European Review for Medical and Pharmacological Sciences 2017; 21: 4437-4445

The effect of saikosaponin D on doxorubicin pharmacokinetics and its MDR reversal in MCF-7/adr cell xenografts

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Abstract. – OBJECTIVE: Multidrug resistance (MDR) is a major cause of chemotherapy failure in the treatment of cancer patients. This study aimed to determine whether saikosaponin D (SSd) can enhance the efficacy of the anticancer drug doxorubicin (Dox) both *in vitro* and *in vivo* and whether SSd can alter Dox pharmacokinetics in the serum of mice.

MATERIALS AND METHODS: MCF-7/adr cells were used to investigate the effect of SSd on reversing MDR. Cell viability was assessed by MTT assay. Pharmacokinetic tests were used to evaluate the effects of SSd on serum Dox disposition. An MCF-7/adr cell xenograft model was established to investigate the effect of SSd on reversing MDR *in vivo*. Tumor growth and weights were measured. Immunohistochemistry staining was used to detect the expression of P-gp (P-glycoprotein), an ATP-dependent efflux pump that mediates MDR in xenograft tumor tissues.

RESULTS: SSd could effectively reverse MDR in MCF-7/adr cells *in vitro* and had no cytotoxic effects on human amniotic epithelial cells (hAEC). There was no significant difference between the Dox pharmacokinetic parameters obtained in the mice that received Dox only and Dox combined with SSd, indicating that SSd did not alter the pharmacokinetic profiles of Dox. Furthermore, the combination of Dox and SSd had a stronger anticancer effect than Dox alone or SSd alone by inhibiting tumor growth and P-gp expression.

CONCLUSIONS: Our results suggest that SSd could effectively reverse MDR *in vitro* and *in vivo* and could be a potential MDR reversal agent for P-gp-mediated MDR in breast cancer therapy.

Key Words:
Saikosaponin D, Multidrug resistance, Pharmacokinetics, P-glycoprotein, Breast cancer.

Introduction

Multidrug resistance (MDR) in cancer is a major cause of chemotherapy failure in the treatment of cancer patients. Cancer cells become resistant to a single drug or a family of drugs with identical mechanisms of action, a phenomenon known as MDR. The cancer cells may acquire broad cross-resistance to mechanistically and structurally unrelated drugs[1,2]. Clinically, the reason for the MDR phenotype in cancer cells has multiple factors. One of the main underlying mechanisms of MDR is the over-expression of P-glycoprotein (P-gp), an ATP-dependent membrane transporter protein encoded by the MDR1 gene[3,4]. P-gp belongs to the superfamily of adenosine triphosphate (ATP)-binding cassette (ABC) transporters and actively pumps a wide range of structurally and functionally unrelated hydrophobic compounds out of the cell, thereby decreasing their intracellular accumulation[5]. P-gp is localized in the kidney, placenta, liver, adrenal glands, intestine and blood-brain barrier cells, where it functions to prevent the absorption of harmful substances and promote their excretion from the body[1,6,7]. Tumor cells often gain MDR through P-gp over-expression, which actively extrudes clinically administered chemotherapeutic drugs[8-11]. For this reason, inhibiting P-gp as a strategy to reverse MDR in cancer patients has been studied extensively. Over the course of research and development, there have been three generations of MDR modulators, also called MDR reversal agents. The first-generation P-gp modulators identified are themselves substrates for P-gp, and thus acted by competing with the cytotoxic compounds for efflux by the P-gp pump. The high serum concentrations of the modulators required for MDR reversal *in vivo* resulted in serious toxicity. The second-generation P-gp modulators have had a better effect as MDR reversal agents than the first-generation compounds, not only *in vitro,* but also *in vivo*. However, they also have some characteristics that limit their use as P-gp modulators.

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For example, they may interact with cytochrome P450 3A4 and lead to unpredictable pharmacokinetic interactions. The third-generation P-gp modulators can combine with P-gp directly. They have a specific affinity to P-gp, are effective MDR modulators and have no obvious effect on chemotherapy pharmacokinetics[12,13]. The sustainable development of these agents may lead to a true therapeutic potential for P-gp-mediated MDR cancer patients. Saikosaponin D (SSd) is one of the major triterpenoid saponins derived from *Bupleurum chinense* DC (BCDC), which exhibits anti-inflammatory, anti-infectious and anti-tumor activities[14-16]. Our previous studies[17] demonstrated that saikosaponin alone was able to reverse MDR in tumor cells *in vitro*. However, the MDR reversal effect of SSd on tumor cells has not been investigated. The aim of this study is to determine whether SSd can alter the pharmacokinetics of anticancer drugs and whether SSd can enhance the efficacy of doxorubicin (Dox) both *in vitro* and in nude mice bearing tumors. SSd is expected to possess a high efficiency, fewer side effects and may represent a new MDR modulator.

Materials and Methods

Cell Lines and Culture

The MDR cell line MCF-7/adr was obtained from the Bogoo Biotechnology Co., Ltd. (Shanghai, China). The human Amniotic Epithelial Cells (hAECs) were generously provided by the Institute for Regenerative Medicine of Jilin University (Changchun, China). All of the cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) in a humidified 5% CO_2 atmosphere at 37°C. MCF-7/adr cells were approximately 142-fold more resistant to Dox. To maintain the MDR phenotype, 1.0 μg/mL of Dox was added to the culture of MCF-7/adr cells and removed 7 days before the experiment.

Drugs and Reagents

Saikosaponin D (SSd) powder with a purity of > 98% was purchased from Jingzhu Biotechnology Co., Ltd. (Nanjing, China). Purified doxorubicin (Dox) was purchased from Hisun Pharmaceutical Co., Ltd. (Zhejiang, China). Verapamil (Ver) was purchased from Hefeng Pharmaceutical Co., Ltd. (Shanghai, China). Mouse anti-human P-gp (P-glycoprotein) monoclonal antibody was purchased from eBioscience (San Diego, CA, USA). MTT reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). The immunohistochemistry kit was purchased from Fuzhou Maixin Biotech (Co., Ltd., Fujian, China).

Animals

Kunming (KM) mice weighing 25 ± 3 g (8-10 weeks of age) were utilized in pharmacokinetic experiments. The mice were obtained from the Center of Experimental Animals, Beihua University. Athymic BALB/c nu/nu mice weighing 18-20 g (4-6 weeks of age) were obtained from the Center of Experimental Animals, Wuhan University. The mice were used for the MCF-7/adr xenografts. All of the animal experiments were performed in strict accordance with the International Standards of Animal Care Guidelines. All of the procedures were performed in accordance with the regulations of the Beihua University Committee on Ethics in the Care and Use of Laboratory Animals.

MTT Assay

Cell viability was assessed by MTT assay. In brief, MCF-7/adr cells or hAECs were seeded in 96-well plates (8×10^3 cells/well) and incubated overnight. Different concentrations of modulators were added to the wells and incubated for 48 h followed by addition of 15 μL MTT solution to each well (5 mg/mL). After 4 h of incubation, the supernatants were removed and 150 μL of dimethyl sulfoxide (DMSO) was added for 10 min. The optical density (OD) at 570 nm of each well was measured with an enzyme immunoassay instrument (Bio-Rad 2550, Bio-Rad, Hercules, CA, USA). The cell survival rate was calculated using the following formula: (%) = (OD of treated group/OD of control group) × 100%. The IC_{50} value was defined as the concentration of drug required to reduce cell survival to 50% and calculated by CalcuSyn software (version 2.0, Biosoft, Cambridge, UK). The reversal fold of MDR was calculated using the following formula: (RF) = IC_{50} value for Dox in MCF-7/adr cells / IC_{50} value for Dox in MCF-7/adr cells treated with SSd[18].

Pharmacokinetic Experiments in Mice

For the pharmacokinetics of SSd, KM mice were randomly divided into 11 groups (n=3 for each group) according to time points. The mice were given 5 mg/kg of SSd by intraperitoneal injection (i.p.). Blood was collected from the eyeball of mice at several time points (0.08, 0.33, 0.67, 1.0, 2.0, 4.0, 8.0, 12, 18, 24 and 48 h) after injection. Plasma samples were isolated and incubated with

0.5 mL methanol overnight at 4°C. Supernatants were obtained after centrifugation at 4000 g for 10 min. SSd concentrations in plasma were analyzed by HPLC and chromatographed on a ZORBAX SB-C18 column (4.6×250 mm, 5 μm particle size). The mobile phase consisted of acetonitrile: water (32:68, v/v), pumped at a flow-rate of 1 mL/min with a determination wavelength of 205 nm[19]. To evaluate the effects of SSd on serum Dox disposition, KM mice were randomly divided into a Dox group and Dox-SSd group. The mice in the Dox group were treated with 5 mg/kg Dox intravenously (i.v.) alone, and the mice in the Dox-SSd group were treated with 5 mg/kg Dox (i.v.) combined with 5 mg/kg SSd (i.p.). Blood was collected from the eyeball of mice at several time points (0.08, 0.25, 0.50, 0.67, 1.0, 2.0, 4.0, 6.0, 8.0, 12 and 24 h) after the drug administration. In brief, these samples were processed and analyzed for Dox concentrations in plasma by HPLC[20]. Pharmacokinetic data assessment was calculated by pharmacokinetics statistics software DAS2.0 (Drug and Statistics, Wannan Medical College, Wuhu, China).

Reversal of MDR in the MCF-7/adr Cell Xenografts

MCF-7/adr cells (1×10^7 cells/per mouse) were injected subcutaneously into the back of nude BALB/c mice (n = 6 for each group). When the volume of the xenograft tumors reached approximately 100 mm^3, the mice were randomly divided into four groups and treated with saline (same volume), Dox (5 mg/kg), SSd (5 mg/kg) and Dox-SSd (5 and 5 mg/kg) by i.p. injection every second day. The tumor size and body weight of each mouse were measured every second day. The tumor volume was calculated using the following equation: $V = \text{length} \times (\text{width})^2 / 2$. Three weeks after injection, the mice were sacrificed. The xenograft tumors were removed and weighed. The inhibitory rate of tumor growth was calculated using the following equation: inhibitory rate = (tumor weight of control group – tumor weight of treatment group) / tumor weight of control group × 100%[21].

Immunohistochemistry Assay

The xenograft tumors were fixed in 10% buffered formalin, embedded in paraffin, and then cut into 5 μm-thick sections. Immunohistochemical staining was performed using the same method as previously described[22]. Briefly, the sections were labeled with a monoclonal antibody against P-gp (1:100) followed by a biotin-labeled secondary antibody and streptomycin anti-biotin peroxidase. Diaminobenzidine (DAB) was used as a chromogen. Finally, sections were counterstained with hematoxylin solution, dehydrated and mounted. P-gp expression was quantified as integrated optical density (IOD) using Image-Pro Plus software 6.0 (Media Cybernetics, Rockwille, MD, USA).

Statistical Analysis

Statistical analysis was conducted in SPSS 13.0 (SPSS Inc., Chicago, IL, USA). All of the experiments were performed in triplicate in at least three independent trials. The results are presented as the mean±SD. Statistical significance was assessed by 2-tailed Student's *t*-test for 2 groups and one-way analysis of variance (ANOVA) for more than 2 groups, followed by the LSD test. $p < 0.05$ was considered significant.

Results

Reversal of MDR by SSd in vitro

To determine the nontoxic concentrations of SSd in MCF-7/adr cells (above 90% cell survival), different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 μg/mL) of SSd were added to cells for 48 h. The results showed that the non-toxic concentrations of SSd were equal to or less than 0.5 μg/mL. Thus, MCF-7/adr cells were incubated with SSd at 0.5, 0.25 and 0.1 μg/mL; a full range of concentrations of Dox to detect the reversal effect of MDR. Ver at 5.0 μg/mL (a non-toxic concentration) was used as a positive control. The results showed that SSd could effectively reverse the MDR of MCF-7/adr cells in a dose-dependent manner and the reverse folds were 4.38-fold, 1.94-fold and 1.56-fold. The reverse fold of Ver was 4.29-fold (Table I). These results suggested that SSd could increase the sensitivity to Dox and reverse MDR *in vitro*.

The Cytotoxic Effect of SSd with Reversal Concentrations in hAEC

To investigate whether reversal concentrations of SSd have cytotoxic effects on normal cells, human amniotic epithelial cells (hAECs) were treated with SSd at 0.5, 0.25 and 0.1 μg/mL for 48 h. Compared with the control group, the survival rates of hAEC were 90.3%, 92.1% and 95.2%, respectively (Figure 1A and 1B, $p > 0.05$). This result revealed that the reversal concentrations of SSd in MCF-7/adr cells had no cytotoxic effects on normal human cells.

Table I. Reversal effects of SSd on MCF-7/adr cells.

Groups	Dose (μg/mL)	IC_{50} value (mean ± SD, μg/mL)	RF of MDR
Dox		192.79 ± 17.14	
Dox+SSd	0.1	123.32 ± 13.75*	1.56
	0.25	99.34 ± 11.29*	1.94
	0.5	43.97 ± 5.80**	4.38
Dox+Ver	5.0	51.49 ± 4.70**	4.29

IC_{50} value, the concentration of drug required to reduce cell survival to 50%; RF, reversal fold, which was calculated from dividing the IC_{50} of Dox alone by the IC_{50} of Dox in combination with SSd or Ver in MCF-7/adr cells. *$p < 0.05$ and **$p < 0.01$ compared with Dox group.

The Pharmacokinetics of SSd in Mice

The above results showed that SSd could effectively reverse MDR *in vitro*. To investigate whether SSd could achieve the required plasma concentration to reverse MDR *in vivo*, the pharmacokinetics of SSd was investigated in KM mice. In preliminary experiments, an injection of 5 mg/kg led to blood drug concentrations at a non-toxic dose and more time to reverse MDR in mice (data not shown). Therefore, the 5 mg/kg dose was used to study the pharmacokinetics of SSd in mice. SSd at 5 mg/kg was injected (i.p.) into KM mice at different time points. SSd concentrations in plasma were analyzed by HPLC. The main pharmacokinetic parameters of SSd were the following: t1/2z: 7.985 ± 0.287 h, AUC ($_{0\text{-}48\ h}$): 8238.967 ± 291.735 mg/L·h, AUC ($_{0\text{-}\infty}$): 8322.089 ± 285.836 mg/L·h, MRT ($_{0\text{-}48\ h}$): 8.455 ± 0.033 h, MRT ($_{0\text{-}\infty}$): 8.966 ± 0.089, and Cmax: 954.667±36.226 mg/L. The peak plasma concentration of SSd achieved was 0.955 μg/mL at 4 h after the administration. Furthermore, at 12 h, the concentration of SSd was 0.16 μg/mL and still sufficient to reverse drug resistance (Figure 2A). These results suggested that 5 mg/kg SSd could achieve a reversal concentration *in vivo*.

Effect of SSd on Pharmacokinetics of Chemotherapeutic Drug Doxorubicin in Mice

To evaluate the effects of SSd on plasma concentrations, pharmacokinetic studies of Dox were performed on KM mice treated with Dox at 5 mg/kg intravenously (i.v.) alone or combined with 5

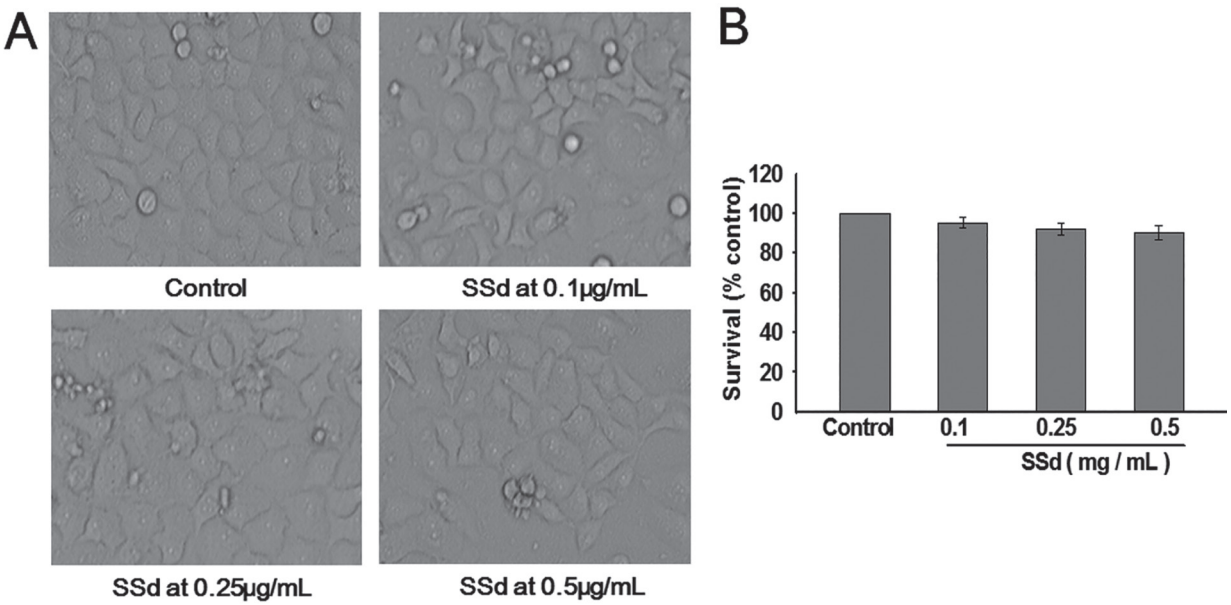

Figure 1. Cytotoxicity of human amniotic epithelial cells (hAEC) induced by SSd. Cells (8×10^3 cells/well) were treated with different concentrations (0.1, 0.25 and 0.5 μg/mL) of SSd for 48 h, and the survival rate was assessed using an MTT assay. ***A.*** The morphology of hAECs after treatment with SSd. ***B.*** The survival rate of hAECs after treatment with SSd.

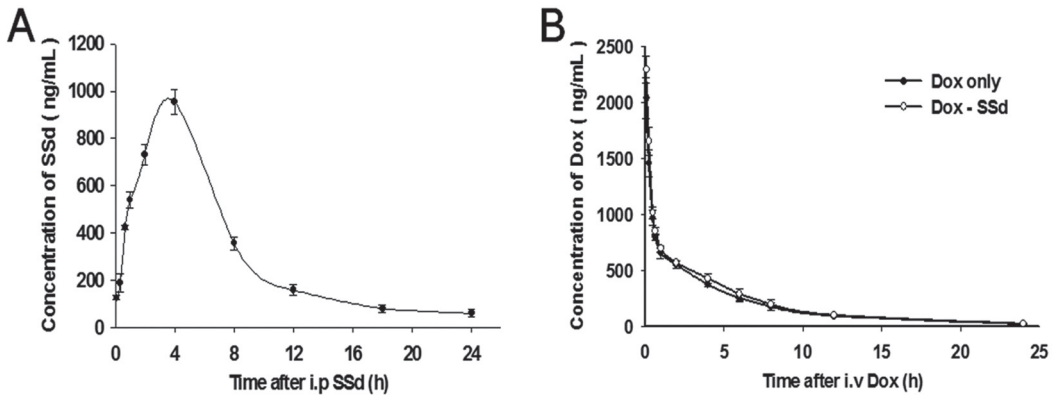

Figure 2. Blood drug time curves in healthy mice. ***A.*** Blood drug time curves of SSd in healthy mice: 5 mg/kg of SSd was injected (i.p.) into each mouse (n = 3). Blood was collected from the eyeball of mice at several time points after injection. Plasma samples were analyzed by HPLC. ***B.*** Blood drug time curves of doxorubicin (DOX) and DOX-SSd in healthy mice: the mice in the experimental group were treated with SSd at 5 mg/kg by i.p. while the mice in the control group received the same volume of saline. All of the mice were injected with Dox at 5 mg/kg by i.v. Blood was collected from the eyeball of the mice. Each sample was assayed in duplicate. The results are expressed as the mean ± SD.

mg/kg SSd (i.p.) at different time points. Dox concentrations in plasma were analyzed by HPLC. The results showed there were no significant differences in the pharmacokinetic profiles between the SSd and Dox-SSd groups (Figure 2B, Table II, $p > 0.05$). The above results suggested that SSd could not cause increases in doxorubicin concentrations in plasma.

Reversal of MDR by SSd in vivo

To investigate whether 5 mg/kg SSd could effectively reverse MDR *in vivo*, MCF-7/adr xenograft mice were treated with the various regimens and tumor growth suppression was observed. The results showed that tumor growth was markedly suppressed in the Dox group, SSd group and Dox-SSd compared with the control group while the tumor growth rate in the Dox-SSd group was much slower than SSd alone and Dox alone (Figure 3A and 3B). Tumor weights were measured at day 21 after treatment, and the average tumor weight of the Dox group, SSd group and Dox-SSd group were much lower than the control group (Figure 3C, $*p < 0.05$, $**p < 0.01$ *vs.* control group). The inhibitory rates of tumor growth based on weight in the Dox group, SSd group and Dox-SSd group were 54.3%, 62.1% and 75.0%, respectively. These results showed that both Dox and SSd could markedly inhibit the growth of the xenograft of MCF-7/adr cells. However, the combination of Dox and SSd had a greater anticancer effect compared to Dox alone and SSd alone. Furthermore, the body weight was not significantly decreased in the drug-treated groups compared with the saline group (data not shown). These results indicated that SSd could reverse MDR *in vivo* without increased toxic side effects.

Table II. Dox pharmacokinetic parameters between two groups of mice pretreated with or without SSd.

Parameters	With SSd	Without SSd	*p*
T1/2α	0.252 ± 0.032	0.222 ± 0.043	> 0.05
T1/2β	4.913 ± 0.19	4.878 ± 0.145	> 0.05
$AUC_{(0-t)}$	5026.642 ± 254.319	5500.242 ± 342.553	> 0.05
$AUC_{(0-\infty)}$	5204.974 ± 231.35	5693.674 ± 332.692	> 0.05
$MRT_{(0-t)}$	5.04 ± 0.128	5.035 ± 0.169	> 0.05
$MRT_{(0-\infty)}$	5.961 ± 0.264	5.946 ± 0.078	> 0.05
K10	0.439 ± 0.031	0.473 ± 0.008	> 0.05

The mice in the experimental group were treated with SSd at 5 mg/kg by i.p. while the mice in the control group received the same volume of saline. All of the mice were injected with 5-mg/kg doxorubicin by i.v. blood was collected from the eyeball of the mice. The data represent the mean ± SD of three independent samples.

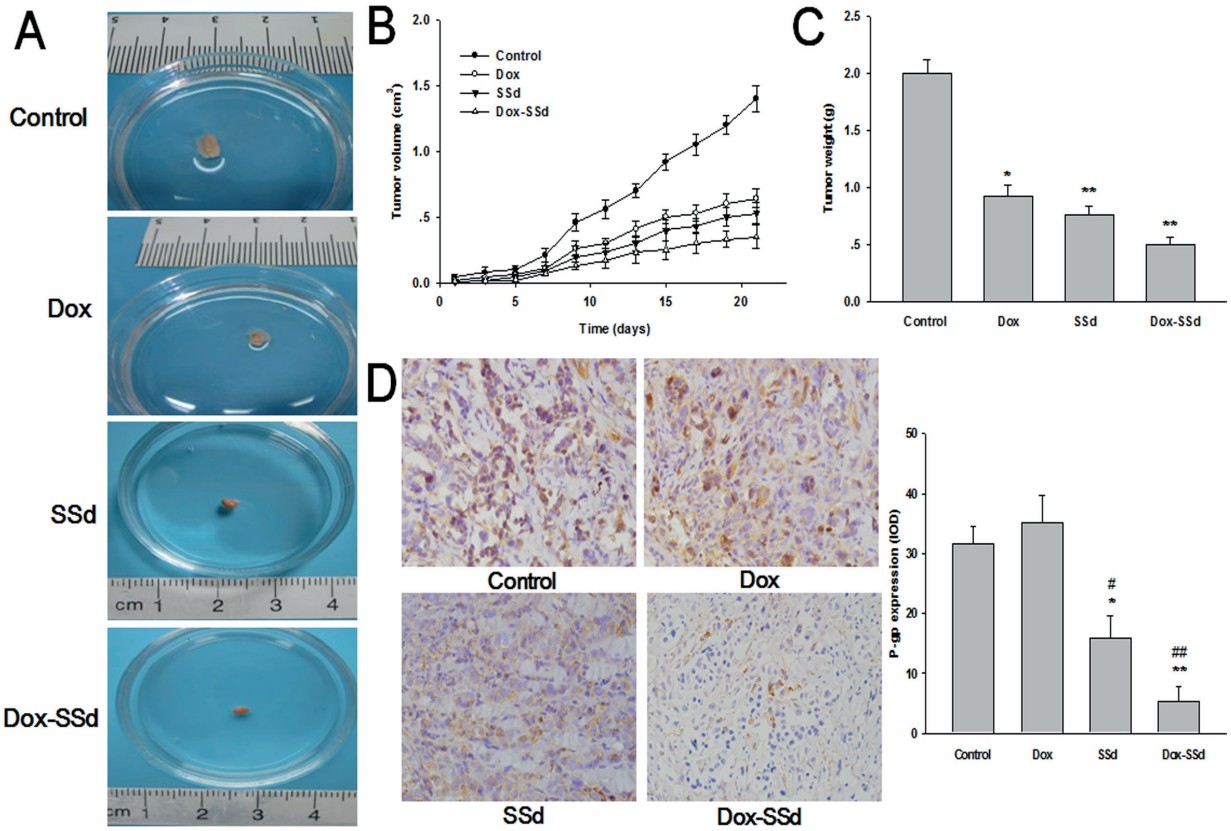

Figure 3. Reversal of MDR by SSd *in vivo*. BALB/c nu/nu mice were implanted subcutaneously (s.c.) with 1×10^7 MCF-7/adr cells. MCF-7/adr xenograft mice were treated with the various drugs. ***A.*** Photographs of xenograft tumors and tumor size: the photos from each group were captured on day 21 after treatment. ***B.*** Growth curves of xenograft tumors: the tumor sizes of each mouse were measured every second day after the injection of various drugs into the MCF-7/adr xenograft mice. ***C.*** Xenograft tumor weight: xenograft tumors were removed from the MCF-7/adr xenograft mice on day 21 and measured. ***D.*** P-gp expression in xenograft tumors: xenograft tumors in each group were stained with a monoclonal antibody against P-gp by immunohistochemistry. The results are expressed as the mean ± SD of at least 3 independent experiments. $^*p < 0.05$, $^{**}p < 0.01$ *vs.* the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* the Dox group

The Expression of P-gp in Xenograft Tumor Tissues

P-gp is the major modulator of MDR; therefore, we examined the expression of P-gp in xenograft tumor tissues by immunohistochemistry staining. The expression of P-gp was significantly decreased in both the Dox-SSd and SSd group compared to the control and Dox group ($^*p < 0.05$, $^{**}p < 0.01$ *vs.* control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* Dox group). Furthermore, P-gp expression in the Dox-SSd group was much lower than in the SSd group (Figure 3D). These results suggest that SSd can reverse MDR *in vivo* by directly inhibiting P-gp expression.

Discussion

P-gp was the first molecule identified as a modulator of MDR. Some studies on cancer cell MDR have shown that P-gp, encoded by the MDR-1 gene, plays an important role, as it pumps anticancer drugs out of the cell to reduce cytotoxicity in cancer cells and enhances the resistance of cancer cells to chemotherapeutics. However, the drug resistance presented by cancer cells can be effectively reversed by several approaches to overcome the activity of P-gp in drug-resistant cells[23-25]. In the past decades, three distinct generations of P-gp modulators have been produced[12,13,25-29]. The first-generation P-gp modulators had a low affinity for P-gp and required high doses, resulting in unacceptably high toxicity, which limited their application. The next generation employed chemically modified first-generation modulators, and the modifications were aimed at eliminating their non-MDR pharmacological activities and made them specific for MDR. However, they usually interfered with the clearance or metabolism and

excretion of anticancer drugs when these MDR modulators and anticancer drugs were co-administered. Thus, they may result in unacceptable toxicity of anticancer drugs that necessitates pharmacologically effective levels in clinical trials. The third-generation P-gp modulators generally did not change the plasma pharmacokinetics of the simultaneously administered anticancer drugs, and therefore they did not need to reduce the anticancer drug dose. The emergence of third-generation novel P-gp modulators as potential anti-MDR molecules is of particular significance. Our experimental results showed that SSd could increase the sensitivity of MCF-7/adr cells to doxorubicin *in vitro* using concentrations of SSd that were not cytotoxic by themselves. The maximum non-toxic dose was 0.5 μg/ml of SSd, which enhanced the cytotoxicity of MCF-7/adr cells to doxorubicin by 4.38-fold. The reversal potency is almost the same as that of verapamil. This finding suggests that SSd is a potent MDR modulator *in vitro*. The identification of a reversal agent with low toxicity and efficient resistance is required. SSd has been used in China as an anti-inflammatory, anti-infectious and anti-tumor drug to treat diseases clinically[14-16]. The dose of SSd used in MDR reversal trials should be safe for humans. The present study also investigated the effect of SSd on human amniotic epithelial cells (hAEC) at the reversal concentrations of 0.5, 0.25 and 0.1 μg/ml. The results indicated that the survival rate of hAEC were 90.3%, 92.1% and 95.2%, respectively (all survival rates above 90%). These data are encouraging with regards to the application of SSd in the clinic as an MDR modulator. Though SSd is a promising MDR modulator, further research is still necessary to determine whether SSd alters the plasma pharmacokinetics of anti-tumor drugs when co-administered. Our data showed that 5 mg/kg could reach sufficient plasma concentrations and led to a reversal of MDR in mice. Furthermore, SSd had no influence on the pharmacokinetics of doxorubicin. These findings suggest that SSd may be a third-generation MDR modulator. Based on the above results, further experiments were conducted *in vivo*. An MCF-7/adr cell xenograft model in nude mice was applied, and the effect of SSd was observed. The results showed that SSd significantly increased the anticancer activity of doxorubicin without a loss of body weight in the combination group. The inhibition rate of the combination group was 75.0% for the growth of the MCF-7/adr cell xenografts. These results suggest that SSd is a potent MDR modulator not only *in vitro* but also *in vivo*. The most common mechanisms for cancer cell MDR include the following: altered cell cycle check points, induction of response genes, alterations in membrane lipids, compartmentalization (in endocytic vesicles), decrease in cell apoptosis, altered drug targets, an increase in efflux pump activity and a decrease in drug absorption[4,30,31]. Although the causes of MDR are multi-factorial, one of the most important mechanisms is the over-expression of P-gp, an ATP-dependent membrane transporter protein encoded by the MDR1 gene, which is frequently related with the survival time and poor prognosis of the cancer patients[32-35]. Its over-expression in tumor cells may reduce intracellular drug accumulation and lessen the cellular toxicity of chemotherapeutics[36,38]. Furthermore, some reports have suggested that the MDR-1^{C3435T} polymorphism might influence MDR-1 and P-gp expression. The expression level of MDR-1 in the CC and CT genotypes were significantly higher than in TT genotype in cancer cells[39,40]. The TT genotype was linked to a weaker expression and activity of P-gp in cancer cells[41]. The present study demonstrated that the expression of P-gp in xenografts treated with SSd was lower than in the control group and the Dox group. The expression of P-gp in xenografts co-treated with Dox and SSd was lower than in xenografts treated with SSd alone. This finding demonstrated that SSd reverses MDR *in vivo* by directly inhibiting the expression of P-gp. MDR-1 3435C>T gene polymorphism may affect drug transport and efficacy. SSd, as a novel P-gp modulator, might have a more beneficial effect on tumor cells carrying MDR-1 3435c.

Conclusions

We demonstrated that SSd increased the sensitivity to doxorubicin and reversed MDR in MCF7/adr cells. SSd could achieve plasma concentrations capable of reversing MDR *in vitro*, which did not affect hAEC. The pharmacokinetic characteristics of Dox were not different in the presence or absence of SSd in mice. Dox combined with SSd had an obvious tumor-suppressing effect on a nude mouse xenograft model by inhibiting the expression of P-gp. Therefore, our results suggest that SSd as a combination therapy may be a promising strategy to overcome P-gp-mediated MDR clinically.

Acknowledgements
This work was supported by a grant from the Items of Science and Technology Department of Jilin Province (20130206050YY; 20150101128JC).

Conflict of Interest
The Authors declare that they have no conflict of interests.

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