ORIGINAL PAPER

The anti-ovarian carcinoma activity of L-amino acid oxidase from *Crotalus adamanteus* venom in vivo and in vitro

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Abstract

Snake venom L-Amino-acid oxidase (svLAAO) has become a critical research target in molecular biology and medical science since its widespread presence and diverse biological roles, including antitumor application. Our research confirmed that *Crotalus adamanteus* (*C. adamanteus*) venom LAAO exhibited potential anti-ovarian cancer activity both in vivo and in vitro. *C. adamanteus* venom LAAO significantly reduced viability of ovarian cancer cells and caused morphological changes that preceded cell death. The results of molecular biology experiments showed that *C. adamanteus* venom LAAO caused expression changes of genes related to apoptotic pathways either intrinsically or extrinsically in ovarian cancer cells. Animal experiments and histological analysis also proved that *C. adamanteus* venom LAAO could effectively inhibit the tissue damage caused by ovarian cancer, and animals treated with *C. adamanteus* venom LAAO showed higher survival time. Catalase blocked the major apoptosis induction of *C. adamanteus* venom LAAO on ovarian cancer cells, suggesting that the cytotoxicity of *C. adamanteus* venom LAAO on ovarian cancer cells was mainly mediated by H_2O_2. These results infer that *C. adamanteus* venom LAAO will have some advantages in new drug research and antitumor drug development in future.

Keywords *Crotalus adamanteus* venom · L-Amino-acid oxidase · Apoptosis · Fas/FasL pathway · Mitochondrial pathway

Introduction

Ovarian cancer is one of the most lethal gynecological diseases worldwide, which exhibits high heterogeneity and rapid progression. Most patients with ovarian cancer are often diagnosed too late for a cure because of the lack of specific early symptoms and effective early detection strategies [1]. Epithelial ovarian cancer (EOC) is the most common type, accounting for 90% of all cases, and it is a devastating disease in which the tumor cells have disseminated beyond the ovaries and pelvic organs at the time of diagnosis [2]. Previous research has suggested that although many patients with EOC have experienced successful initial therapy, the overall survival rate has only modestly improved, and EOC remains a deadly disease due to extensive peritoneal dissemination, chemotherapy resistance, immune escape, and immune counterattack of tumor cells [3]. To date, conventional treatments and currently available drugs are often difficult to curb the recurrence and metastasis of ovarian cancer. Therefore, novel and highly potent natural bioactive therapeutics targeting carcinogenesis, survival, growth, apoptosis, and transformation of ovarian cancer cells may be a promising strategy to control cancer development and reduce the risk of side effects or complications of chemoradiotherapy.

SvLAAO can catalyze the stereospecific oxidative deamination of L-amino acid to α-keto acids along with the generation of hydrogen peroxide (H_2O_2), which has attracted great attention due to its numerous clear biological or pharmacological functions, such as hemorrhage, antiparasitic and cytotoxicity [4–6]. Tan found that svLAAO can inhibit tumor cell proliferation and induce tumor cell apoptosis, and believed that the anticancer activity of svLAAO is attributed to its H_2O_2 cytotoxicity [7]. Nonetheless, the exact physiological role and mechanism of svLAAO against tumors are poorly understood. To further explore the anti-tumor activity

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of svLAAO, we used LAAO from *C. adamanteus* venom as a therapeutic agent to investigate its apoptosis-inducing effect and mechanism of action on epithelial-derived ovarian cancer cells.

Materials and methods

Reagents, cells, and animals

The *C. adamanteus* LAAO and catalase were from Sigma-Aldrich. Cell culture reagents were from Life Technologies. Real-time fluorescence quantitative PCR reagents were from Vazyme Biotech Co., Ltd. Western blotting-related chemicals were from Dingguo Changsheng Biotech Co. Ltd.

Human ovarian cancer cell line CAOV3 and OVCAR3 were from Xiangf Biotech and Dingguo Changsheng Biotech Co. Ltd, respectively. CAOV3 and OVCAR3 were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 100 unit/mL penicillin/streptomycin at 37 °C in a humidified incubator with 5% CO_2.

Swiss-Kunming female mice (16 ± 2 g) were from the Laboratory Animal Centre of Army Medical University. All animals were under controlled environmental conditions (12 h light/dark cycle at 22 ± 1 °C, relative humidity 40–70%) and received balanced feed and water ad libitum during the experiments. All animal experiments were carried out in strict accordance with Methods for the Management of Experimental Animals in Chongqing (No. 195).

Cell viability, morphological alteration, and apoptosis

CCK-8 assays for cytotoxicity

CCK-8 was utilized to evaluate the cytotoxic effect of svLAAO on CAOV3 and OVCAR3 cells. Before the CCK-8 experiments, we treated cells with different concentrations of svLAAO (1, 3, 5, and 7 µg/mL), and then observed at different time points (6, 12, 24, and 36 h) to preliminarily select the experimental time point and concentration according to the changes of cell morphology. And then, two types of ovarian cancer cells were seeded with a volume of 100 µL cell suspension (8000 cells/well) into 96-well plates and incubated the plate for 12 h in a humidified incubator at 37 °C. After a 12 h culture, the medium was changed freshly and cultured for another 12 h. Next, cells were washed with PBS and incubated with a medium containing 1, 3, 5, and 7 µg/mL svLAAO for 24 h. Finally, 10 µL CCK-8 solution was added to each well and incubated at 37 °C for 2.5 h. The cytotoxicity was determined by absorbance analysis at 450 nm using a microplate reader. The effective concentration (IC50) at which svLAAO treatment reduced cell viability by 50% was determined by the dose–response curve of GraphPad Prism 8 software.

Acridine orange (AO)/propidium iodide (PI) double staining test

Based on the experimental results of CCK-8 and the observation of cells under the light microscope, we selected 5 µg/ml svLAAO as the optimal stimulation concentration, and 24 h as the suitable incubation time for the follow-up experiments. For morphological studies, cells were incubated with 5 µg/mL svLAAO with or without different concentrations of catalase, an H_2O_2 scavenger. To comply with the subsequent molecular experiments, cell samples were collected and controlled the number of cells within 5.0×10^5. First, 5.0×10^5 cells were seeded in 6-well microplates and cultured for 24 h in RPMI-1640 medium with 10% FBS. Second, we changed the medium containing different concentrations of catalase enzyme (0.025–0.15 mg/mL catalase) and pre-incubated for 1 h. Third, 5 µg/mL svLAAO was added into the above wells and continued to incubate for 24 h. Fourth, cells were trypsinized, washed twice with PBS, and then resuspended with 500 µL of staining buffer. Next, we added 5 µL AO and 10 µL PI staining solution into the cell suspension, mixed gently, and incubated for 15 min at 4 °C in a dark environment. Finally, the cells were washed again with PBS and verified by a fluorescence microscope. Cell survival status was evaluated by analyzing the results of cell staining and morphological changes under the fluorescence microscope.

Molecular biological experiments

Cells were seeded (5.0×10^5 cells per well) in 6-well microplates and cultured to 70–80% confluence before experiments. Next, cells were treated with 5 µg/mL svLAAO and different concentrations of catalase at the logarithmic growth phase. The sampling scheme of svLAAO and catalase are the same as the "AO/PI double staining test" (See 2.2.2). And untreated cells served as blank control. At last, stimulated ovarian cancer cells were harvested at 24 h time point for further gene and protein expression detection. Each experiment was performed three times independently.

RNA isolation and qRT-PCR

Total RNA was extracted from cells with TaKaRa kit according to the manufacturer's instructions. Total RNA (1 µg) from each group was used to generate cDNA. GAPDH served as internal control, and all primers used were presented in Supplementary Table 1. The primer sequences were from http://pga.mgh.harvard.edu/primerbank/; the concentrations of all primers were 400 nmol/L. The PCR

cycling conditions were performed as follows: 30 s at 95 °C for pre-denaturation; 40 cycle reactions (10 s at 95 °C for DNA polymerase activation, 30 s at 60 °C for annealing/extension); and the melting steps (65 °C to 95 °C, increment 0.5 °C). The comparative CT method was used to determine the relative quantitation of gene expression for each gene compared with the housekeeping gene GAPDH.

Measurement of apoptosis-related protein expressions

After treatments, cells were collected, washed with ice-cold PBS, and then lysed with RIPA buffer plus protease inhibitors for 30 min. Next, cell lysates were centrifuged at $12,000 \times g$ for 5 min at 4 °C, and the supernatant was denatured in boiling water in SDS-PAGE Sample Loading Buffer for 5 min. Equivalent amounts (40 μg) of total protein were separated by 10 or 12% SDS-PAGE gels, transferred to PVDF membranes, and then immunoblotted with antibodies of apoptosis-related proteins. GAPDH was utilized for loading control, and protein expression levels were normalized to GAPDH levels. All western blots were scanned and quantified with an ECL western blotting detection system.

Animal model construction of ovarian cancer

All mice were acclimated for 1 week before the initiation of experiments, feeding with a standard diet and water ad libitum. Mice were randomly divided into two large groups: (1) blank control group (without any treatment, 3 mice); (2) experimental group: (1) CAOV3 cell suspension injection group (6 mice), (2) OVCAR3 cell suspension injection group (6 mice). The experimental group of mice has inoculated intraperitoneally with 3.0×10^5 CAOV3 or OVCAR3 cell suspension (without additives). After being injected, all mice continued a standard diet for 4 weeks under standard conditions, and then abdominal dissection of each group was performed to observe tumor development.

Histological analysis

After successful construction of ovarian cancer mouse model, to evaluate the effect of svLAAO treatment on mice with ovarian cancer, mice were randomly divided into five groups (6 mice per group): blank control group (healthy mice, without any treatment), ovarian cancer-bearing group (untreated with svLAAO), 0.5, 1.0, and 1.5 μg/g body weight svLAAO-treated groups. SvLAAO treatment was initiated 30 days after tumor cell implant, and repeated at 24 h intervals for a total of four applications. Mice were dissected 7 days after the first injection with svLAAO, observed visceral tissue, and took out gastric tissue. The gastric tissues were immediately fixed in 4% paraformaldehyde and dehydrated with ethanol of gradient concentration, then cleared with xylene, embedded in paraffin. The paraffin-embedded tissue blocks were sectioned into thin, 2.5 μm slices and fixed on standard glass microscope slides which were utilized for histological study. Finally, the sections were stained with 0.25% (w/v) hematoxylin and eosin (H&E) and photographed under an optical microscope. The protocol was performed twice, and a total of 30 animals were used in all evaluation groups at each study moment. Another 24 ovarian cancer-bearing mice, treated or not with svLAAO, were monitored for survival curve evaluation.

Statistical analysis

Statistical analysis was performed using PASW Statistics version 18.0. The average and standard deviation were calculated and expressed as Mean ± SD. Comparison of experimental data and significance of differences between groups were analyzed by one-way ANOVA test.

Results

Cytotoxic effect assessment of *C. adamanteus* venom LAAO on ovarian cancer cells

Primary results showed that cytotoxicity of svLAAO on CAOV3 or OVCAR3 cells was elevated in a dose-dependent manner at different points in time (data not shown). Especially, at 24 h time point, cell morphology has been significantly changed in 3, 5, and 7 μg/mL svLAAO treatment groups, and a large number of cells appeared apoptosis or death (Fig. S1). Cells were incubated with different concentrations of svLAAO (1.0–7.0 μg/mL) and a concentration-dependent decrease in cell viability was noted (Fig. 1A). The concentration that reduced cell viability to 50% (IC50) was calculated as 6.725 μg/mL in CAOV3 cells and 6.364 μg/mL in OVCAR3 cells.

Cell morphology was analyzed by AO/PI staining after 24 h treatment with 5 μg/mL svLAAO and different catalase concentrations (0.025–0.15 mg/mL) (Fig. 1B). Cell DNA stained evenly and morphological structure was normal in the blank control group; In 5 μg/mL svLAAO group (0 Cat.), the cell membrane blebbing, the chromatin condensation, the nucleus was cleaved into small spots (white arrows), and living cells decreased significantly, suggesting that apoptotic cell death may have been triggered. Cells incubated with 5 μg/mL svLAAO and catalases (0.025–0.15 mg/mL), the proportion of viable cells increased significantly, which suggested that H_2O_2 plays an important role in svLAAO cytotoxicity.
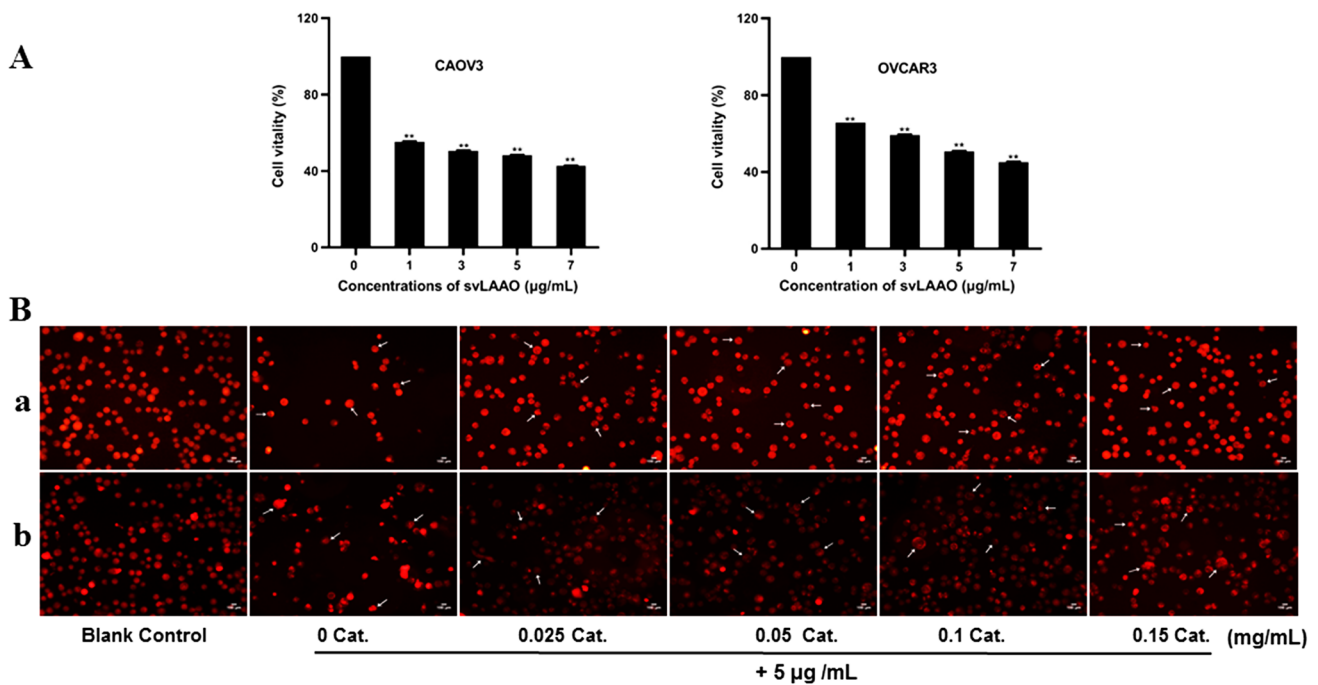
A

B

Fig. 1 SvLAAO cytotoxicity against ovarian cancer cells. **A** Concentration–response of svLAAO cytotoxicity. Cells were incubated with different concentrations of svLAAO (1.0–7.0 μg/mL) for 24 h and cell viability was tested using CCK-8 reagent. **B** Morphological alteration in svLAAO-treated ovarian cancer cells. The CAOV3 (*a*) and OVCAR3 (*b*) cells were incubated with svLAAO and different concentrations of catalase, and assessed at 24 h time point by AO/PI staining. White arrows indicate apoptosis cells or necrotic cells. Data are representative results of three independent experiments as Mean ± SD (**$p < 0.01$). Scale bar = 100 μm

Activation of the Fas/FasL and Mitochondrial pathway by *C. adamanteus* venom LAAO

As illustrated in Fig. 2, the mRNA levels of apoptosis-related genes were altered in both cell lines after incubation with 5 μg/mL svLAAO for 24 h. In CAOV3 cells (Fig. 2A), 5 μg/mL svLAAO-incubation group compared with blank control group, the mRNA levels of Fas,
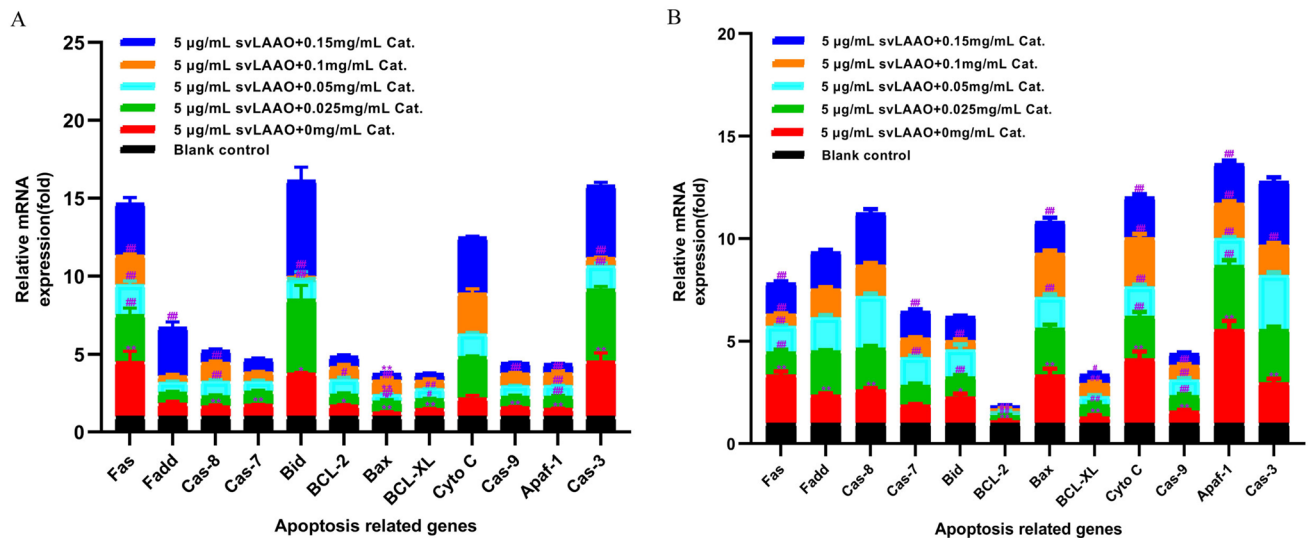

Fig. 2 The mRNA expression changes of Fas/FasL and mitochondrial pathway-related genes were detected in ovarian cancer cells. The CAOV3 **A** and OVCAR3 **B** cells were exposed to 5 μg/mL svLAAO and different concentrations of catalase for 24 h. GAPDH served as control. *,**Statistically significant difference as compared to blank control group (0 μg/mL svLAAO), $n = 3$, *$p < 0.05$, **$p < 0.01$, #,##Statistically significant difference as compared to 5 μg/mL svLAAO + 0 mg/mL catalase group, $n = 3$, #$p < 0.05$, ##$p < 0.01$

Bid, Caspase 3 were increased obviously; and Caspase 8, Caspase 7, BCL-2, Bax, BCL-XL, Caspase 9, and Apaf-1 were decreased substantially; however, there was no significant difference in mRNA expression of Fadd and Cyto C. In OVCAR3 cells (Fig. 2B), 5 μg/mL svLAAO-incubation group compared with blank control group, the mRNA expressions of Fas, Fadd, Caspase 8, Bid, Bax, Cyto C, Apaf-1, and Caspase3 were up-regulated significantly; BCL-2, BCL-XL and Caspase 9 mRNA levels were down-regulated markedly; but Caspase 7 mRNA level was no obvious difference.

The western blot results (Fig. 3A, B, Figs. S2, S3) showed that 5 μg/mL svLAAO-incubation group compared with blank control group (Con.), the protein level of Fas and Cyto C was raised distinctively both in CAOV3 and OVCAR3 cells; the protein level of Caspase 8 and Caspase 9 was decreased significantly; the Caspase 7 and Caspase 3 protein levels were up-regulated markedly in CAOV3 cells, and down-regulated significantly in OVCAR3 cells, respectively; in CAOV3 cells, lower protein expressions of Fadd and Bid were detected; however, in OVCAR3 cells, the Fadd protein level was not significantly different from blank control level, and the Bid protein expression was distinctively increased compared to its blank control. These results suggested that, at the transcription level and protein level, there are certain differences in some gene expression changes caused by svLAAO at 24 h time point. Furthermore, our results also hinted that the apoptosis-inducing effect of svLAAO on ovarian cancer cells may be linked to the type of cells.

Next, we incubated CAOV3 or OVCAR3 cells with 5 μg/mL svLAAO and different concentrations of catalase for 24 h. As indicated in figures (Figs. 2, 3A, B, Figs. S2, S3), to a certain extent, the mRNA and protein expressions of apoptosis-related genes triggered by 5 μg/mL svLAAO at

24 h time point were eliminated or partially counteracted by catalase.

Antitumor effect of svLAAO in vivo

The immunocompetent female mice were used to assess the tumorigenicity of tumor cells. There were not any visible tumors in the peritoneal or thoracic cavities after intraperitoneal (i.p) injection by gross inspection. However, vesicular bulges appeared on the surface of the small intestine after injection of ovarian cancer cells for 1 month (Fig. 4B), and a small amount of ascitic fluid was observed as well compared to blank control. The results of ovarian cancer-bearing group treated with different concentrations of svLAAO (0.5, 1.0, 1.5 μg/g body weight) showed that the blister-like bumps on the surface of the small intestine were markedly reduced or disappeared when compared to untreated tumor-bearing group. Here, we present only a part of the mouse anatomical materials of the svLAAO-treated groups (1.5 μg/g body weight svLAAO treatments, Fig. 4(a–e)). Our results indicated that svLAAO can effectively inhibit the invasion of tumor cells to the small intestine of ovarian cancer-bearing mice. And survival observation showed that the treatment of tumor-bearing mice treated with 1.5 μg/g body weight svLAAO induced a significant improvement in survival over the tumor-bearing control mice (Fig. 4A).

Histological analysis of gastric tissues of ovarian cancer-bearing mice indicated that the structure of the gastric tissue was greatly damaged in tumor groups, and the cell arrangement was sparse, the level was unclear, the structure was confusing, the cell shape was irregular, and the cell structure was mostly unclear and incomplete (Fig. 4C(g, i)). However, cells of normal mouse gastric tissue were closely arranged, well-organized, orderly in the structure, regular in

Fig. 3 The protein expression alteration of Fas/FasL and mitochondrial pathway-related genes was detected in ovarian cancer cells. The CAOV3 **A** and OVCAR3 **B** cells were incubated with 5 μg/mL svLAAO and different concentrations of catalase for 24 h, and the cell lysates were examined by western blot. GAPDH served as control. Results for western blot are one of the three independent experiments
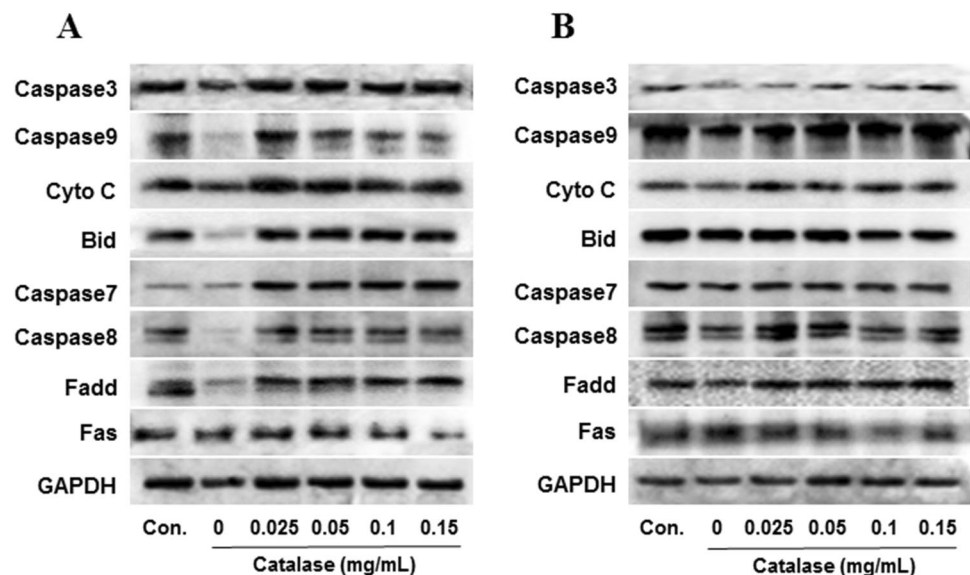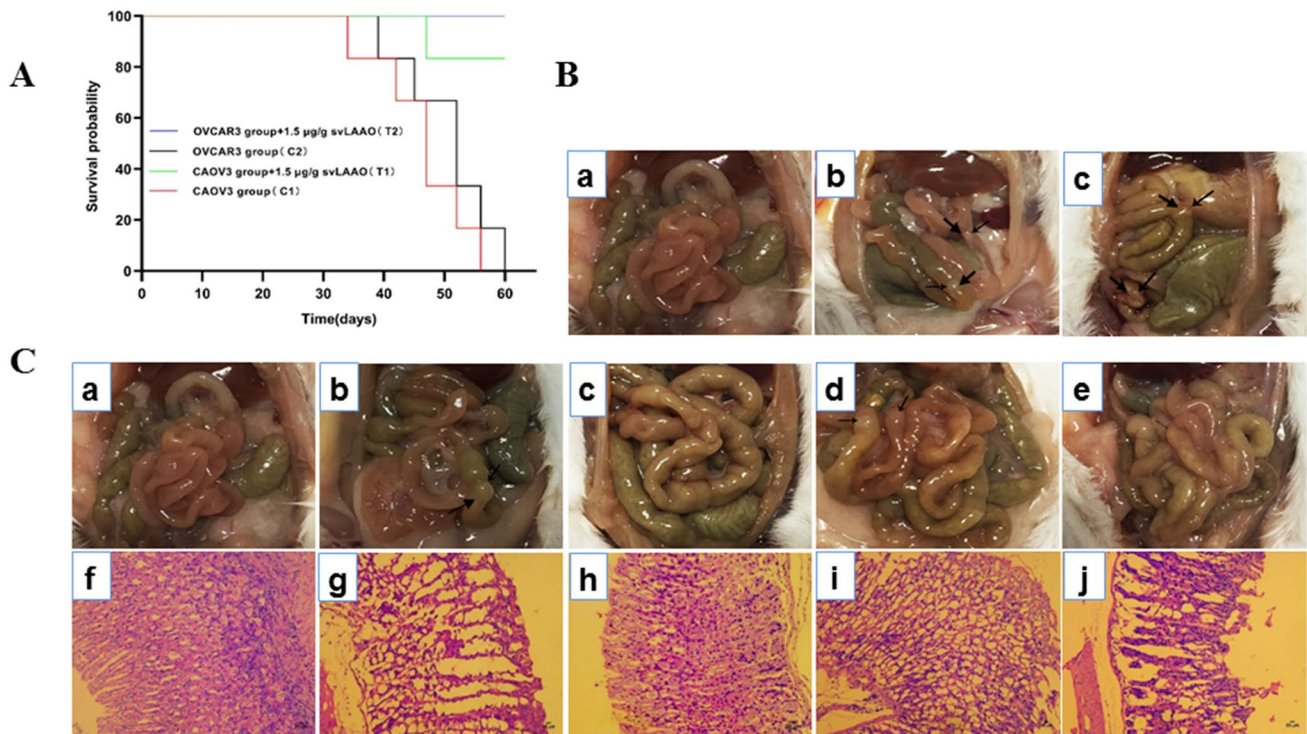
Fig. 4 Antitumor effect of *C. adamanteus* venom LAAO on ovarian cancer in vivo. **A** Survival percentage mice ($n=24$) inoculated with ovarian cancer cells (3.0×10^5 cells, intraperitoneally) and treated with *C. adamanteus* venom LAAO on 30th, 31st, 32nd and 33rd days. The survival observation period of ovarian cancer-bearing mice was 60 days. Statistics: T1>C1, T2>C2, $p<0.01$. **B** Mice inoculated intraperitoneally with ovarian cancer cells. (*a*) blank control, (*b*) CAOV3 cell injection group, (*c*) OVCAR3 cell injection group. These samples were harvested 30 days after i.p. injection of ovarian cancer cells. **C** Morphological and histological analysis of the viscera of ovarian cancer-bearing mice after treatment with 1.5 µg/g body weight svLAAO. (*a–e*) Morphological analysis of mouse small intestine. (*a*) blank group, (*b*) CAOV3 group, (*c*) CAOV3 cells + 1.5 µg/g body weight svLAAO group; (*d*) OVCAR3 group, (*e*) OVCAR3 cells + 1.5 µg/g body weight svLAAO group. (*f–j*) Histological analysis of mouse gastric tissues. (*f*) blank group, (*g*) CAOV3 group, (*h*) CAOV3 cells + 1.5 µg/g body weight svLAAO group, (*i*) OVCAR3 group, (*j*) OVCAR3 cells + 1.5 µg/g body weight svLAAO group. Mice were dissected 7 days after the first injection with svLAAO. Black arrows represent the invasion of ovarian cancer cells into the small intestine of mice. Scale bar = 20 µm

the shape, and the nucleus is visible (Fig. 4C(f)). To some extent, the structure of gastric tissue in tumor-bearing mice was improved after i.p. injection of different concentrations of svLAAO (Fig. 4C(h, j), Fig. S4).

Discussion

The basic principle of antitumor drugs is to reduce cancer cells, promote the benign transformation of cancer cells or cause cancer cell apoptosis or necrosis, but minimize the harm to healthy cells. Apoptosis is a normal physiological process and plays an important role in maintaining homeostasis within an organism. Substantial evidence indicates that apoptosis is controlled by multiple extracellular and intracellular signals, and its dysregulation is implicated in the pathogenesis of human tumors [8]. The following two principal pathways generally control apoptosis of tumor cells: the death receptor pathway and the mitochondrial-mediated

pathway [9, 10]. The activated tumor cell membrane-bound death receptors or mitochondrial perturbation may lead to the activation of downstream caspases, which may disrupt the cytoskeleton, shut down DNA replication and repair, degrade chromosomal DNA, gradually disintegrate cells into apoptotic bodies, and eventually trigger apoptosis of cells [11, 12].

Earlier studies showed that alterations in gene expressions of Fas family or BCL-2 family might play a crucial role in EOC [13]. The Fas/FasL system is recognized as a primary mechanism for apoptosis induction in cells, which may be involved in the occurrence, development, and chemoresistance of ovarian cancer [14]. Fas can initiate the extrinsic apoptosis pathway to induce the cell death signal cascade by autocrine-paracrine, and eventually leads to cell apoptosis [15]. The existing experimental results proved that the low-level expression of Fas may be more favorable to the survival of tumor cells and escape from human immune monitoring [16]. Here, we present evidence that *C. adamanteus* venom

LAAO may lead to a higher rate of programmed cell death due to the activation of the Fas/FasL apoptosis pathway by upregulated Fas mRNA and protein levels. Once Fas is activated, which could trigger off an intracellular chain reaction, leading to continuous activation of genes related to the Fas signaling pathway [17]. On the one hand, the activated executioner caspases, such as caspase-3 and -7, split-specific substrates to execute the apoptotic dissolution of the cell [9]. On the other hand, in the mitochondrial pathway, the anti-apoptotic BCL-2 family members (such as BCL-2 and BCL-XL) and pro-apoptotic BCL-2 family members (such as Bax and Bid) resident in the cytoplasm translocated to mitochondria when intracellular death-inducing signaling triggered [18]. BCL-2 and BCL-XL stabilize in the outer membrane of mitochondria and inhibit the release of Cytochrome C (Cyto C) from mitochondria, which play an important role in tumor occurrence and progress by regulating the permeabilization of the outer mitochondrial membrane and prolonging the survival of malignant cells [10]. BCL-2 expression was correlated with a survival advantage in ovarian cancer [13]. The Bid is a member of the BCL-2 family and recognized as one of the most effective inducers of mitochondrial priming in ovarian cancer, which can induce the release of apoptogenic factors during cell death [19]. Activated caspase-8 cleaves cytoplasmic Bid to truncated Bid (tBid, a potent inducer of cell apoptosis), which represents the connection between the extrinsic and intrinsic apoptosis pathways, is translocated to the mitochondrial membrane [20]. TBid targets with BCL-2 protein and has been proposed as a promising molecular target for killing cancer cells, triggering the release of Cyto C from mitochondria to the cytosol [21]. The Cyto C in cytosol triggered the assembly of the Apaf-1/caspase-9 holoenzyme that formed an "apoptosome", and in turn, activated downstream caspase-3/-7, and eventually led to the permeability transition at the inner membrane in response to activation of cell surface death receptors [22]. Our analysis indicated that *C. adamanteus* venom LAAO activated apoptosis-regulated genes Fas/FasL and mitochondrial pathways in ovarian cancer cells. But the expression trends of some genes were not consistent between mRNA and protein levels, which may be related to the complex transcriptional processes and post-translational modifications of genes.

Although most models of ovarian cancer use mice with non-functioning immune systems, a normal immune mouse model can be used for exploring the role of the immune system in the occurrence and progression of the disease [23]. We found that ovarian cancer cells can survive and grow in mice with intact immune systems. And intestinal symptoms in ovarian cancer-bearing mice were eliminated by *C. adamanteus* venom LAAO. Histological analysis also showed that *C. adamanteus* venom LAAO significantly ameliorated the structural damage to tumor-mouse gastric tissues. Numerous studies have demonstrated that the damage caused by svLAAO to normal cells is usually negligible compared with that of tumor cells [4, 24]. This implies that svLAAO has a good prospect in anti-tumor research and application.

Accumulated research disclosed that the relevant action mechanisms of cytotoxicity of svLAAO likely depend on the oxidative stress arising from the production of H_2O_2 [25]. There's a popular supposition that H_2O_2 plays a critical role in svLAAO-induced cell apoptosis by generating membrane oxidation stress that can lead to disruption of normal cell physiology, of which activity can be inhibited by adding catalase or other H_2O_2 scavengers [26]. We observed that *C. adamanteus* venom LAAO impaired viability of ovarian cancer cells; furthermore, the number of living cells increased greatly and cell morphology was also restored to a certain extent when the cells were co-treated with catalase and svLAAO. In addition, the change of mRNA and protein levels triggered by svLAAO can be partially or completely inhibited by catalase. However, there exists a very considerable difference between svLAAO and exogenous H_2O_2 for cell apoptosis mechanism. The differences are mainly embodied in several aspects: the morphological changes of cells induced by svLAAO and exogenous H_2O_2 were different, and the apoptosis induced by svLAAO can be inhibited or abolished with antioxidant or catalase, but not inhibited the apoptosis of exogenous H_2O_2 [27]. Our results confirmed that catalase couldn't completely inhibit the variation in gene expression caused by svLAAO, which further confirmed that there may be other ways for svLAAO to induce apoptosis of tumor cells. Of course, in addition to the well-known cytotoxicity of svLAAO, it is also involved in the regulation of tumor cell cycle processes, which is an essential link in regulating tumor cell apoptosis [28]. Our studies illustrated that *C. adamanteus* venom LAAO has anti-tumor potential in vivo and in vitro, which may be new material for the study of anti-ovarian cancer mechanisms and the development of the drug, but more research is required to elucidate the action mechanisms of svLAAO in the tumor cell death process.

Declarations

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