS) and fixed for 10 min at room temperature (RT) with 4% paraformaldehyde. The samples were permeabilized with 0.2% TritonX-100 for 5 min. And then blocked with 10% bovine serum albumin (BSA) in PBS for 30 min. Antibodies against cytochrome c, p65 and p50 in the 1% blocking solution were added to the sample and incubated for overnight at 4°C. Following three 10-min washes with PBS, fluorescein isothiocyanate- and rhodamine conjugated secondary antibodies were added in 1% blocking solutions and incubated for 1 hr. Subsequently, the stained samples were mounted with 4',6-diamidino-2-phenylindole (DAPI)-containing Vectashield solution (Vector Laboratories Inc.) to counterstain cell nuclei. After five additional 5-min washes, samples were examined with a Leica DM 14000B confocal microscope.

Animal studies

All animals were maintained, and animal experiments were done in SPF Laboratory Animal Center at Dalian medical university. The animals used in this study were female nu/nu mice (4-6 weeks old). To evaluate the therapeutic efficacy of solasonine in a human U87 orthotopic glioma mouse model, U87 cells (2×10^6 in 100 μL PBS) were injected subcutaneously near the axillary fossa of the nude mice using a 27-gauge needle. The tumor cell-inoculated mice were randomly divided into three treatment groups that each contained five mice. Two weeks later, when the tumor diameters reached 3 mm × 4 mm, group A was treated with PBS; group B and group C with solasonine by intraperitoneal injection every day. Tumors were measured with a caliper every 2 days, and the tumor volume was calculated using the formula $V = 1/2 (\text{width}^2 \times \text{length})$. Body weights were also recorded. On day 30 after tumor cell inocu-

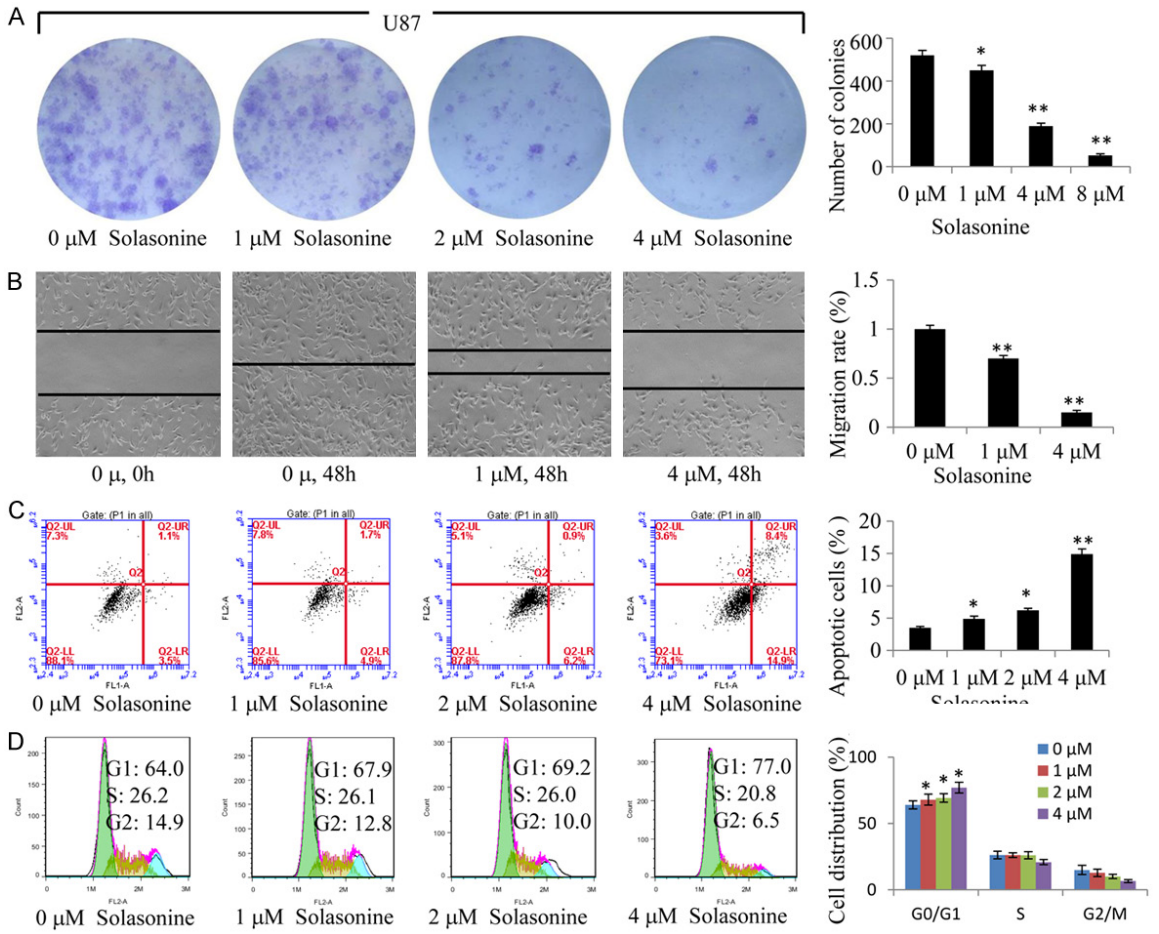

Figure 2. Solasonine suppressed cell colony formation and migration, promoted apoptosis and altered cell cycle. A: The tumor cell U87 MG-induced colony formation was analyzed, and the colony formation rate was calculated. B: Solasonine inhibits U87 MG migration in wound healing assay. U87 MG were plated, scratched and then incubated with solasonine with indicated doses. Cell migration was measured by manual counting. C: The apoptosis analyzed with FCAS. The experiments were repeated for three independent times. Results represent the mean ± SD of three experiments. DMSO treated group. D: U87 MG cell cycle analysis were performed after 24 h of solasonine treatment by FACS vantage flow cytometer. The experiments were repeated three times. Results represent the mean ± SD of three experiments. *P < 0.05 and **P < 0.01 vs. DMSO-treated group.

lation, all experimental mice were terminated with ether anesthesia and the total weight of the tumors in each mouse was measured. To determine p65 NF-κB expression, the tumor tissues were harvested and freshly fixed with 10% neutral formalin and desiccated and embedded in paraffin. 4 μm sections were stained with hematoxylin and eosin, p-p65 NF-κB (1:150) antibody, and examined under a light microscope. The images were examined under a Leica DM 4000B fluorescence microscope equipped with a digital camera.

All animal maintenance and procedures were carried out in strict accordance with the recommendations established by the Animal Care and Ethics Committee of Dalian Medical University as well as the guidelines by the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Animal Care and Ethics Committee of Dalian Medical University. In animal study, all efforts were made to minimize suffering of mice. All mice were humanely sacrificed by ether anesthesia inhalation before death.

Statistical analysis

All experiments were repeated three times. Data are represented as mean ± standard deviation (SD). Analysis of variance and Student's

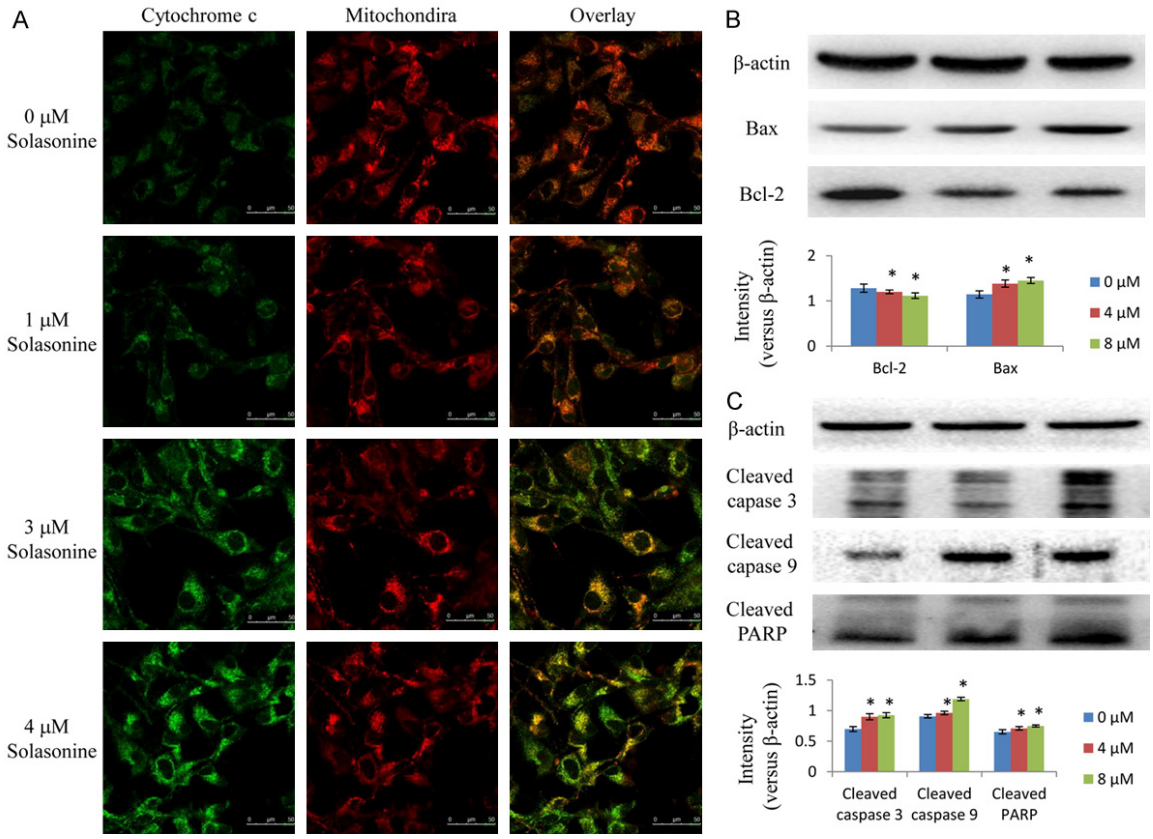

Figure 3. Solasonine promoted cytochrome c release and downstream caspase activation. (A) The release of cytochrome c in U87 MG cells was determined by immunofluorescence imaging analysis to monitor cytochrome c release from the mitochondrial intermembrane space into the cytosol. Cytochrome c (green) shows a continuously enhanced pattern with the use of increasing doses of solasonine and co-localizes with the mitochondria (red) in U87 MG cells. The levels of the Bax and Bcl-2 (B), cleaved caspase-3/9 and cleaved PARP proteins (C) in U87 MG cells was analyzed by western blotting. Bar = 50 μm. n = 3. (*P < 0.05, significant differences between solasonine treatment groups and control groups).

t-test were used to compare the values of the test and control samples *in vitro* and *in vivo*. P < 0.05 was considered to be a statistically significant difference. SPSS 17.0 software was used for all statistical analysis.

Results

Solasonine inhibited glioma cell proliferation and changed cell morphology

The anti-tumor effects of solasonine were detected in U87 MG, U251 and U118 MG cells. Firstly, we quantitatively examined the effect of solasonine (**Figure 1A**) on cell morphology and morphological observation indicated that solasonine could obviously impact the growth and the morphology of U87 MG cells at indicated doses (**Figure 1B**). By MTT assay, solasonine inhibited the proliferation of the three cell lines in a concentration-dependent manner (**Figure 1C**). Data showed that the IC_{50} values of solasonine for U87 MG cells, U251 and U118 MG cells were 3.97, 6.68 and 13.72 μM, respectively.

Solasonine suppressed cell colony formation and migration, together promoted apoptosis and altered cell cycle

Consistent with cell proliferation inhibition, solasonine also significantly inhibited colony formation and resulted in a remarkable decrease at colony formation ratio (**Figure 2A**). Wound-healing assay further revealed the inhibition effect of solasonine on tumor cell mobility in U87 MG cells (**Figure 2B**). Subsequently, the apoptosis of U87 MG induced by solasonine was detected. By Annexin V-FITC apoptosis detection, it was observed that the solasonine-induced apoptosis in U87 MG cells was incre-

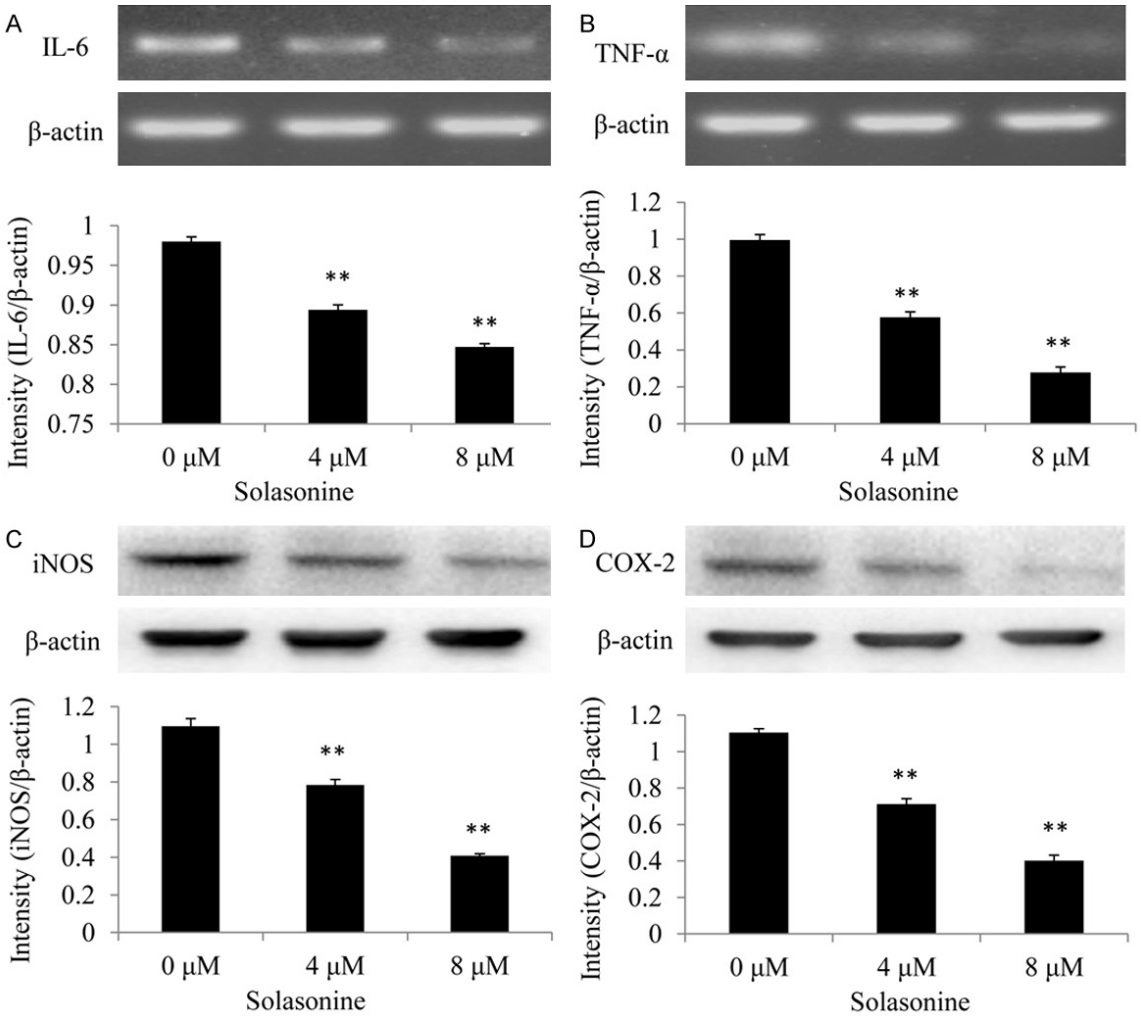

Figure 4. Effect of solasonine on the expression of proinflammatory mediators in U87 MG cells. Human U87 MG cells were treated with solasonine at the indicated doses. At 48 h after treatment, expression levels of IL-6 and TNF-α gene were analyzed by RT-qPCR (A, B), expression levels of iNOS and COX-2 protein were analyzed by western blotting (C, D) in U87 MG cells, respectively. The data are presented as mean ± SD of three tests. (**P < 0.01, significant differences between solasonine treatment groups and control groups).

ased in a dosage-dependent manner (**Figure 2C**). To understand the inhibitory mechanism of solasonine on cell growth, cell cycle analysis was performed. The U87 MG cells were exposed to the indicated concentrations of solasonine for 24 h, and as shown in **Figure 2D**, cells were arrested in G1 phase, the percentage of cells in S phase and G2/M phase decreased.

Solasonine induced apoptosis via modulating cytochrome c and caspase signaling

Induction of apoptosis in target cells is a key mechanism for anti-cancer therapy. We next seek to determine the dose-dependent effect of solasonine on apoptosis. The results indicated that solasonine treatment resulted in a significant dose-dependent induction of apoptosis in U87 MG cells. Through immunofluorescence imaging analysis (**Figure 3A**), cytochrome c (green) shows a continuously enhanced pattern with the use of increasing doses of solasonine and co-localizes with the mitochondria (red) in U87 MG cells. And cytochrome c that release from the mitochondrial intermembrane space into the cytosol increased obviously with increase doses of solasonine. Next, the levels of the Bax and Bcl-2, together the cleaved caspase-3/9 and cleaved PARP proteins in U87 MG cells was further analyzed by Western blotting (**Figure 3B**, **3C**). Intriguingly, high Bax/Bcl-2

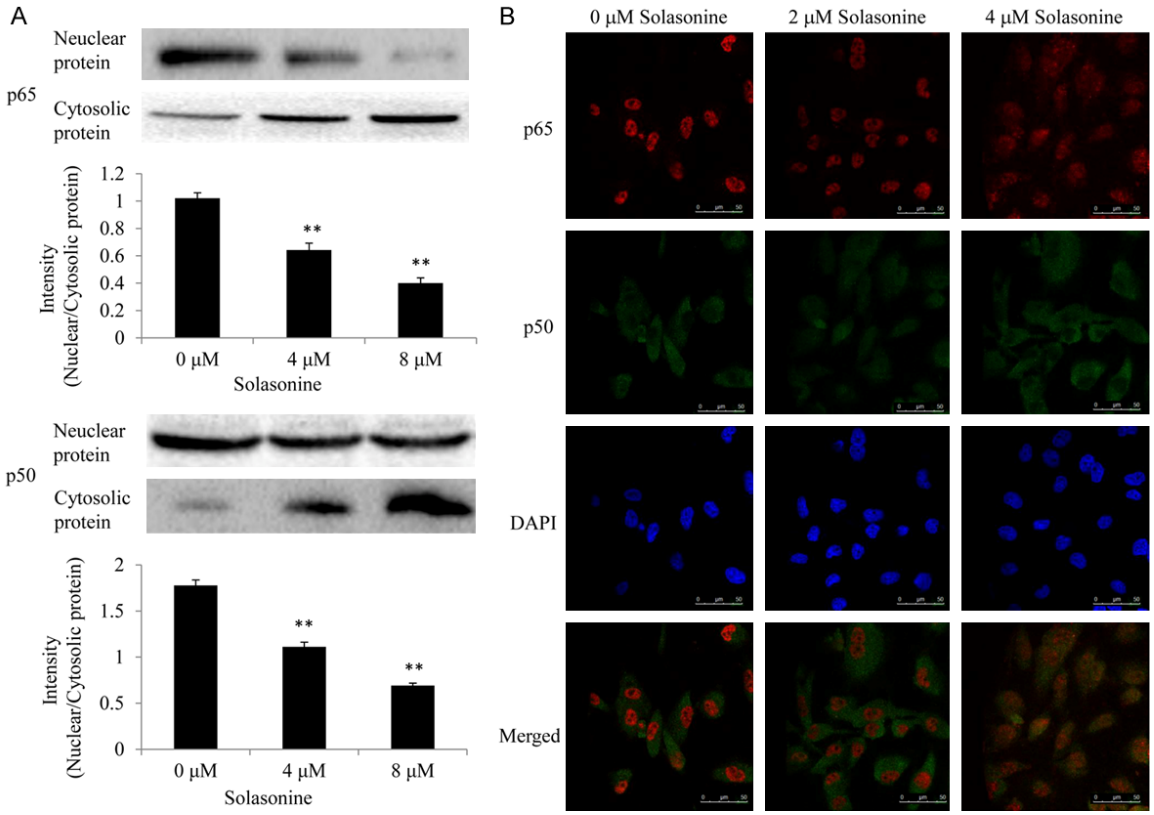

Figure 5. Solasonine inhibited NF-κB translocation from cytoplasm to nuclei in U87 MG cells. A: Expression levels of nuclear and cytosolic NF-κB were analyzed by western blotting respectively, and quantitative analysis was exerted to compare the translocation level of p65 and p50 between at the indicated doses of solasonine. B: At 24 h after treatment, the subcellular localization of p50 and p65 and the colocalization of p65 with p50 were examined by confocal microscopy analysis with a confocal microscope. Cells with typical morphology were presented. Bar = 50 μm. n = 3. (**P < 0.01 compared with the control cultures).

ratio is the most important index of apoptosis mediated through the intrinsic mitochondrial mechanism [25], and solasonine could induce high Bax/Bcl-2 ratio. Besides, solasonine could markedly increase the expression levels of the cleaved caspase-3, caspase-9 and PARP proteins as compared with the control group (**Figure 3C**). These results demonstrated that solasonine could induce glioma cell apoptosis through triggering cytochrome c release from mitochondria and facilitating the downstream apoptosome assembly and caspase activation in the cytosol.

Solasonine decreased the expression of proinflammatory mediators

To assess the anti-inflammatory activity of solasonine, the expression of proinflammatory mediators in U87 MG cells were further examined. At 48 h after treatment, expression levels of IL-6 and TNF-α gene were analyzed by RT-qPCR (**Figure 4A**, **4B**), expression levels of iNOS and COX-2 protein were analyzed by Western blotting (**Figure 4C**, **4D**) in U87 MG cells, respectively. It was observed that solasonine reduced the protein expression of iNOS and COX-2, and decreased the gene expression of IL-6 and TNF-α in a dose-dependent manner. All these confirmed that in the treatment groups with either 4 or 8 μM solasonine, proinflammatory mediators expressions were significantly reduced compared with the vehicle group. These initial results indicated that solasonine could be of strong anti-inflammatory activity *in vitro* in glioma.

Solasonine inhibited NF-κB translocation from cytoplasm to nuclei

NF-κB is present in the cytosol in an inactive state. To further investigate the relationship between solasonine-induced cell proliferation and the activated signaling pathways in glioma,

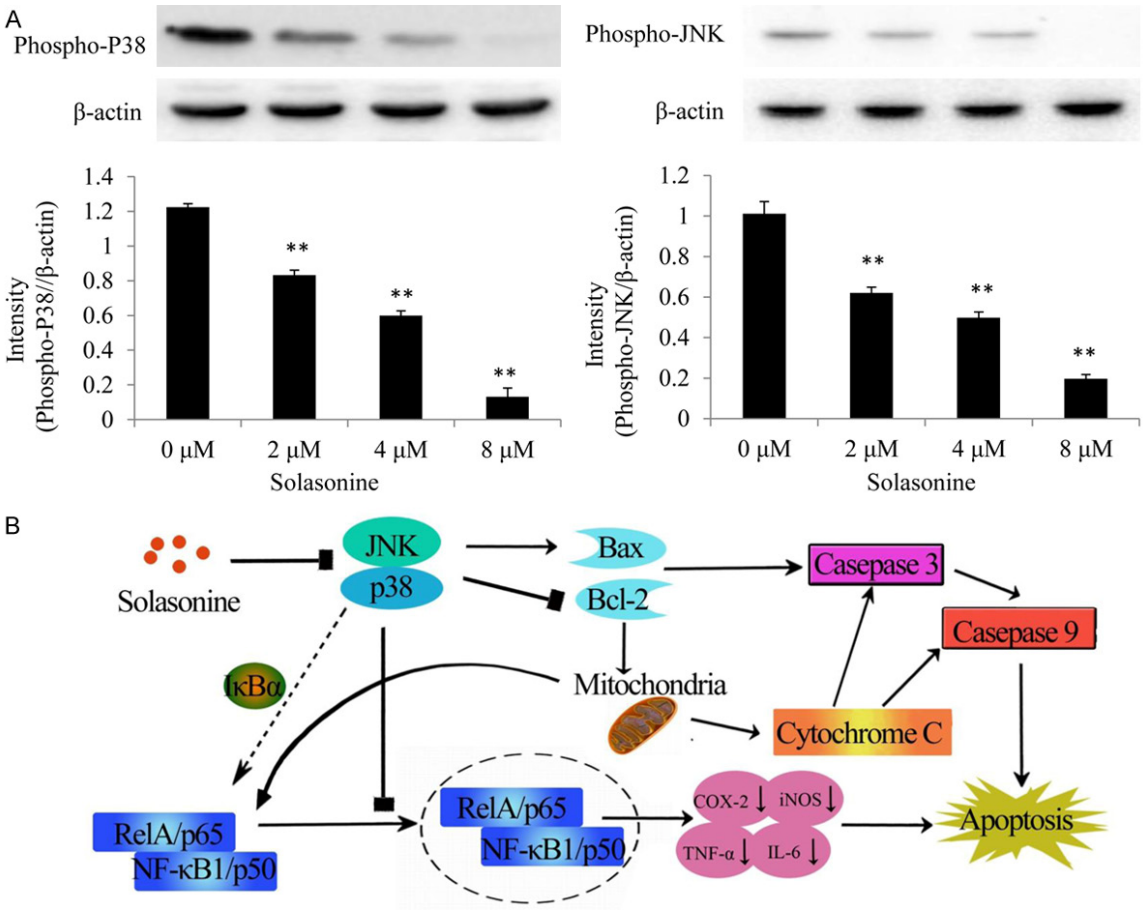

Figure 6. Solasonine suppressed the inflammatory response through partial regulation of MAPK signaling, by targeting p-p38 and p-JNK in glioma. A: Solasonine suppressed phosphorylation of P38 and JNK MAPKs in U87 MG cells. (**P < 0.01 compared with the control cultures). B: The anti-inflammatory mechanism of solasonine regarding its effect on NF-κB signaling pathway.

U87 MG cells were stimulated with different concentrations of solasonine. Constitutive translocation of NF-κB p50/p65 to the cell nucleus were detected in U87 MG cells were detected by Western blotting (**Figure 5A**). We next performed immunofluorescence assay to confirm the nuclear localization of NF-κB and the colocalization of p50 and p65 in U87 MG cells by confocal microscopy analysis with a confocal microscope (**Figure 5B**). The results confirmed that solasonine inhibited NF-κB signaling pathway in U87 MG cell, leading to the inhibition of nuclear translocation of the p50-p65 subunits of NF-κB, which could be followed by p65 phosphorylation. Thus the effect of solasonine on p65 phosphorylation was further explored in the following section regarding the *in vivo* study. The results indicate that the inhibition of U87 MG cell proliferation by dopamine might be mediated by inhibiting NF-κB translocation from cytoplasm to cell nuclei.

Solasonine suppressed phosphorylation of p38 and JNK MAPKs

The phosphorylation status of these MAPKs was investigated by Western blotting analysis (**Figure 6A**). Solasonine suppressed phosphorylation of p38 and JNK MAPKs in U87 MG cells. All these indicate that solasonine suppresses the inflammatory response through partial regulation of MAPK signaling, by targeting p-p38 and p-JNK in glioma. Besides, the anti-inflammatory mechanisms of solasonine regarding its effect on inflammatory signaling pathway were concluded in graphical representation (**Figure 6B**). The molecular activity of solasonine will be our next area of investigation.

Solasonine inhibited the growth of glioma xenografts in nude mice

In order to detect the inhibitory effect of solasonine on U87 MG cells in vivo, we performed

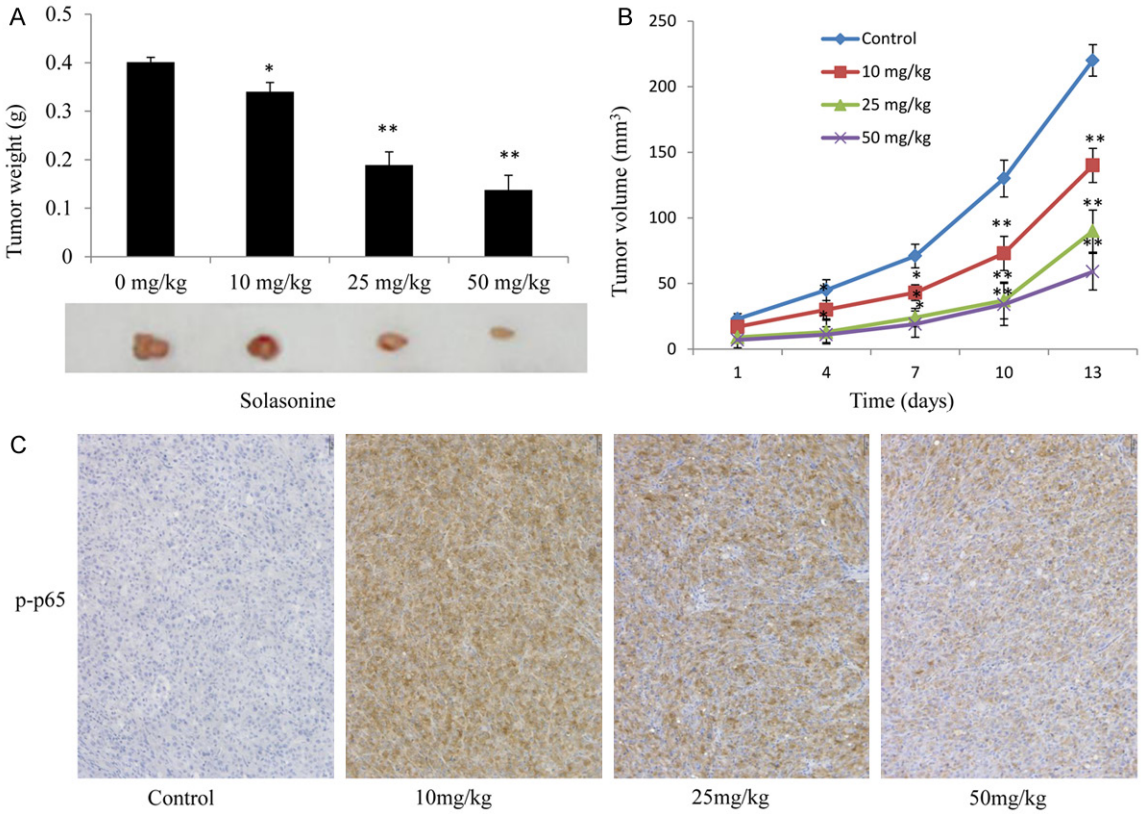

Figure 7. Solasonine inhibited tumor growth in glioma mouse models. An orthotopic mouse model of human U87 MG was used to evaluate the effect of solasonine. A: Photographic illustration of tumors from control and solasonine-treated nude mice on the day of sacrifice (day 13), and the tumor weights of them were showed in the graph. B: Tumor volumes were also measured. C: Immunohistochemical analysis of p-p65 protein expression in tumor samples. Neutral formalin fixed tumor samples were prepared from animals and analyzed by immunohistochemical staining with rabbit anti-rabbit second antibody using the Vectastain Elite ABC kit, and examined under a microscope. (*P < 0.05 and **P < 0.01 compared with the control groups).

elementary tumor xenografts in nude mice. In these 13 days, each group sacrificed one mouse. On the 13th day, the weights of the tumors were weighed after dissection. As shown in **Figure 7A**, **7B**, solasonine treatments resulted in significant tumor suppression compared with the control group. Besides, since p65 phosphorylation could lead to the nuclear translocation of NF-κB, we further examined the effect of solasonine on p65 phosphorylation. The immunohistochemical staining assay was used to determine the expression of p-p65 (the activated form of p65). The expression levels of p-p65 were significantly decreased with solasonine treatment *in vivo*, as compared with the vehicle group (**Figure 7C**).

Discussion

Currently there is little known about the biological effects of solasonine *in vivo* and *in vitro*. Solasonine could be of better drug ability, but only few researches have studied on its anticancer activities. In the present study, we found that solasonine could effectively inhibit glioma cells growth and enhance apoptosis induction dose-dependently with IC_{50} of only 6 μM. Furthermore, we showed that the effects of solasonine on glioma cells growth and apoptosis were mediated through inhibiting NF-κB signaling pathway. Here, to the best of our knowledge, it might be the first time to report the treatment of solasonine on glioma growth and to demonstrate the underlying mechanisms both *in vitro* and *in vivo*.

In our study, considering that U87 MG cell line is more applicable for morphological observation and has a higher ability to form xenograft in nude mice, we performed almost *in vitro* experiments in U87 MG cells to study the molecular mechanism of the anticancer effect of solasonine.

Recent literature [26, 27] from the past decade have pointed to a complex relationship between inflammation and tumor growth. Data now support mechanisms through which an inflammatory microenvironment may drive tumorigenesis [28, 29]. Models of inflammation-induced cancers of the body begin with a normal cell acquiring a mutation that confers survival advantages relative to its neighbors, and similar mechanisms have been reported in the context of gliomas. As immunotherapy gains traction as a brain cancer therapeutic [30], it is especially important to consider the role of inflammation in gliomagenesis. Very complex and seemingly competing mechanisms of inflammation may both suppress and stimulate a glioma's inflammatory microenvironment, the balance of which drives malignancy. Despite inflammation's dichotomous effects on gliomagenesis, the summation of evidence suggests that the net effect of an inflammatory microenvironment is immunosuppressive, a characteristic which has hampered the success of various glioma immunotherapies [31, 32]. All these indicate that anti-inflammatory mechanism could be potential for discovering new drugs for glioma treatment.

The intracellular pathways involved in inflammatory reactions are complicated, thus, the precise mechanism of action by which solasonine inhibits its cellular targets was investigated. Glioma tumor growth is advanced by an inflammatory microenvironment involving various inflammatory mediators like COX-2, iNOS, TNF-α and IL-6, leading to enhanced vasodilation, proliferation [33], migration [34], and the rapid growth of gliomas [35]. Thus we explore whether solasonine could inhibit the expression of inflammatory mediators. Besides, it is known that NF-κB is a central regulator of the inflammatory process and plays a critical role in inflammation. Not only is NF-κB aberrantly activated in glioma but it also serve as a nodal point where a group of upstream signaling pathways converge. And such node subsequently regulate several downstream effectors associated with tumor progression [36]. Since NF-κB regulates the expression of a group of proinflammatory mediators mentioned above, we further seek to explore the effect of solasonine on NF-κB activity. We found that solasonine could inhibit translocation of the NF-κB p65/p50 proteins from cell cytoplasm to nucleus. All these could indicate the involvement of anti-inflammatory pathway in the anticancer effect of solasonine.

MAPK pathways are pivotal in regulation of inflammation and production of inflammatory mediators. It has been reported that the activation of NF-κB is triggered by MAPKs ERK, JNK, and p38 MAPK [37]. However, other reports showed a negative regulation between NF-κB and MAPKs [38]. Therefore, the relationship between NF-κB and MAPKs is complex and appears to depend on the cell type and stimulus. In U87 MG cells, solasonine also ameliorates inflammatory states through inhibition of JNK and p38 phosphorylation, but ERK MAPK phosphorylation. This indicates that solasonine works with different mechanisms in different cell lines. The present results showed that solasonine specifically targets p-p38 and p-JNK, which is a very interesting finding. Studies assessing the molecular mechanisms by which the small structural identity of solasonine produces specific recognition and inhibition of target MAPKs are underway. In conclusion, as illustrated above, we showed that solasonine suppressed inflammation in glioma cells via suppression of NF-κB, and p-p38 and p-JNK MAPKs.

In conclusion, we have shown that solasonine inhibits glioma growth through down-regulation of the canonical NF-κB signaling pathway, as well as via suppression of MAPK signaling mediated through p38 and JNK. These findings might prove useful in establishing new drugs for anticancer therapy especially for malignant glioma for which current treatment options are still limited.

Acknowledgements

This work is supported by grants from National Natural Science Foundation of China (Nos. 81-372714, 81672480), Liaoning Provincial Natural Science Foundation of China (No. 2016-02244), Distinguished Professor Project of Liaoning Province, Special Grant for Translational Medicine, Dalian Medical University (No. 2015002).

Disclosure of conflict of interest

None.

Address correspondence to: Bo Zhang, Department of Neurosurgery, The Second Affiliated Hospital of

Dalian Medical University, 467 Zhong Shan Road, Dalian 116023, P. R. China. Tel: +8617709872377; Fax: +86-411-8467-2130; E-mail: zhangbodlykdx@126.com

References

[1] Nussbaumer S, Bonnabry P, Veuthey JL and Fleury-Souverain S. Analysis of anticancer drugs: a review. Talanta 2011; 85: 2265-2289.

[2] Cragg GM and Newman DJ. Antineoplastic agents from natural sources: achievements and future directions. Expert Opin Invest Drugs 2000; 9: 1-15.

[3] Dall' Agnol R and Von Poser GL. The use of complex polysaccharides in the management of metabolic diseases: the case of solanum lycocarpum fruits. J Ethnopharmacol 2000; 71: 337-341.

[4] Lin CC, Lin WC, Yang SR and Shieh DE. Anti-inflammatory and hepatoprotective effects of solanum alatum. Am J Chin Med 1995; 23: 65-69.

[5] Vieira G Jr, Ferreira PM, Matos LG, Ferreira EC, Rodovalho W, Ferri PH, Ferreira HD and Costa EA. Anti-inflammatory effect of solanum lycocarpum fruits. Phytother Res 2003; 17: 892-896.

[6] Grace MH and Saleh MM. Hepato-protective effect of daturaolone isolated from solanum arundo. Pharmazie 1996; 51: 593-595.

[7] Ibarrola DA, Hellio'n-Ibarrola M and Montalbetti Y. Isolation of hypotensive compounds from Solanum sisymbriifolium Lam. J Ethnopharmacol 2000; 70: 301-307.

[8] Kim HM and Lee EJ. Solanum lyratum inhibits anaphylactic reaction and suppress the expression of L-histidine decarboxylase mRNA. Immunopharmacol Immunotoxicol 1998; 20: 135-146.

[9] Maruo VM, Bernardi MM and Spinosa HS. Toxicology evaluations of long-term consumption of solanum lycocarpum St. Hill fruits in male and female adult rats. Phytomedicine 2003; 10: 48-52.

[10] Maruo VM, Soares MR, Bernardi MM and Spinosa HS. Embryotoxic effects of solanum lycocarpum St. Hill fruits consumption using preimplantation and organogenesis in rats. Neurotoxicol Teratol 2003; 25: 627-631.

[11] Blankemeyer JT, McWilliams ML, Rayburn JR, Weissenberg M and Friedman M. Developmental toxicology of solamargine glycoalkaloids in frog embryos. Food Chem Toxicol 1998; 36: 383-389.

[12] Haraguchi M, Uchimura RT, Motidome M and Gottlieb O. Aproveitamento dos estero'ides dos frutos da lobeira. Cien Cult 1978; 32: 81-82.

[13] Munari CC, de Oliveira PF, Campos JC, Martins Sde P, Da Costa JC, Bastos JK and Tavares DC. Antiproliferative activity of solanum lycocarpum alkaloidic extract and their constituents, solamargine and solasonine, in tumor cell lines. J Nat Med 2014; 68: 236-41.

[14] Kleihues P and Ohgaki H. Primary and secondary glioblastomas: from concept to clinical diagnosis. Neuro Oncol 1999; 1: 44-51.

[15] Olson JJ, Nayak L, Ormond DR, Wen PY, Kalkanis SN; AANS/CNS Joint Guidelines Committee. The role of cytotoxic chemotherapy in the management of progressive glioblastoma: a systematic review and evidence-based clinical practice guideline. J Neurooncol 2014; 118: 501-555.

[16] Lefranc F, Sadeghi N, Camby I, Metens T, Dewitte O and Kiss R. Present and potential future issues in glioblastoma treatment. Expert Rev Anticancer Ther 2006; 6: 719-732.

[17] Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, Wolinsky Y, Kruchko C and Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. Neuro Oncol 2015; 17 Suppl 4: iv1-iv62.

[18] Grivennikov SI, Greten FR and Karin M. Immunity, inflammation, and cancer. Cell 2010; 140: 883-99.

[19] Aggarwal BB, Vijayalekshmi RV and Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 2009; 15: 425-30.

[20] Yeung YT, McDonald KL, Grewal T and Munoz L. Interleukins in glioblastoma pathophysiology: implications for therapy. Br J Pharmacol 2013; 168: 591-606.

[21] Linos E, Raine T, Alonso A and Michaud D. Atopy and risk of brain tumors: a meta-analysis. J Natl Cancer Inst 2007; 99: 1544-50.

[22] Rahman A and Fazal F. Blocking NF-κB: an inflammatory issue. Proc Am Thorac Soc 2011; 8: 497-503.

[23] Chung TW, Moon SK, Chang YC, Ko JH, Lee YC, Cho G, Kim SH, Kim JG and Kim CH. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J 2004; 18: 1670-81.

[24] Karin M, Yamamoto Y and Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004; 3: 17e26.

[25] Kikuchi T, Nihei M, Nagai H, Fukushi H, Tabata K, Suzuki T and Akihisa T. Albanol A from the root bark of Morus alba L. induces apoptotic cell death in HL60 human leukemia cell line. Chem Pharm Bull (Tokyo) 2010; 58: 568-571.

[26] Grivennikov SI, Greten FR and Karin M. Immunity, inflammation, and cancer. Cell 2010; 140: 883-899.

[27] Sowers JL, Johnson KM, Conrad C, Patterson JT and Sowers LC. The role of inflammation in brain cancer. Adv Exp Med Biol 2014; 816: 75-105.
[28] Mantovani A, Allavena P, Sica A and Balkwill F. Cancer-related inflammation. Nature 2008; 454: 436-444.
[29] Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006; 441: 431-436.
[30] Vlahovic G, Fecci PE, Reardon D and Sampson JH. Programmed death ligand 1 (PDL1) as an immunotherapy target in patients with glioblastoma. Neuro Oncol 2015; 17: 1043-1045.
[31] Charles NA, Holland EC, Gilbertson R, Glass R and Kettenmann H. The brain tumor microenvironment. Glia 2011; 59: 1169-1180.
[32] Joseph P Antonios, Horacio Soto, Robert M Prins, Isaac Yang, Noriyuki Kasahara, Linda M Liau and Carol A Kruse. Chronic inflammation drives glioma growth: cellular and molecular factors responsible for an immunosuppressive microenvironment. Neuroimmunol Neuroinflammation 2014; 1: 66-76.
[33] Munoz-Fernandez MA and Fresno M. Involvement of nitric oxide on the cytokine induced growth of glial cell. Biochem Biophys Res Commun 1993; 194: 319-25.
[34] Yeh WL, Lu DY, Liou HC and Fu WM. A forward loop between glioma and microglia: glioma-derived extracellular matrix-activated microglia secrete IL-18 to enhance the migration of glioma cells. J Cell Physiol 2012; 227: 558-68.
[35] Cobbs CS, Whisenhunt TR, Wesemann DR, Harkins LE, Van Meir EG and Samanta M. Inactivation of wild-type p53 protein function by reactive oxygen and nitrogen species in malignant glioma cells. Cancer Res 2003; 63: 8670-3.
[36] Sen E. Targeting inflammation-induced transcription factor activation: an open frontier for glioma therapy. Drug Discov Today 2011; 16: 1044-51.
[37] Guha M and Mackman N. LPS induction of gene expression in human monocytes. Cell Signal 2001; 13: 85-94.
[38] Ahmed KM, Dong S, Fan M and Li JJ. Nuclear factor-kappaB p65 inhibits mitogen-activated protein kinase signaling pathway in radioresistant breast cancer cells. Mol Cancer Res 2006; 4: 945-955.