The role of endogenous reactive oxygen species in oxymatrine-induced caspase-3-dependent apoptosis in human melanoma A375 cells

Yingfan Zhang[a], Huanhai Liu[b], Jiyang Jin[c], Xiaohai Zhu[a], Lixuan Lu[a] and Hua Jiang[a]

Rapid increases in incidence and mortality of human malignant melanoma are observed worldwide; thus, the development of new effective chemicals to control melanoma is urgent. In this study, the cytotoxic effect of oxymatrine, a natural quinolizidine alkaloid, against three human melanoma cell lines (A375, Sk-Mel-28, MM96L) and the underlying mechanisms were investigated. Oxymatrine killed all three human melanoma cell lines in a dose-dependent manner. The compound also dose-dependently caused apoptosis in human melanoma A375 cells. In addition, oxymatrine induced a remarkable change in mitochondrial membrane potential and triggered the release of cytochrome *c* from mitochondria to cytosol. Furthermore, this small compound resulted in a marked activation of capase-3, caspase-9, and poly (ADP-ribose) polymerase, while caspase-3 inhibitor Z-DEVD-FMK significantly reversed the proapoptotic effect of oxymatrine in A375 cells. Moreover, oxymatrine also dose-dependently increased the generation of reactive oxygen species in A375 cells, and *N*-acetylcysteine, a reactive oxygen species production inhibitor, almost completely blocked oxymatrine-induced apoptosis. In conclusion, our findings suggest that oxymatrine triggers oxidative stress, resulting in the collapse of the mitochondrial transmembrane potential, which in turn leads to cytochrome *c* release and apoptosis through the intrinsic caspase-9/caspase-3 pathway in human melanoma A375 cells. *Anti-Cancer Drugs* 21: 494–501 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2010, **21**:494–501

Keywords: A375 cells, apoptosis, melanoma, oxymatrine, reactive oxygen species

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Received 31 October 2009 **Revised form accepted** 21 December 2009

Introduction

Apoptosis is arguably the most potent defense against cancer because it is the mechanism used by metazoans to eliminate deleterious cells. Many chemopreventive agents seem to target signaling intermediates in apoptosis-inducing pathways. Inherently, the process of carcinogenesis selects against apoptosis to initiate, promote, and perpetuate the malignant phenotype. Thus, targeting apoptosis pathways in premalignant and malignant cells may be an effective method of cancer prevention [1]. It is obvious that mitochondrial dysfunction plays an important role in apoptosis [2,3]. An increase in reactive oxygen species (ROS) and a consequent loss of mitochondrial membrane potential are reported as typical phenomena in the process of apoptosis related to mitochondria [4–6]. ROS, which is known to affect mitochondrial membrane potential, triggers mitochondria-associated cell apoptosis [7–9]. Both the mitochondrial structural and functional integrity account for redox balance. When the balance is disrupted, excess ROS is produced, followed by oxidative stress [10]. In tumor cells, the oxidative stress can be alleviated by several antioxidants, such as reduced glutathione (GSH) and superoxide dismutase. GSH depletion-mediated cell death is also induced by accumulation of ROS in mitochondria, which is an early signal of mitochondria-dependent ROS-induced cell apoptosis [11].

Oxymatrine is one of the alkaloids extracted from the Chinese herb *Sophora japonica* (*Sophora flavescens Ait*.) with the activities of protecting ischemia reperfusion injury, inhibiting acute lung injury, and preventing lung fibrosis and tumors [12–17]. However, the mechanism of action of oxymatrine on human malignant melanoma is as yet unknown. Considering the variety of pharmacological activities of oxymatrine, we investigate whether oxymatrine exerts potent antitumor activity on human malignant melanoma. The findings of this study suggest that oxymatrine triggers oxidative stress of human melanoma A375 cells, resulting in the collapse of mitochondrial transmembrane potential, which in turn leads to cytochrome *c* release and apoptosis through the intrinsic caspase-9/caspase-3 pathway. The antioxidant *N*-acetyl cysteine (NAC), a ROS production inhibitor, almost reverses oxymatrine-induced apoptosis. Collectively, our results show that the

0959-4973 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328336e927

accumulation of ROS plays an important role in oxymatrine-induced mitochondrial signaling pathway. This may herald a novel approach for further studies of oxymatrine as a candidate for the treatment of human tumors, especially melanoma.

Materials and methods

Cell lines

Human melanoma A375, Sk-Mel-28, and MM96L cells and human keratinocytes HaCaT cells were maintained in Dulbecco's modified Eagle's medium (4.5 g/l glucose; Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal calf serum (Gibco, Invitrogen, Carlsbad, California, USA) plus 2 mmol/l of glutamine and 50 U/ml of penicillin. All of the cell lines were purchased from the Shanghai Institute of Cell Biology (Shanghai, China), and were grown at 37°C in a 5% (v/v) CO_2 atmosphere.

Drugs and reagents

Oxymatrine (purity > 99%, obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) was dissolved at a concentration of 20 mmol/l in 100% dimethyl sulfoxide (DMSO) as a stock solution, stored at −20°C, and diluted with medium before each experiment. The final DMSO concentration did not exceed 0.1% throughout the study (all the control groups are composed of 0.1% DMSO). 3-(4,5-Dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), the GSH synthesis inhibitor L-buthionine-(*S,R*)-sulfoximine, and NAC were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). NAC was freshly dissolved in medium at a stock concentration of 40 mmol/l. The pH was adjusted to 7.4, and the NAC was then sterilized by 0.22 μm filtration and diluted to different concentrations. All the NAC treatments mentioned in this study are pretreatments. The 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) and the 5-(and-6)-carboxy-2′-7′-dichlorofluorescin diacetate (carboxy-DCFDA) were purchased from Invitrogen. Stock solutions of carboxy-DCFDA (15 mmol/l) were dissolved in DMSO, and were then stored at −20°C. The annexin V-fluorescein isothiocyanate/propidium iodide (PI) kit was purchased from BD Biosciences (San Jose, California, USA), the caspase-3 specific inhibitor Z-DEVD-FMK was purchased from Calbiochem (San Diego, California, USA), the ProteoExtract Cytosol/Mitochondria Fractionation Kit was purchased from Merck Bioscience (Bad Soden, Germany), and the ApoGSH Glutathione Detection Kit was purchased from BioVision Research Products (Mountain View, California, USA). Anti-caspase-3, anti-cleaved caspase-3, anti-caspase-8, anti-cleaved caspase-9, and anti-poly(ADP-ribose) polymerase (PARP) were purchased from Cell Signaling Technology (Beverly, Massachusetts, USA). Anti-cytochrome *c*, anti-cytochrome *c* oxidase subunit IV (COX IV), and anti-α tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA). All other chemicals were purchased from Sigma Chemical Co.

Cytotoxicity assay

The cytotoxic activity of oxymatrine in three melanoma cell lines was measured using the MTT assay. Cell lines were seeded in 96-well microtiter plates (4000 cells/well). After treatment (5–40 μmol/l of oxymatrine for 72 h) in 96-well plates, MTT solution (5 mg/ml in RPMI 1640 medium; Sigma-Aldrich) was added (10 μl/well), and the plates were incubated for a further 4 h at 37°C. The purple formazan crystals were dissolved in 100 μl of DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Sunrise, Tecan, Austria) at 570 nm. Assays were performed in triplicate in three independent experiments. The concentration of drug inhibiting 50% of cells (IC_{50}) was calculated using the software of dose-effect analysis with microcomputers.

Cell apoptosis assay

The cells were staining with annexin V–fluorescein isothiocyanate/PI and then measured by flow cytometry [18]. Samples were analyzed by FACSCalibur flow cytometer (Becton Dickinson, San Jose, California, USA). Annexin V^+/PI^- cells were considered as apoptotic cells.

Subcellular fractionation

The proteins in the A375 cells were separated into cytosolic and mitochondrial fractions using the ProteoExtract Cytosol/Mitochondria Fractionation Kit (Merck Bioscience, Bad Soden, Germany) according to the manufacturer's instructions [19]. To check the selectivity of proteins from subcellular fractionation, tubulin and COX-IV were used as marker proteins representing the cytosolic and mitochondrial fractions, respectively.

Western blot

Proteins were extracted in lysis buffer (30 mmol/l Tris, pH 7.5, 150 mmol/l sodium chloride, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l sodium orthovanadate, 1% Nonidet P-40, 10% glycerol, and phosphatase and protease inhibitors). The proteins were then separated by SDS–PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were probed with antibodies overnight at 4°C, and then incubated with a horseradish peroxidase-coupled secondary antibody. Detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK).

Cell mitochondrial membrane potential assay

A375 cells were treated with or without oxymatrine (5, 10, 20 μmol/l) for 12 hand then harvested, and the disruption of mitochondrial transmembrane potential was measured using fluorochrome dye JC-1 by flow cytometry as reported earlier [20].

Detection of intracellular ROS

The production of intracellular ROS was measured in the cells using the oxidation-sensitive fluorescent dye carboxy-DCFDA [21]. An increase in green fluorescence intensity is used to quantify the generation of intracellular ROS. After adding carboxy-DCFDA at a final concentration of 15 μmol/l to the culture medium, the cells were incubated at 37°C for an additional 30 min and then harvested, washed with PBS, and measured immediately by FACSCalibur flow cytometer (Becton Dickinson).

Intracellular glutathione levels

GSH levels were determined by the ApoGSH Glutathione Detection Kit (Biovision) according to the manufacturer's instructions [22]. Briefly, the treated cells were harvested at 6 h posttreatment, and the samples were prepared by repeated freeze–thaw cycles. The fluorescence value was measured by a fluorescent mutilabel counter (Safire, Tecan, Austria). The total glutathione amount was calculated by applying the sample readings to the standard curve.

Statistical analysis

Data were expressed as mean values ± standard deviation (SD). They were statistically evaluated by the Student's *t*-test when only two value sets were compared, and one-way analysis of variance followed by Dunnett's test when the data involved three or more groups. *P* less than 0.05 or 0.01 was considered statistically significant, indicated by * and **, respectively.

Results

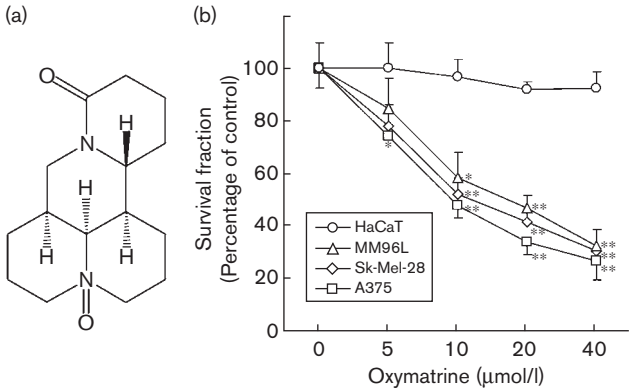
Oxymatrine killed human melanoma cells *in vitro*

The cytotoxic effect of oxymatrine was determined in three human melanoma cell lines (A375, Sk-Mel-28, MM96L). The dose–response curves are shown in Fig. 1b. All three cell lines exhibited dose-dependent sensitivity to oxymatrine (0–40 μmol/l) at 72 h, with IC_{50} values ranging from 9.1 to 16.4 μmol/l. The results suggested that oxymatrine had promising antimelanoma activity. It is important to note that oxymatrine at the concentrations mentioned above did not affect nontumor human keratinocytes HaCaT cell viability by the MTT uptake assay (Fig. 1b). Oxymatrine selectively killed human melanoma cell lines without affecting nontumor human keratinocytes *in vitro*, indicating that the selective antitumor action of oxymatrine observed here.

Oxymatrine triggered significant apoptosis in A375 cells

To assess whether the cytotoxic effect of oxymatrine was caused by apoptotic cell death, we determined the apoptosis of A375 cells using annexin V/PI binding assay followed by flow cytometry. The cells were treated by oxymatrine 12 h after the treatment. As illustrated in Fig. 2a, oxymatrine induced dose-dependent increasement in apoptotic A375 cells: approximately 50% of the cells were at early apoptosis in the group treated with oxymatrine (20 μmol/l) , compared with 7.7% of the group treated with the vehicle. Annexin V and PI-double positive cells were observed with approximately 9.1% of total cells in the groups treated with oxymatrine, indicating a low level of necrosis induced by oxymatrine.

Fig. 1



Oxymatrine caused significant cytotoxicity in human melanoma cells *in vitro*. (a) The chemical structure of oxymatrine. (b) Oxymatrine killed three human melanoma cell lines. Three human melanoma cells (A375, Sk-Mel-28, MM96L) and the nontumorigenic human keratinocyte HaCaT cells were treated with oxymatrine (5–40 μmol/l) for 72 h, and cytotoxicity was analyzed by MTT assay. Data represent the mean ± SD of three different experiments with triplicate sets in each assay. *$P<0.05$, **$P<0.01$ versus group not treated with drug.

Oxymatrine-induced apoptosis in A375 cells was dependent on caspase-3

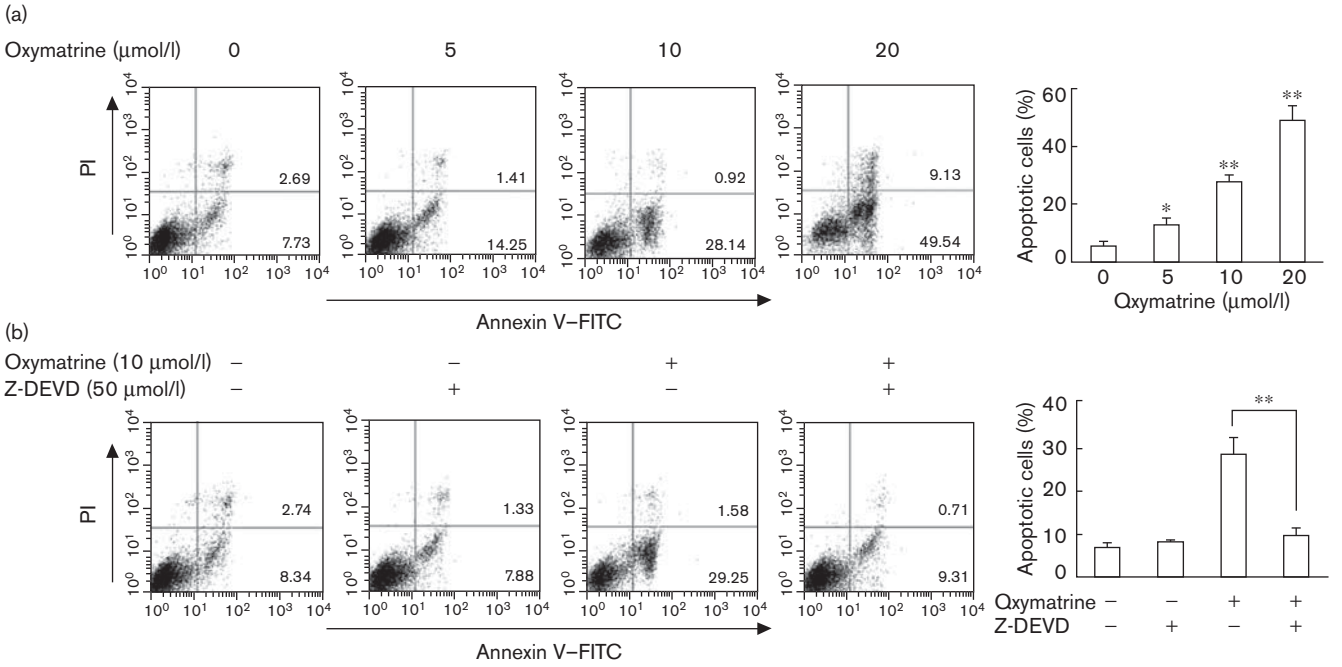
To further confirm whether oxymatrine-induced cell apoptotic death was indeed caspase-dependent, we treated A375 cells with oxymatrine (10 μmol/l) in the presence or absence of Z-DEVD-FMK (50 μmol/l), a caspase-3 inhibitor. Using oxymatrine-treated cells as the control, approximately 30% of A375 cells underwent apoptosis 12 h after the treatment. Compared with the group treated with oxymatrine, the addition of caspase-3 inhibitor Z-DEVD-FMK markedly reduced the apoptotic response to approximately 9.3%, as shown in Fig. 2b. Taken together, these findings showed that oxymatrine treatment could trigger caspase-3-dependent apoptosis of A375 cells.

Oxymatrine-induced apoptosis in A375 cells was through the intrinsic mitochondrial pathway

The molecular events activating the apoptotic cell death after oxymatrine treatment were studied further. The dose-dependent effect of oxymatrine was examined in A375 cells. As assessed by western blot, the activation of caspase-3 was obvious after oxymatrine treatment (Fig. 3a). Consequently, PARP was also found to be cleaved in oxymatrine-treated cells, corroborating the induction of apoptosis in A375 cells by oxymatrine.

To date, there are two known alternative pathways to initiate apoptosis: the intrinsic, characterized by cytochrome *c* release

Fig. 2



Oxymatrine induced caspase-3-dependent apoptosis of human melanoma A375 cells. (a) Apoptosis in A375 cells was assessed at 12 h after treatment with 5–20 μmol/l of oxymatrine by annexin V–fluorescein isothiocyanate (FITC)/propidium iodide (PI) binding and measured by flow cytometry analysis. Numbers indicate the percentage of cells in each quadrant. *$P<0.05$, **$P<0.01$ versus group not treated with drug. (b) A375 cells were treated with 50 μmol/l of Z-DEVD-FMK, a caspase-3 specific inhibitor, for 1 h before treatment with 10 μmol/l of oxymatrine for 12 h. Apoptosis was assessed by flow cytometry as mentioned above. **$P<0.01$.

and caspase-9 activation, and the extrinsic, involving activation of caspase-8 and 10 [23]. To assess which pathway is operative, we tested the expression level of cytochrome *c*, caspase-9, and caspase-8. As shown in Fig. 3a, immunoblot with an antibody against caspase-9 showed a large increase in activated caspase-9 after oxymatrine treatment. In contrast, no cleavage of caspase-8 was found in oxymatrine-treated cells (Fig. 3a). Consistently, cytochrome *c* was released from mitochondria to cytosol (Fig. 3b), indicating activation of the intrinsic pathway.

In mitochondria, cytochrome *c* plays an essential role in the generation of mitochondrial transmembrane potential [24]. The release of cytochrome *c* observed in A375 cells prompted us to evaluate alteration in mitochondrial transmembrane potential by fluorochrome JC-1 staining. As can be observed in Fig. 3c, JC-1 incorporation was more significantly decreased in oxymatrine-treated cells than in control cells, indicating the loss of mitochondrial membrane potential caused by oxymatrine. These data strongly indicated that oxymatrine-induced apoptosis in A375 cells was executed by the intrinsic mitochondrial pathway.

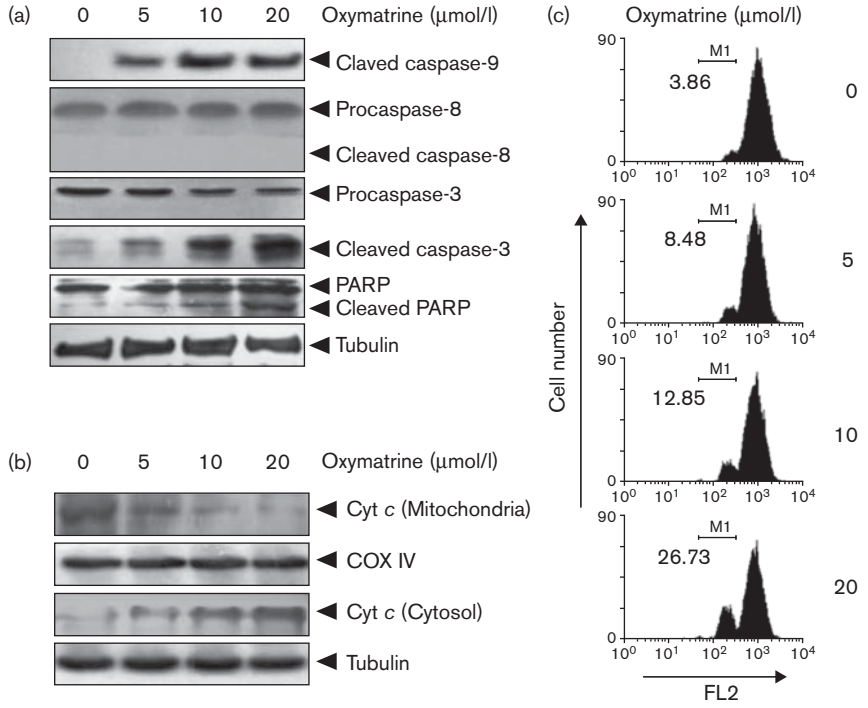
Oxymatrine increased the level of ROS and decreased the level of GSH in A375 cells

As a loss of mitochondrial membrane potential is associated with the generation of ROS [25], we detected the level of ROS in A375 cells treated with various concentrations of oxymatrine for 6 h with the cellular oxidation of (H_2DCFDA), a probe that is oxidized to green fluorescent DCF by various peroxide-like ROS and nitric oxide-derived reactive intermediates. As shown in Fig. 4a, the level of ROS in cells treated with oxymatrine was increased in a concentration-dependent manner, with a 3.0-fold increase for 40 μmol/l of oxymatrine treatment compared with that of vehicle-treated cells. These data showed that oxymatrine significantly increased ROS production in A375 cells, which may promote mitochondrial dysfunction and trigger mitochondria-mediated apoptosis. Excessive production of ROS could disturb the homeostasis between GSH and ROS. We tested the effect of oxymatrine on the intracellular GSH level in A-375 cells. As shown in Fig. 4b, treatment with 10, 20, and 40 μmol/l of oxymatrine decreased the level of GSH by 39.7, 52.3, and 67.1%, respectively, compared with that of vehicle-treated cells.

Antioxidant NAC almost reversed oxymatrine-mediated apoptosis in A375 cells

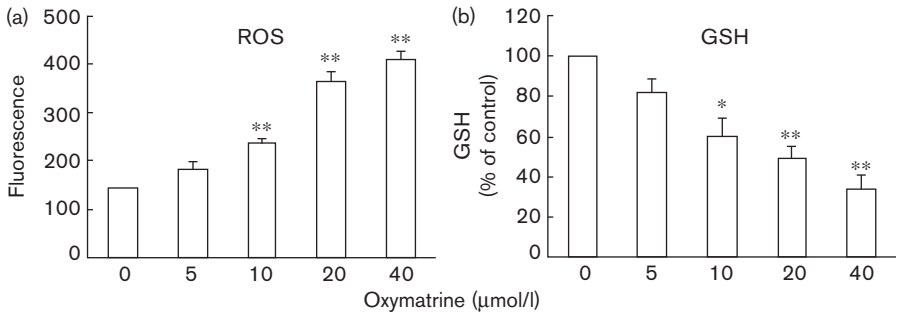
To illustrate the role of ROS in oxymatrine-induced apoptosis, A375 cells were treated with oxymatrine in the presence or absence of antioxidant NAC. As shown in Fig. 5a and b, the ratio of apoptosis was changed from 31.1 ± 3.9 to 7.2 ± 1.4%, indicating that the addition

Fig. 3



Oxymatrine dose-dependently collapsed mitochondrial membrane potential and altered the expressions of apoptosis-related proteins in A375 cells. (a) A375 cells were treated with or without oxymatrine (5, 10, 20 μmol/l) for 24 h, and the cells were then harvested and lysed. The expressions of apoptosis-related proteins were analyzed by western blotting. The data shown here are from one of three different experiments. (b) A375 cells were treated with or without oxymatrine (5, 10, 20 μmol/l) for 24 h, and the cells were then harvested and separated into cytosolic and mitochondrial fractions using the commercial fractionation kit. The expressions of cytochrome *c* (Cyt *c*) in cytosol and mitochondria were analyzed by western blotting. The data shown here are from one of three different experiments. (c) A375 cells were treated with or without oxymatrine (5, 10, 20 μmol/l) for 12 h, and the cells then were harvested and the disruption of mitochondrial transmembrane potential was measured using fluorochrome dye JC-1 by flow cytometry. The data shown here is one of three different experiments.

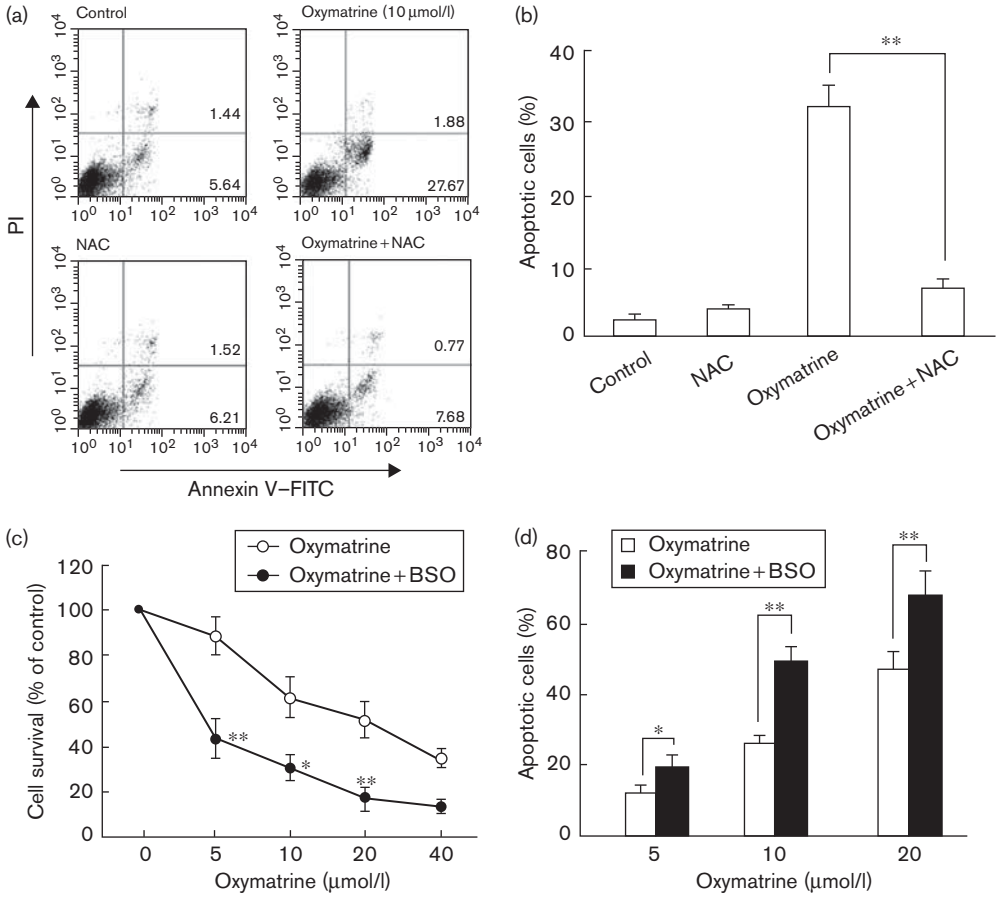
Fig. 4



Oxymatrine increased reactive oxygen species (ROS) and decreased glutathione (GSH) levels in A375 cells. Cells were treated with oxymatrine (5, 10, 20, 40 μmol/l) for 6 h, and the intracellular levels of ROS (a) and GSH (b) were then detected. Intracellular levels of ROS and GSH markedly increased and decreased in oxymatrine-treated cells, respectively, compared with those of vehicle control. Values are means ± SD for three independent experiments performed in triplicate. $*P<0.05$, $**P<0.01$ versus group not treated with drug.

of antioxidant NAC almost completely reversed apoptotic cell death brought about by oxymatrine. Conversely, preincubation with the GSH synthesis inhibitor L-buthionine-(*S,R*)-sulfoximine, which led to GSH depletion, significantly increased the sensitivity of cancer cells to oxymatrine (Fig. 5c) and enhanced the proapoptotic effect of oxymatrine (Fig. 5d). These observations show that oxymatrine-induced cell apoptotic death is a result of oxidative stress from an imbalance between ROS generation and degradation.

Fig. 5



Oxymatrine-induced apoptosis in A375 cells can be almost reversed by antioxidant *N*-acetyl cysteine (NAC). (a) Cells were pretreated with 1 mmol/l of NAC for 1 h, and then treated with or without 10 μmol/l of oxymatrine for 12 h; apoptosis was then assessed by annexin V–fluorescein isothiocyanate (FITC)/propidium iodide (PI) binding and flow cytometry analysis. (b) The statistical data of apoptosis are from three independent experiments. $^{**}P<0.01$. (c) A375 cells were treated for 12 h with DL-buthionine (*S*,*R*)-sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, followed by treatment with oxymatrine for 24 h. Data are expressed as percentage of the control. $^{*}P<0.05$, $^{**}P<0.01$ versus corresponding the group not treated with BSO. (d) A375 cells were treated for 12 h with BSO, followed by treatment with oxymatrine for 12 h. Apoptosis was assessed by flow cytometry. $^{*}P<0.05$, $^{**}P<0.01$.

Discussion

Human malignant melanoma is a highly metastatic skin cancer with numerous genetic and epigenetic mechanisms that suppress apoptosis [26,27]. Despite the development of new therapeutic modalities of therapy, the outcomes of patients with advanced-stage melanoma are extremely poor [27]. Current standard treatment includes single-agent dacarbazine, which improves clinical response but not the average survival duration [28,29]. These poor clinical responses, together with the toxicity associated with such treatment, mean that the identification of other less toxic biological molecules is imperative. Unfortunately, few antineoplastic compounds were identified in a recent specific search for agents active in this disease. In this respect, more and more researchers are focusing on the effects of natural active compounds in cancer treatment. In this study, oxymatrine, which was previously found to exert an antineoplastic effect on hepatoma, was clearly shown to kill all three human melanoma cell lines, A375, Sk-Mel-28, and MM96L. However, it was interesting that oxymatrine at the concentrations mentioned above hardly affected the viability of the benign non-tumor human keratinocyte HaCaT cells, suggesting the selective antitumor action of oxymatrine to some degree. Similarly, Yang *et al.* [30] reported that gambogic acid could selectively induce apoptosis of human hepatoma SMMC-7721 cells while had relatively less effect on human normal embryon hepatic L02 cells owing to the higher distribution and longer retention time of gambogic acid in tumor cells compared with the normal cells. The detailed mechanisms by which oxymatrine kills melanoma cells but not benign cells require further investigation.

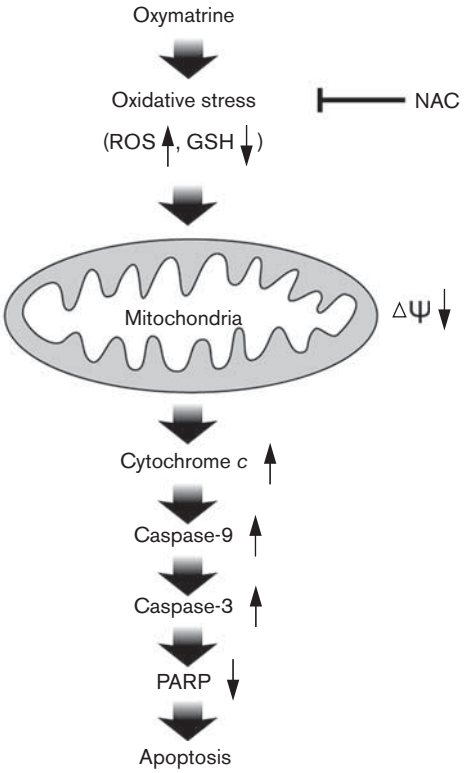
Next we selected one melanoma cell line, A375, and investigated the mechanism underlying the cytotoxic

effect of oxymatrine. The results in Fig. 2 show that oxymatrine can trigger caspase-3-dependent apoptosis in human melanoma A375 cells. We delineated a series of molecular events initiating the signaling cascade involved in oxymatrine-induced apoptosis in A375 cells. First, we showed that the treated cells showed enhanced activation of caspase-3, which was associated with elevated cleavage of PARP. It is believed that caspase-3 can be activated only by upstream initiator caspases, which are capable of autocatalytic activation [31]. Second, activation of caspase-9 and 8 was tested in oxymatrine-treated cells. The results of immunoblot showed that caspase-9 was obviously activated during the process; nonetheless caspase-8 remained unchanged. Meanwhile, cytochrome *c* was released from mitochondria to cytoplasm, a hallmark of the initiation of mitochondria-mediated apoptosis. Furthermore, loss of mitochondrial transmembrane potential, serving as a key step in the mitochondria-dependent apoptosis, provided further proof of the intrinsic apoptotic cascade induced by oxymatrine.

ROS are known to induce the collapse of mitochondrial membrane potential, and therefore trigger a series of mitochondria-associated events including apoptosis [32]. Loss of mitochondrial membrane potential also induces apoptosis by causing the release of proapoptotic factors, such as cytochrome *c* from the mitochondrial inner space to cytosol. Cytochrome *c* in cytosol exerts its apoptogenic effects by participating in the activation of caspase-9, which in turn activates the executioner caspase-3 [31]. As one of the identified substrates of caspase-3, PARP is involved in the repair of DNA damage induced by certain anticancer agents or radiation. During apoptosis, caspase-3 cleaves PARP into two fragments, p89 and p24, thus suppressing PARP activity [33]. As shown in this study, cytochrome *c* released from mitochondria to cytosol, caspase-9 was activated, and PARP was cleaved in oxymatrine-treated A-375 cells. All these changes have been shown to occur during apoptosis, and should be intrinsically lethal. ROS could be scavenged by the redox-related enzymes, such as GSH, catalase, superoxide dismutase, and thioredoxin, which can protect cells against ROS-induced toxicity [34]. A low GSH level is sometimes associated with mitochondrial dysfunction and apoptosis induction, thereby decreasing the chemoresistance of the tumors [35]. Our results also showed that oxymatrine dramatically stimulated GSH depletion. In addition, oxymatrine treatment with an antioxidant NAC protected the cells from apoptosis in A375 cells. We conclude that oxymatrine induces melanoma cell apoptosis through the caspase-3-dependent and ROS-dependent mitochondrial pathway.

In conclusion, our findings suggest that oxymatrine triggers oxidative stress in human melanoma A375 cells, resulting in the collapse of the mitochondrial transmembrane potential, which in turn leads to cytochrome *c* release and apoptosis through the intrinsic caspase-9/caspase-3 pathway. Understanding the signaling pathway involved in the process will hopefully shed light on strategies to improve therapeutic approaches to melanoma chemical therapy. The findings indicate that oxymatrine shows promise as a therapeutic agent against human melanoma. On the basis of the results of this study, the mechanism by which oxymatrine induces apoptosis in human melanoma A375 cells is summarized in Fig. 6.

Fig. 6



Overview of pathways for oxymatrine-induced apoptosis in human melanoma cell line A375. GSH, glutathione; NAC, *N*-acetyl cysteine; ROS, reactive oxygen species.

Acknowledgements

This study was supported by the Natural Science Foundation of Shanghai (No. 08JC1407100, 09ZR14100 00) and the Program for Outstanding Medical Academic Leader of Shanghai (H. Jiang). The authors state no conflict of interest.

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