

Full-length article

A novel artemisinin derivative, 3-(12- β -artemisininoxy) phenoxy succinic acid (SM735), mediates immunosuppressive effects *in vitro* and *in vivo*¹

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Key words

artemisinin; non-steroidal anti-inflammatory agents; SM735; immuno-suppressive activity

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Abstract

Aim: To study the immunosuppressive activity of SM735 {[3-(12- β -artemisininoxy)] phenoxy succinic acid}, a synthetic artemisinin derivative with nonsteroidal anti-inflammatory drug structure, with the aim of finding potential immunosuppressive agents. **Methods:** Concanavalin A (ConA), lipopolysaccharide (LPS), and mixed lymphocyte reaction (MLR), were used to induce the proliferation of splenocytes, and [³H]-thymidine incorporation was used to evaluate the proliferation of splenocytes. Cytokine production was promoted with ConA, LPS, or PMA plus ionomycin, and was detected with the enzyme-linked immunosorbent assay. Dinitrofluorobenzene (DNFB) and sheep red blood cells (SRBC) were used to induce delayed-type hypersensitivity and quantitative hemolysis of SRBC (QHS) mouse models, as criteria for the evaluation of *in vivo* immune activity. **Results:** SM735 strongly inhibited the proliferation of splenocytes induced by ConA, LPS, or MLR, with IC₅₀ values of 0.33 μ mol/L, 0.27 μ mol/L, and 0.51 μ mol/L, respectively. When compared with a CC₅₀ value of 53.1 μ mol/L, SM735 had a favorable safety range. SM735 dose-dependently inhibited proinflammatory cytokine production [including interleukins (IL)-12, interferon (IFN)- γ and IL-6] induced by LPS or PMA plus ionomycin. Upon ConA stimulation, SM735 suppressed IFN- γ in a dose-dependent manner, but did not affect IL-2 secretion. SM735 also strongly suppressed both T-cell-mediated delayed-type hypersensitivity (DTH) and B-cell-mediated QHS reactions. **Conclusion:** SM735 had strong immunosuppressive activity *in vitro* and *in vivo*, suggesting a potential role for SM735 as an immunosuppressive agent, and established the groundwork for further research on SM735.

Introduction

Artemisinin is extracted from the herb *Artemisia annua* L, and various forms of the drug are used as anti-malarial agents^[1,2]. Artemisinin is a potent anti-malarial agent with low toxicity, and its derivatives have improved efficacy in malaria treatment relative to artemisinin itself. In addition to anti-malarial effects, anti-lymphocytic effects have also been reported for artemisinin derivatives^[3,4]. Since the 1980s, the immunosuppressive actions of artemisinin and its deriva-

tives have been studied in China. Artemisinin derivatives have also been tested for the treatment of dermatoses such as photoallergic skin diseases and systemic lupus erythematosus, and promising results have been reported^[5-7].

Researchers have demonstrated that the anti-malarial activity of artemisinin and its derivatives is associated with their endoperoxides^[8,9]. When catalyzed by the ferrous ion in heme, cleavage of peroxy bonds produces free radicals and thus causes damage to malarial DNA and proteins. However, the underlying mechanism of the anti-lymphocytic

action of artemisinin and its derivatives is poorly understood.

Despite their anti-lymphocytic mechanisms being poorly understood, we hypothesized that artemisinin derivatives might be promising immunosuppressive agents, because some clinical studies have shown that they are effective in the treatment of immune dysfunction diseases. When considering an optimal molecular structure (higher efficacy and lower toxicity) for artemisinin derivatives for use as immunosuppressants, we noticed that nonsteroidal anti-inflammatory drugs (NSAIDs) remained the first-line therapy for most people with arthritis and other inflammatory diseases. Compared with other anti-inflammatory or immunosuppressive drugs such as steroids and cyclosporine, the molecular structures of NSAIDs are relatively simple and easy to synthesize. In addition, NSAIDs have few side effects, and a low level of toxicity, which are clinically desirable features. Given the relevance of the structure of NSAIDs to their immunosuppressive activity, we became interested in combining artemisinin and NSAIDs to develop a new class of immunosuppressive agents. Therefore, we synthesized and studied more than 100 different artemisinin derivatives. As a result of this screening program and subsequent medicinal chemistry studies, we obtained some derivatives that have significant immunosuppressive activity. Previous studies indicated that SM735 [3-(12- β -artemisininoxy)]phenoxy succinic acid, $C_{25}H_{32}O_9 \cdot 1/2H_2O$, MW: 485.5, Figure 1) is a representative compound, with markedly lower toxicity and higher immunosuppressive activity than artemisinin^[10]. Here, we further investigate its immunological characteristics both *in vitro* and *in vivo*.

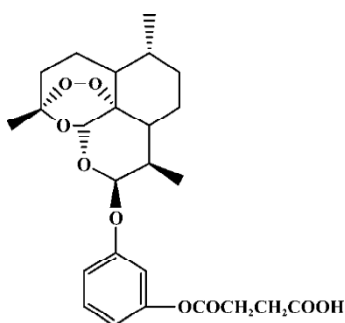


Figure 1. Chemical structure of 3-(12- β -artemisininoxy) phenoxy succinic acid (SM735).

The discovery of cyclosporine (cyclosporin A; CsA) and its successful utilization in organ transplantation was a milestone in clinical transplantation. CsA has produced a new era in transplantation in terms of both efficiency and quality of life for patients. In addition, research into the mecha-

nisms by which CsA acts has been rewarding in that we now have a better understanding of the mechanisms leading to T lymphocyte activation^[11,12]. Recently, Noori *et al* reported that the immunosuppressive activity of artemisinin was even greater than that of CsA, as indicated by both *in vivo* and *in vitro* studies^[13]. Therefore, in the present study, we demonstrate that SM735 has strong immunosuppressive effects *in vitro* and *in vivo*, and explore its possible mechanism of action.

Materials and methods

Reagents Concanavalin A, lipopolysaccharide (*Escherichia coli* 055:B5), ionomycin, phorbol 12-myristate 13-acetate (PMA), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and 3,3',5,5'-tetramethylbenzidine were purchased from Sigma (St Louis, MO, USA). Mitomycin-C was purchased from Kyowa Hakko (Tokyo, Japan). RPMI-1640 and fetal bovine serum (FBS) was purchased from Gibco (Life Technology, NY, USA). 1-Fluoro-2,4-dinitrobenzene (DNFB) was purchased from Merck (Hong Kong, China). Mouse enzyme-linked immunosorbent assay (ELISA) kits of interleukin (IL)-2, IL-12p40, interferon (IFN)- γ and IL-6 were purchased from Pharmingen (San Diego, CA, USA).

Cyclosporine (Sandimmun) was purchased from Novartis Pharma AG, Switzerland. Cyclophosphamide was purchased from Hualian Pharma (Shanghai, China). SM735 [3-(12- β -artemisininoxy)phenoxy succinic acid] was synthesized by us. Before use, SM735 was dissolved in pure dimethyl sulfoxide (Me_2SO ; 100 g/L) as a stock solution, and stored at 4 °C. The stock solution was diluted to the needed concentrations with RPMI-1640 supplemented with 10% FBS. The final concentration of Me_2SO in the culture medium was less than 0.01%, which had no influence on the assays^[10,14]. For *in vivo* experiments, both SM735 and the reference drugs were dissolved in 0.5% Tween-80, 0.33% Me_2SO in saline.

Animals and housing conditions Inbred 7–9-week-old BALB/c, C57BL/6 mice were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Certificate No. 99-003). The mice were housed in specific pathogen-free conditions with a room temperature of 24 ± 2 °C, a 12-h light/dark cycle, and provided with sterile food and water *ad libitum*. All mice were allowed to acclimatize in our facility for 1 week before any experiments were started. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica.

Preparation of splenocytes Splenocytes were prepared

aseptically from inbred 7–9-week-old BALB/c, C57BL/6 mice, and cultured in RPMI-1640 supplemented with 10% endotoxin-free, heat-inactivated FBS, 100 kU/L penicillin, 100 mg/L streptomycin, 10 mmol/L *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 50 μ mol/L 2-mercaptoethanol (2-ME).

Lymphocyte proliferation and cytotoxicity assay

Lymphocyte proliferation was carried out as described elsewhere^[15]. Briefly, 7–8-week-old male BALB/c mice were killed and splenocytes were prepared aseptically to a single-cell suspension. Splenic lymphocytes were stimulated with ConA (5 mg/L) or lipopolysaccharide (LPS 10 mg/L), plus the required concentrations of drugs. The cell cultures were then incubated for 48 h at 37 °C in a humidified 5% CO₂ incubator. Cells were pulsed with 0.5 μ Ci/well [³H]-thymidine 8 h prior to the end of the culture. After 48 h, cells were harvested onto a glass fiber filter using a HARVESTER96® (TOMTEC, USA) 96-well cell harvester and incorporated radioactivity was counted with a Beta Scintillation counter (1450 MicroBeta Trilux, PerkinElmer Life Sciences, Boston, MA, USA).

Cytotoxicity was assessed by using the MTT assay. Briefly, 15 μ L of 5 g/L MTT was pulsed 4 h prior to the end of the culture (in a total volume of 160 μ L), and then 80 μ L solvent [10% sodium dodecylsulfate (SDS), 50% *N,N*-dimethyl formamide, pH 7.2] was added to dissolve the precipitate. The solution was incubated for another 7 h and *OD*_{570 nm} was read by using a microplate reader (Bio-Rad, Model 550, Tokyo, Japan).

Mixed lymphocyte reaction proliferation assay The mixed lymphocyte reaction (MLR) was carried out as previously described, with some modifications^[16]. Briefly, spleen cells from 7–9-week-old BALB/c mice were prepared in a 1×10^{10} cells/L suspension, which was inactivated for 2 h with 50 mg/L of mitomycin C. Cells were washed and co-cultured with freshly prepared splenocytes from C57/BL6 mice (the ratio of stimulator to responder was 1.0) in a final concentration of 1.5×10^9 cells/L for 96 h. The desired concentrations of each compound were added in cultures for immunological activity assays. [³H]Thymidine (0.5 μ Ci/well) was pulsed 24 h prior to the end of the culture and then cells were harvested onto a glass fiber filter for measurement of incorporated radioactivity.

Cytokine production and analysis Splenocytes (5×10^6) were prepared from 7–9-week-old BALB/c mice, added to 1 mL of RPMI-1640 media, and then were incubated with 5 mg/L of ConA, 10 mg/L of LPS, or 10 μ g/L of PMA plus 1 μ mol/L of ionomycin, for 24 h of culture in 48-well microculture plates. The supernatants were harvested and stored

at –80 °C before the assay was carried out. Cytokine levels in supernatants were measured by ELISA, according to the manufacturer's instructions (Pharmingen, San Diego, CA, USA). 3,3',5,5'-Tetramethylbenzidine was used to develop the color reaction. The absorbance was read at 450 nm by a microplate reader (Bio-Rad, model 550, Tokyo, Japan). Cytokine concentrations were calculated based on a standard curve created using standard murine cytokines.

DNFB-induced delayed-type hypersensitivity response Female BALB/c mice were randomized into 6 groups and sensitized with 20 μ L of 0.5% DNFB dissolved in acetone-olive oil (4:1) on each hind foot on d 0 and d 1. Me₂SO vehicle, CsA and SM735 were administered to each group ($n=10$) by intraperitoneal injection on 4 consecutive days (d 7–d 10). On d 9 mice were challenged with 10 μ L of 0.2% DNFB on both sides of the right ear, using the method described by Phanuphak *et al*, with some modifications^[17]. The extent of ear swelling was expressed as the difference between the weight of punches taken from the left and right ears by using an 8-mm punch 48 h after the second challenge.

Quantitative hemolysis of sheep red blood cells Female BALB/c mice were immunized by ip injection with 0.2 mL of 16.7% sheep red blood cells (SRBC) on d 0. Me₂SO vehicle, 25 mg/kg of cyclophosphamide and SM735 were administered to each group ($n=6$) by ip injection on 4 consecutive days (d 1–d 4). On d 5, mice were killed, and mixed suspensions of 2×10^9 spleen cells/L were made. A total of 1 mL of cell suspension was incubated with 1 mL of 0.5% SRBC and 1 mL of 1:10 dilution of guinea pig complement for 0.5 h at 37 °C. The suspension was then centrifuged (3 min at 3000 \times g) and the extent of hemolysis in the supernatant was determined at 520 nm, according to the method used by Simpson and Gozzo with some modifications^[18].

Statistical analysis Three independent experiments were performed with similar results. We counted the 50% cytotoxic concentration (CC₅₀) and the 50% inhibitory concentration (IC₅₀) values using the Origin software package (Microcal Software). Student's *t*-test and one-way analysis of variance (ANOVA) with Newman-Keuls multiple comparisons on post-tests were used to analyze data and compare groups. $P < 0.05$ was considered significant.

Results

Cytotoxicity of SM735 for murine splenocytes To determine the safe dose range of SM735, we first examined the cytotoxic effect of the compound. As Figure 2A shows, in a 48-h culture, SM735 had a typical S-shaped concentration-toxicity relationship for murine splenocytes. SM735 at

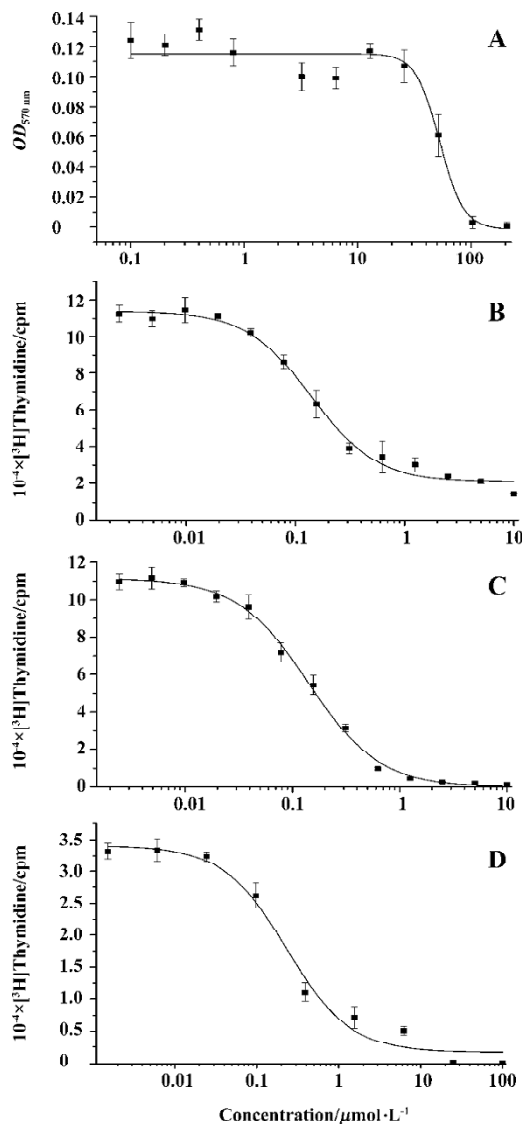


Figure 2. (A) Cytotoxicity of SM735 was tested in BALB/c mice splenocytes using the MTT assay on a 48-h culture, with a CC_{50} value of $53.1 \pm 7.8 \mu\text{mol/L}$. (B) SM735 inhibited ConA-induced splenocyte proliferation in a 48-h culture, with an IC_{50} value of $0.33 \pm 0.06 \mu\text{mol/L}$. (C) SM735 inhibited LPS-induced splenocyte proliferation in a 48-h culture, with an IC_{50} value of $0.27 \pm 0.02 \mu\text{mol/L}$. (D) SM735 suppressed the mixed lymphocyte reaction, with an IC_{50} value of $0.86 \pm 0.18 \mu\text{mol/L}$. Mitomycin C-inactivated splenocytes from BALB/c mice were co-cultured with splenocytes from C57BL/6 mice for 96 h in the presence of SM735. Three independent experiments were performed with similar results. Mean \pm SEM. The data were fitted with sigmoidal functions and the IC_{50} values were counted by using the Origin software package. Correlation indices: (A) $R^2=0.960$; (B) $R^2=0.993$; (C) $R^2=0.997$; (D) $R^2=0.984$.

a dose of $25 \mu\text{mol/L}$ showed no cytotoxicity (inhibitive rate $<10\%$, $P>0.05$). The 50% cytotoxic concentration (CC_{50}) value of SM735 was $53.1 \pm 7.8 \mu\text{mol/L}$ for a 48-h culture.

SM735 inhibits mitogen-induced murine splenocyte proliferation Concentration-dependent suppression of murine splenocyte proliferation in response to ConA and LPS was observed when SM735 was added to cell cultures. Figure 2B, 2C shows that proliferation induced by ConA or LPS was significantly inhibited by SM735 exposure. Both concentration-dependencies fitted typical S-shaped curves. Comparisons with a vehicle control showed that this effect was statistically significant ($P<0.05$) when concentrations of SM735 were above $0.04 \mu\text{mol/L}$ in response to ConA and above $0.02 \mu\text{mol/L}$ in response to LPS. In response to ConA, the IC_{50} value of SM735 inhibition of lymphocyte proliferation was $0.33 \pm 0.06 \mu\text{mol/L}$, and in response to LPS, the IC_{50} value was $0.27 \pm 0.02 \mu\text{mol/L}$.

SM735 inhibits splenocyte proliferation in MLR Mitomycin C-inactivated splenocytes from BALB/c mice ($H-2^d$) were applied as allogeneic stimuli to proliferating splenocytes of C57BL/6 mice ($H-2^b$). As shown in Figure 2D, SM735 strongly suppressed T cell proliferation in MLR in a dose-dependent manner, with an IC_{50} value of $0.86 \pm 0.18 \mu\text{mol/L}$ for a 96-h co-culture. When concentrations of SM735 were above $0.08 \mu\text{mol/L}$, comparisons with the vehicle control showed that this effect was statistically significant ($P<0.05$). The IC_{50} values were comparable to those when ConA-stimulated T cell proliferation was suppressed.

Effects of SM735 on cytokine production We used ConA alone, LPS alone, or PMA plus ionomycin as stimuli to promote cytokine secretion in mouse splenocytes. Then the effects of SM735 on the production of proinflammatory cytokine IL-6, and Th1-type cytokines IL-2, IL-12, and IFN- γ , were examined. The results are summarized in Table 1. The production of IL-12, IFN- γ , and IL-6 was significantly decreased in a concentration-dependent manner when the splenocyte cultures were exposed to SM735, upon stimulation with LPS or PMA plus ionomycin. We also detected a strong inhibitive effect of SM735 on IFN- γ production induced by ConA. Unexpectedly but interestingly, we did not observe a significant effect of SM735 on IL-2 production.

SM735 suppresses DNFB-induced delayed-type hypersensitivity reaction Figure 3 illustrates the dose-dependent inhibitory effect of SM735 on DNFB-induced DTH ear swelling. When SM735 was administered for 4 consecutive days, at doses of 7.5, 15, and 30 mg/kg, SM735 significantly suppressed ear swelling by 19.6%, 35.5%, and 55.4%, respectively. The inhibition was comparable to that of CsA, which exerted 55.2% suppression at a dose of 50 mg/kg.

SM735 suppresses anti-SRBC specific immunoglobulin production Quantitative hemolysis of SRBC (QHS) is a model of primary antibody production in response to anti-

Table 1. Effects of SM735 on cytokine production by stimulated splenocytes. Splenocytes from BALB/c mice were stimulated for 24 h. Cytokine production was measured in cell-free supernatants using ELISA. $n=3$. Data are mean \pm SEM. ^b $P<0.05$, ^c $P<0.01$ vs stimulated-cells without SM735.

SM735/ mol·L ⁻¹	Stimulus	Cytokine production/ μ g·L ⁻¹		IL-6
		IL-2	IFN- γ	
–	–	27 \pm 2	83 \pm 7	
–	ConA	2321 \pm 33	1523 \pm 22	
1 \times 10 ⁻⁷	ConA	2250 \pm 77	1219 \pm 33 ^c	
1 \times 10 ⁻⁶	ConA	2343 \pm 154	976 \pm 18 ^c	
1 \times 10 ⁻⁵	ConA	2053 \pm 162	322 \pm 18 ^c	
		IL-12	IFN- γ	IL-6
–	–	145 \pm 17	97 \pm 21	397 \pm 27
–	LPS	707 \pm 8	240 \pm 13	1013 \pm 43
1 \times 10 ⁻⁷	LPS	592 \pm 33 ^b	207 \pm 13 ^b	840 \pm 10 ^c
1 \times 10 ⁻⁶	LPS	565 \pm 18 ^c	172 \pm 2 ^c	792 \pm 42 ^c
1 \times 10 ⁻⁵	LPS	292 \pm 26 ^c	103 \pm 7 ^c	522 \pm 1 ^c
–	PMA+Ion	699 \pm 29	844 \pm 36	3256 \pm 176
1 \times 10 ⁻⁷	PMA+Ion	586 \pm 52 ^b	768 \pm 6 ^c	2886 \pm 26 ^b
1 \times 10 ⁻⁶	PMA+Ion	487 \pm 37 ^c	557 \pm 31 ^c	2663 \pm 52 ^c
1 \times 10 ⁻⁵	PMA+Ion	198 \pm 35 ^c	144 \pm 21 ^c	1465 \pm 35 ^c

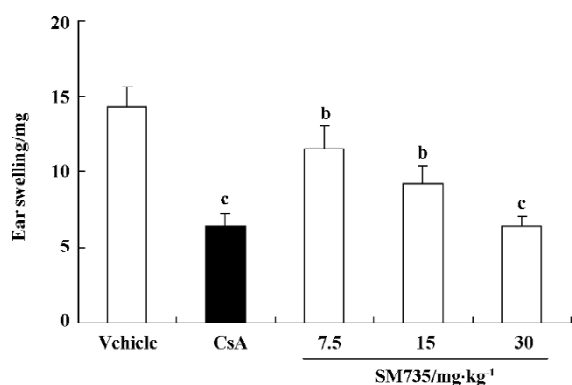


Figure 3. BALB/c mice were initially sensitized with DNFB on d 0 and d 1, and then challenged with DNFB on d 9. Vehicle, CsA or SM735 was administered on d 7–d 10. Ear punches were taken 48 h post challenge, and ear swelling was calculated as the difference between the weights of the punches taken from the left ear (DNFB treated) and the right ear (untreated). $n=10$. Mean \pm SD. ^b $P<0.05$, ^c $P<0.01$ vs vehicle group. Three independent experiments were performed with similar results.

genic stimulation. Because CsA specifically blocks T cell activation by suppressing IL-2 production, with little effect on B cells, we chose cyclophosphamide (CTX) as a reference drug in the present study^[19]. As Figure 4 shows, administration of 15 and 30 mg/kg SM735 for 4 consecutive days significantly suppressed QHS in a dose-dependent manner. The inhibitive rates were 15.3% ($P<0.05$) and 25.4% ($P<0.01$), respectively, slightly less potent than the 31.8% inhibition effected by CTX ($P<0.01$).

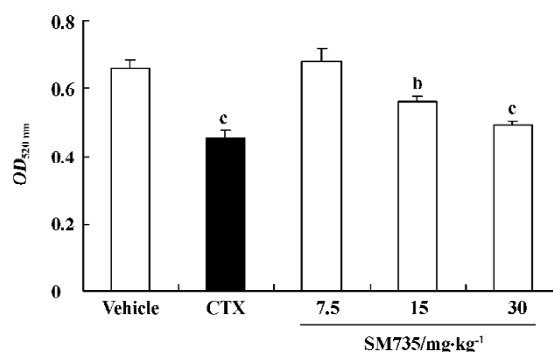


Figure 4. BALB/c mice were immunized with SRBC for testing immunoglobulin secretion. Vehicle, 25 mg/kg CTX and SM735 were administered to each group for 4 consecutive days. Supernatant hemolysis at 520 nm was read to assess the immunoglobulin-secreting activity of B cells. $n=6$. Mean \pm SD. ^b $P<0.05$, ^c $P<0.01$ vs vehicle group.

Discussion

Patients who receive organ grafts are principally treated with immunosuppressive agents. In addition, there are a number of other autoimmune chronic inflammatory disorders that might be able to be treated with immunosuppressive drugs. In the 1970s and 1980s, the discovery and clinical use of CsA and tacrolimus (FK506) enabled the successful transplantation of major organs in human patients. However, it seems now that neither are able to be tolerated in the long term^[20,21]. These drugs cause systemic immunosuppression that greatly increases the risks of tumors arising and lethal fungal infections occurring. Therefore, new immunosuppressants are required that possess better therapeutic effects or can be combined with currently used drugs to reduce long-term tolerance and side effects.

Previous studies have indicated that artemisinin possesses anti-lymphocytic activities. There are numerous reports of clinical cases in which autoimmune diseases have been treated with artemisinin and artesunate; therapeutic

effects were achieved in these cases, thus encouraging us to study artemisinin and its derivatives. To improve the efficacy of the drug, we optimized artemisinin by linking it with various NSAID structures. Here, we report that a newly synthesized artemisinin derivative, SM735, possesses potent immunosuppressive activity both *in vitro* and *in vivo*.

Lymphocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses. Treatment with SM735 can dose-dependently reduce both the T and B cell proliferation promoted by mitogens. Because it has a relatively high CC_{50} value, SM735 has a favorably large safety range.

A mixed lymphocyte reaction is induced by allogeneic stimuli, and T lymphocytes are the main responders, indicating an immune response very similar to that which occurs in post-transplantation graft-versus-host diseases^[22,23]. MLR is often used clinically for tissue typing to identify the compatibility of donor organs and recipients. Furthermore, the suppression of MLR by immunosuppressants also helps improve the success of transplantation^[24,25]. Here we show that SM735 also potently inhibited MLR, with an IC_{50} value comparable to that for the inhibition of T cell proliferation. At concentrations above 10 $\mu\text{mol/L}$, SM735 almost completely abrogated MLR. This suggests that SM735 would probably act as an immunosuppressant in physiological conditions.

Cytokines are important modulators and effectors in the immune system. In particular, multiple proinflammatory cytokines have been proved to be closely associated with many autoimmune diseases. It is critical to silence proinflammatory cytokines to maintain successful immunosuppression^[26-28]. In the present study, we used mitogen ConA, bacterial LPS, and PMA plus ionomycin, which agitated the whole cell population by activating protein kinase C, to promote cytokine production in splenocytes. The results showed that SM735 significantly inhibited IL-12, IFN- γ and IL-6 production with LPS or PMA plus ionomycin stimulation. When using ConA as a stimulant, we also observed a dose-dependent inhibition of IFN- γ production. Unexpectedly, the IL-2 level was not obviously altered. Although the two cornerstone immunosuppressants, CsA and FK506, are putatively regarded as IL-2 inhibitors, it seems that SM735 acts totally differently, on some event(s) downstream of IL-2-mediated naive T cell activation. IL-2 is widely considered to be a key cytokine in T-cell-dependent immune responses. However, the main non-redundant activity of this cytokine centers on the regulation of T cell tolerance, and recent studies have indicated that a failure in the production of CD4⁺CD25⁺ regulatory T cells is the underlying cause of autoimmunity in the

absence of IL-2^[29]. Thus, the fact that SM735 does not inhibit IL-2 indicates that further research is necessary on the induction of long-term immune tolerance by SM735.

To examine the immunoregulatory effects of SM735 *in vivo*, we used a mouse DTH model and the QHS model. Ear swelling in DTH is primarily the result of antigen-specific CD4⁺ T cell activation^[30]. Administration of SM735 for 4 consecutive days significantly suppressed ear swelling, indicating that SM735 is capable of inhibiting the T-cell-dependent immune response *in vivo*. In our experiments, the effect of SM735 was comparable to that of cyclosporine at a curative dose. The QHS model reflects the antibody-producing capacity of plasma cells in response to SRBC^[18,19]. The suppressive effect of SM735 in QHS indicated that it also suppressed antibody-secreting B cells *in vivo*.

In conclusion, our results demonstrate that SM735, a newly synthesized artemisinin derivative, has strong immunosuppressive effects *in vitro* and *in vivo*. Because artemisinin and its derivatives potentially have low toxicity and few side effects^[4,31], our study suggests the possibility of developing SM735 and other artemisinin derivatives as novel safe immunosuppressants, probably with a different mechanism from that of CsA or FK506. However, further studies are necessary to elucidate the details of the mechanism.

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