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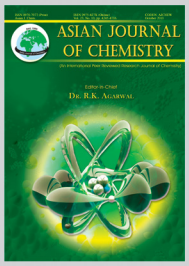

Inhibitory Activities of Daidzein Derivatives Against Human Vascular Smooth Muscle Cells Proliferation and Migration

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In order to explore the application possibility of daidzein derivatives (DDs), 3-(4'-phenylsulphonylphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD1) and 3-(4'-hydroxyphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD2), their absorption inhibitory activities against human vascular smooth muscle cells (VSMCs) proliferation and migration were investigated in present study. Results showed that the partition coefficients of DD1 and DD2 were much better than that of daidzein in octanol-water solvent. Final absorptions of DD1, DD2 and daidzein by human umbilical vascular smooth muscle cells were 71.67, 61.44 and 54.58 %, respectively. The inhibitory rate of DD1, DD2 and daidzein of 100 μM measured against vascular smooth muscle cells proliferation were 84.47, 68.53 and 66.35 %, respectively. Human umbilical vascular smooth muscle cells migration ability was dose-dependently inhibited by daidzein derivatives and daidzein. At the concentration of 100 μM, the inhibitory effect of migrated cell number which cells were treated with DD1, DD2 and daidzein were respectively at 85.00, 73.75 and 67.50 % compared with untreated control. Meanwhile, the cell migration distance of them were significantly inhibited as 81.17, 71.43 and 63.64 % of untreated control, respectively ($p < 0.05$). All those results from the experiment suggested that these derivatives of daidzein as functional food ingredients might contribute to the prevention of cardiovascular diseases *via* inhibiting vascular smooth muscle cells proliferation and migration.

Key Words: Daidzein derivative, Vascular smooth muscle cell, Proliferation, Migration, Inhibition.

INTRODUCTION

Vascular smooth muscle cells (VSMCs) proliferation is considered as a key characteristic in the development of atherosclerosis and can occur in response to many different humoral and mechanical stimuli[1,2], such as low-density lipoprotein[3], chronic inflammation[4,5] among others. In general, as one of main cells in blood vessel wall, VSMCs are commonly quiescent. They remain in the G_0/G_1 phase and proliferate at the low index (< 0.05 %)[6,7]. Once the blood vessels injury, the activated VSMCs would migrated to the intima of blood vessels and then proliferated there, thus resulting in arterial stenosis and organ dysfunction[8,9]. Therefore, inhibition of VSMCs proliferation and migration provides a possible solution in the maintenance of vascular homeostasis and thus to prevent development of atherosclerosis[10].

Recently, many studies have reported that natural bioactive compounds from plants could inhibit VSMC proliferation. Nie *et al.*[10], reported that avenanthramides existing exclusively in oats were effective in reducing mitogen-stimulated VSMC proliferation in a cell culture system *in vitro*. Moon *et al.*[11] investigated that quercetin present in the human diet could reduce TNF-α-stimulated VSMC proliferation *via* inhibiting ERK activation, arresting G1-phase and reducing cyclin D1/CDK4 activities.

Daidzein is a class of naturally occurring polyphenolic compounds in various plants such as *Hippophae rhamnoides* L., *Kudzuvine* root and soybean among others. It exhibits antioxidation[12], antiinflammatory, antiestrogens[13], antiviral and anticarcinogenic activities[14]. Despite it exhibits various biological activities *in vitro*, daidzein has not been widely employed in therapeutic medicine due to its poor solubility and first-pass effect[15,16]. Qiu[15] studied on the pharmacokinetic of daidzein in rats and found that daidzein has relatively lower bioavailability which was only of 12.8 % when calculated with free daidzein. The biological activities of polyhydroxylated compounds do not seem to depend only on their chemical structure[17], but also on their degree of lipophilicity, which could enhance their uptake into cells or influence their interaction with proteins and enzymes[18]. Mellou *et al.*[19] synthesized acylated derivatives of flavonoid with enzymatic reactions and reported that the introduction of an acyl group into glucosylated

flavonoids significantly improved their antioxidant and antimicrobial activities. Katsoura *et al.*[20] also found that biocatalytic preparation of cholestane saponins could increase their antitumor potency. Therefore, the synthesis and research of compound derivatives has gradually been got more and more attention.

In order to improve daidzein availability in functional food and therapeutic medicine, we have designed and synthesized the derivatives of daidzein (DDs), 3-(4'-phenylsulphonylphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD1) and 3-(4'-hydroxyphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD2) (Fig. 1) according to the principles of design[21,22] in the previous study. Their pharmacokinetic was systematically investigated using a Wistar rat model in the previous study. The relative bioavailabilities of derivatives, DD1 and DD2, were observed as 42.93 and 21.56 %, respectively[23]. Therefore, the pharmacokinetics of these derivatives was evidently improved compared with daidzein at the same molar dose. To the best of our knowledge, there are few reports describing the absorption of these daidzein derivatives and their inhibitory activities against human VSMCs proliferation and migration. In the present study, the absorption of DD1 and DD2 by VSMCs and their conversion in VSMCs were measured. The effects of these derivatives compared with daidzein on VSMC proliferation and migration were subsequently examined.

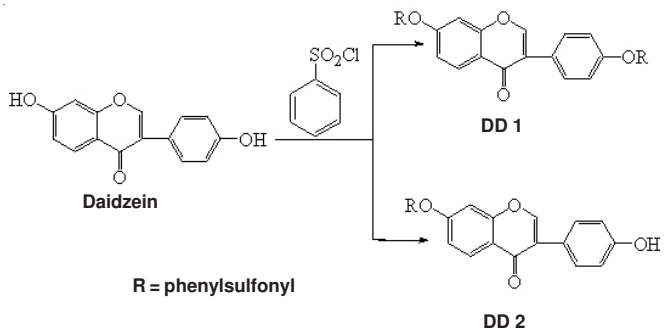


Fig. 1. Synthesis of daidzein sulfonic acid esters. 3-(4'-Phenylsulphonylphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD1) and 3-(4'-hydroxyphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD2)

EXPERIMENTAL

General procedures: Human umbilical cord was offered by Jiangxi Provincial People's Hospital from volunteers. Human bromodeoxyuridine (BrdU) enzyme linked immunosorbent assay (ELISA) kit was purchased from Uscnlife Science and Technology Co. Ltd. (Wuhan, Hubei, CN). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Ameresco Inc. (Solon, Ohio, USA). Dulbecco's modified Eagle's medium (DMEM) and other tissue culture reagents were purchased from Invitrogen Co. (Inchinnan, Scotland, UK). Ethical approval for this study was granted by the University of Nanchang Research Ethical Committee. Research was carried out in compliance with fundamental ethical principles.

Synthesis of derivatives of daidzein: Derivatives of daidzein *i.e.*, 3-(4'-phenylsulphonylphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD1) and 3-(4'-hydroxyphenyl)-7-phenylsulphonyl-4*H*-chromen-4-one (DD2), were synthesized from daidzein and benzenesulphonic acid chloride in our lab[24,25]. Their structures were confirmed by IR, MS, ^{1}H NMR spectral data, elemental analysis and X-ray diffraction analysis.

Cell culture: Human umbilical vascular smooth muscle cell (HUVSMC) was obtained from umbilical artery by the attached explant method. Briefly, the artery from fresh human umbilical cord was separated from the connective tissue and transferred into a petri dish containing free-serum medium. The separated artery was stripped off the adventitia and endarterium with forceps, then cut to pieces into 1 mm^3 and transferred into cell culture flasks. After incubation for 2 h at 37 ºC, culture flasks were supplemented with 5 mL of DMEM containing 20 % (v/v) fetal bovine serum (FBS) and kept incubating under the humidified atmosphere. Cells were cultured in DMEM supplemented with heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, 588 µg/mL L-glutamine and 0.16 % $NaHCO_3$ and incubated under a humidified atmosphere of 95 % O_2 and 5 % CO_2 at 37 ºC. Primary cultured HUVSMCs were maintained in DMEM with 20 % FBS and then were generally subcultured for generations and used between generations 3 and 6, which were maintained in DMEM with 10 % FBS.

Absorption assay: Human umbilical vascular smooth muscle cells were triplicately plated overnight in wells of 6-well plates. After free-serum starvation for 8 h, the cells were pretreated with different concentrations of DD1, DD2 or daidzein in DMEM with 20 % FBS and incubated for 0.5, 1, 6, 12, 24 and 48 h at 37 ºC. The cells and their medium were collected at different time points and inter mixed with methanol and ethyl acetate. After centrifuging at 800 × g for 10 min, collected top layer was dried under nitrogen gas and re-dissolved in 200 µL methanol. Finally, the value of daidzein derivatives (DDs) or daidzein in HUVSMCs and cultural medium was measured using an Agilent 1100 HPLC System (Agilent Technologies Inc., Santa Clara, California, USA). The Diamonsil C_{18} (200 mm × 4.6 mm, 5 µm) column (Dima Co. Ltd., Orlando, Florida, USA) was used for the HPLC analyses with methanol-phosphoric acid (0.1 %) and methanol (1:1, v/v) as mobile phase. The flow rate was at 0.5 mL/min. Column temperature was set at 25 ºC. The detection UV wavelength was set at 248 and 262 nm. The value of DDs in HUVSMCs and cell medium was performed by Agilent 1100 HPLC offline software.

Cell proliferation assay: Human umbilical vascular smooth muscle cells were plated overnight in triplicate in wells of 96-well plates at a density of 4×10^3 per well. After free-serum starvation for 8 h, DD1, DD2 or daidzein of different concentrations were added to plate wells with DMEM containing 20 % FBS and co-incubated for 48 h at 37 ºC. Finally, alive cells was determined with the MTT assay, the cell viability was measured by trypan blue exclusion test and the value of cell protein was determined with Bradford assay.

MTT assay: MTT assay was carried out as described by Chang *et al.*[26]. Briefly, proliferous HUVSMCs were obtained as mentioned above. After treatment with DD1, DD2 or daidzein for 48 h, each well was washed three times with phosphate-buffered saline (PBS) to remove unabsorbed derivatives and daidzein. MTT solution was then added to each well to a final

concentration of 0.5 mg/mL in cell medium and incubated for 4 h at 37 ºC. The MTT-formazan crystals were formed by metabolically viable cells and then dissolved in 150 µL of dimethylsulfoxide. Finally, the absorbance levels were measured at a wavelength of 570 nm with a Bio-Rad model 3550-UV microplate reader (Bio-Rad Laboratories Inc., Berkeley, California, USA).

Cell attachment assay: Human umbilical vascular smooth muscle cells were plated in triplicate in wells of 96-well plates at a density of 4×10^3 per well. Meanwhile, DD1, DD2 or daidzein of different concentrations were added to plate wells with DMEM containing 10 % FBS and co-incubated for 8 h at 37 ºC. Finally, the attached cell was measured with the MTT assay.

Bromodeoxyuridine incorporation into cell DNA: The effect of cell DNA synthesis was evaluated by BrdU incorporation into cell DNA using a human BrdU ELISA kit following the method of the manufacturers' instructions. After HUVSMCs pretreatment with DD1, DD2 or daidzein for 48 h, each well of 96-well plate was added 100 µL BrdU of 100 µM, covered with the plater sealer and incubated for 2 h at 37 ºC. Instead of liquid, 100 µL of fix denant were added to each well and incubated for 1 h at 37 ºC. After washing three times with the buffer to each well, 100 µL of anti-BrdU-POD was added and incubated for 1 h at 37 ºC with a new plater sealer. The washing process was repeated for 5 times, 90 µL of 3,3',5,5'-tetramethylbenzidine were then added to each well. After incubation for 0.5 h at room temperature, the reaction was stopped by adding 50 µL of 2 % H_2SO_4. The absorbance was measured at 450 nm within 0.5 h using a Bio-Rad model 3550-UV microplate reader (Bio-Rad Laboratories Inc., Berkeley, California, USA).

Cell migration assay: Human umbilical vascular smooth muscle cells were plated on glass slide of culture dishes and incubated for 24 h. Then half of cells on glass slide were removed with cell scraper. After free-serum starvation for 8 h, DD1, DD2 or daidzein of different concentrations were added to culture dishes with DMEM containing 20 % FBS and incubated for 48 h. Cell migration under microscope were observed by Giemsa staining. Migration ability was calculated with migrated cell number and migration distance.

Statistical analysis: All experiments were performed in triplicate and values were expressed as means ± SD. The statistical analysis was performed using one-way analysis of variance (ANOVA) and Student's *t*-test with SPSS 11.0 for windows. Coefficients of relationship were considered statistically significant when the *p*-values were less than 0.05.

RESULTS AND DISCUSSION

Solubility and octanol-water partition coefficients of daidzein and diadzein derivatives: The octanol-water partition coefficients of diadzein derivatives and daidzein were determined as described by Lipinski[24]. Solubility and partition coefficients of daidzein, DD1 and DD2 are given in Table-1. The solubility of DD1 and DD2 in methanol were found 16.57 and 97.22 times higher than that of daidzein, respectively. It should be mentioned that the solubility of DD1 was 1535.00 µg/mL in ethyl acetate solvate, which suggests that DD1 has a much better lipophilicity than DD2 of 1240 µg/mL and daidzein of 0.13 µg/mL. In addition, the octanol-water partition coefficients of DD1 and DD2 were obtained as 3.57 and 1.97, respectively, which were significantly better than that of daidzein both in lipophilicity.

Conversion of diadzein derivatives in human umbilical vascular smooth muscle cells: The conversion of derivatives into daidzein and the value thereof were measured using an HPLC method. The HPLC chromatogram showed that the peak sequence were daidzein, DD2 and DD1 at 17.99, 22.73 and 23.94 min, respectively (Fig. 2).

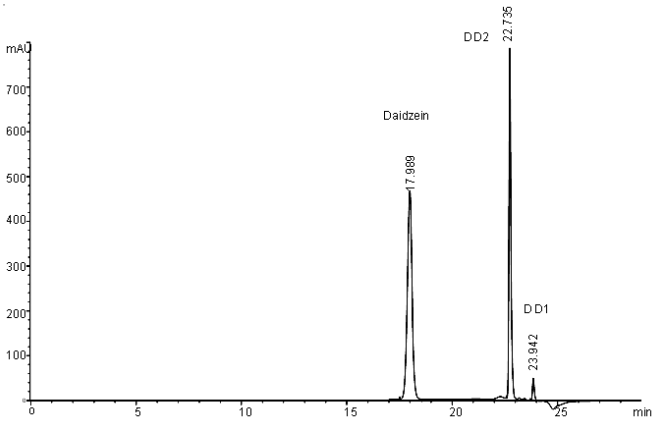


Fig. 2. HPLC chromatograms of daidzein and its derivatives samples of cell lysis buffer

As shown in Fig. 3, the values of daidzein, DD1 or DD2 in cell lysis buffer decreased with the time. Meanwhile, new compounds appeared in cells treated with DDs and daidzein. With increasing time, reduced daidzein was observed in cells treated with daidzein, indicating that daidzein was absorbed and metabolized in HUVSMCs in a time-depended manner (Fig. 3a). The value of DD1 decreased with increasing time. Meanwhile, DD2, daidzein and other new unknown compounds were observed in cells treated with DD1. It suggested that DD1 was metabolized to DD2, daidzein and other new unknown compounds in the cells (Fig. 3b). DD2 were also found to be transformed to daidzein and other new unknown compounds in cells treated with DD2 as shown in Fig. 3c. It indicated that DD1 or DD2 was absorbed and metabolized in HUVSMCs.

Absorption of daidzein or diadzein derivatives in human umbilical vascular smooth muscle cells: The decrement of daidzein or DDs in cell culture medium was regarded

TABLE-1
SOLUBILITY AND OCTANOL-WATER PARTITION COEFFICIENTS OF DAIDZEIN AND DAIDZEIN DERIVATIVES

Compound	Solubility (µg/mL)				log P
	Water	Methanol	Hexane	Ethyl acetate	
Daidzein	n.d	97.60 ± 1.24	Not detected	0.13 ± 0.11	Not detected
DD1	0.30 ± 0.11	562.00 ± 58.00	0.77 ± 0.21	1535.00 ± 107.00	3.57 ± 0.75
DD2	0.53 ± 0.31	9489.00 ± 11.00	0.37 ± 0.12	1240.00 ± 30.00	1.97 ± 0.45

TABLE-2
VALUES OF DAIDZEIN AND DAIDZEIN DERIVATIVES IN CELL CULTURE MEDIUM AND THE ABSORPTIONS OF THEM IN HUVSMCS

	Time (h)		0.5	1	6	12	24	48
Daidzein	Value (μg)		33.02 ± 0.50	30.27 ± 0.42	30.20 ± 0.42	30.14 ± 0.40	23.09 ± 0.09	16.35 ± 0.17
	Absorption (%)		8.27	15.919	16.101	16.282	35.872	54.58
DD1	Value of DD1 and metabolite	DD1	37.20 ± 0.27	32.42 ± 0.30	30.34 ± 0.44	30.90 ± 0.0.43	13.11 ± 0.40	11.18 ± 0.27
		DD2	0.54 ± 0.05	1.59 ± 0.09	1.64 ± 0.08	1.41 ± 0.07	1.33 ± 0.06	1.26 ± 0.05
		Daidzein	n.d	n.d	n.d	n.d	0.12	0.04
		Total in DD1	37.74	34.01	31.98	32.32	14.57	12.46
	Absorption (%)		14.24	22.70	27.32	26.55	66.91	71.67
DD2	Value of DD2 and metabolite	DD2	29.74 ± 0.43	27.54 ± 0.38	26.36 ± 0.40	25.49 ± 0.39	19.22 ± 0.38	18.51 ± 0.40
		Daidzein	n.d	n.d	n.d	n.d	0.07 ±	0.01 ±
		Total in DD2	29.74	27.54	26.36	25.49	19.33	18.51
	Absorption (%)		38.04	42.64	45.08	46.90	59.75	61.44

Note: The conversions of DDs and daidzein were calculated with the following formula: $M_{DD1} = (Mr_{DD1}/Mr_{DD2}) \times M_{DD2} = 1.355\ M_{DD2}$; $M_{DD1} = (Mr_{DD1}/Mr_{daidzein}) \times M_{daidzein} = 2.099\ M_{daidzein}$; $M_{DD2} = (Mr_{DD2}/Mr_{daidzein}) \times M_{daidzein} = 1.549\ M_{daidzein}$.

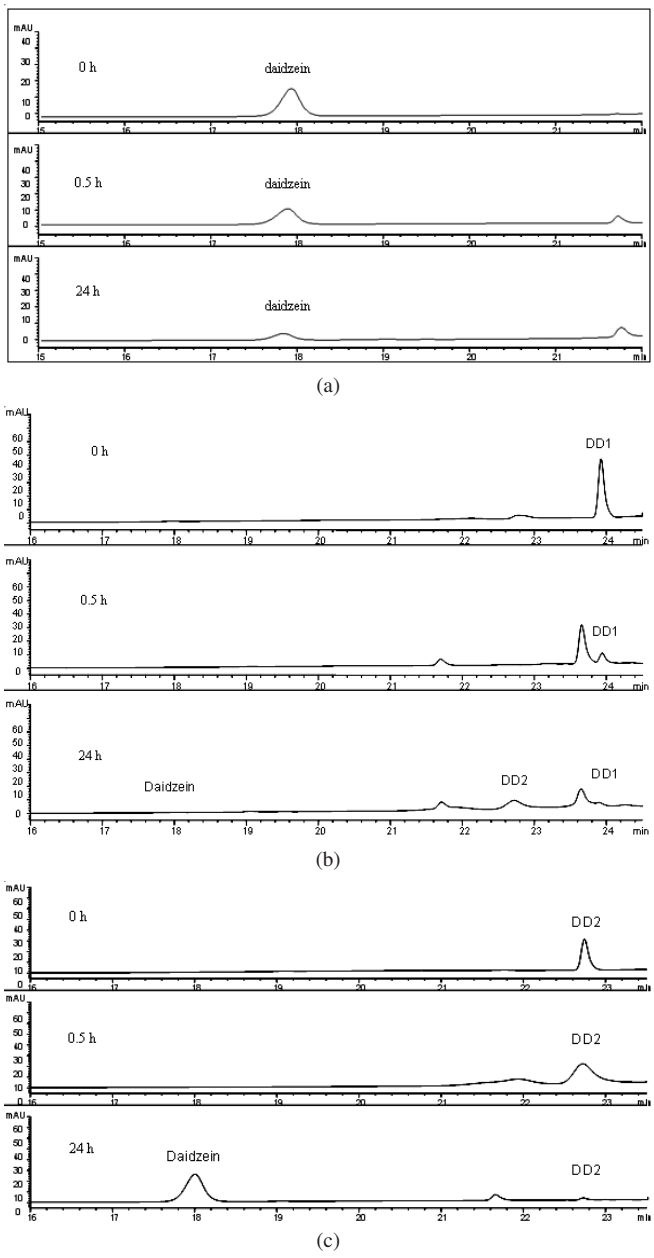


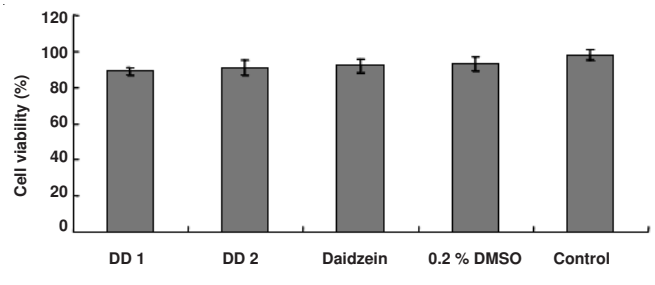
Fig. 3. Conversion of DDs in HUVSMCs determined using an HPLC method. At different incubation time points, the values of daidzein (a), DD1 (b) or DD2 (c) in HUSMCs were measured using an Agilent 1100 HPLC system

as the absorption of them by HUVSMCs (Table-2). As time progressed, the value of daidzein, DD1 or DD2 in cell culture medium decreased. Compared to the additive amount, finally absorption of DD1 and DD2 were 71.67 and 61.44 %, respectively, but the percentage measured for daidzein was only 54.584 %. It indicated that DD1 or DD2 has a much better absorption by HUVSMCs than that of daidzein.

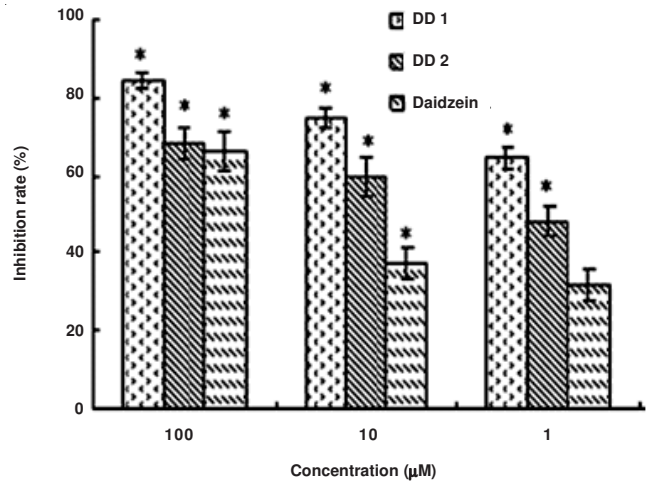
Effects of diadzein derivatives on serum-induced proliferation of human umbilical vascular smooth muscle cells: Diadzein derivatives inhibited HUVSMCs proliferation in a dose-dependent manner. Fig. 4 shows that DD1, DD2 and daidzein are not significantly cytotoxic to cell viability (Fig. 4a), but inhibit HUVSMCs proliferation (Fig. 4b). The absorbance levels of cells treated with different concentrations of DD1, DD2 or daidzein were reduced, which indicated that HUVSMCs proliferation was dose-dependently inhibited (Fig. 4b). The inhibitory rate of DD1, DD2 and daidzein against HUVSMCs proliferation were 84.47, 68.53 and 66.35 % of the control at a concentration of 100 μM, respectively. At a concentration of 1 μM, the value of DD1 and DD2 inhibiting cell proliferation respectively were 64.62 and 48.24 % compared to the untreated control, but the percentage for daidzein was only 31.56 %.

Diadzein derivatives decreased the value of total protein and DNA synthesis in human umbilical vascular smooth muscle cells: As shown in Fig. 5, stimulation of quiescent HUVSMCs with 20 % FBS induced a significant increase compared to the control ($p < 0.05$) in the value of total protein and DNA synthesis. Besides, DD1, DD2 and daidzein dose-dependently decreased the value of total protein (Fig. 5a) and BrdU incorporation into DNA (Fig. 5b) in serum-induced cells. At a concentration of 10 and 100 μM, respectively, both of DD1 and DD2 inhibited cell protein and DNA syntheses ($p < 0.05$). Their inhibitory intensities on protein value and DNA synthesis were both in the following order: DD1 > DD2 > daidzein. All derivatives were not significantly cytotoxic to cellular attachment as determined by the cell attachment assay compared to the control (Fig. 6). This indicated that DDs inhibited serum-induced cell protein and DNA synthesis but not cellular attachment.

Effects of diadzein derivatives on serum-induced migration of human umbilical vascular smooth muscle cells: Fig. 7 showed that the migration number and distance

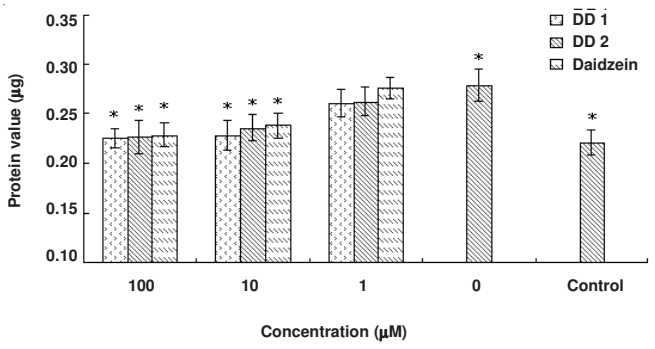


(a)



(b)

Fig. 4. Effects of DDs on serum-induced viability and proliferation of HUVSMCs. (a) The viability of HUVSMCs exposed to DD1, DD2 or daidzein; (b) The inhibition of DDs and daidzein on cells proliferation. *Significantly difference *versus* untreated control



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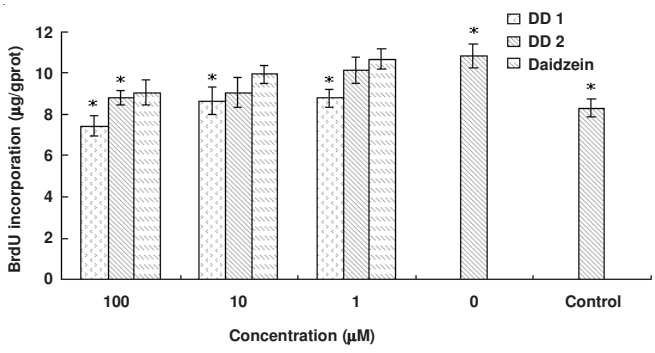


(b)

Fig. 5. Effects of DDs on serum-induced total protein and DNA synthesis of HUVSMCs. (a) protein level of HUVSMCs; (b) DNA synthesis of HUVSMCs. *Significantly difference *versus* untreated control

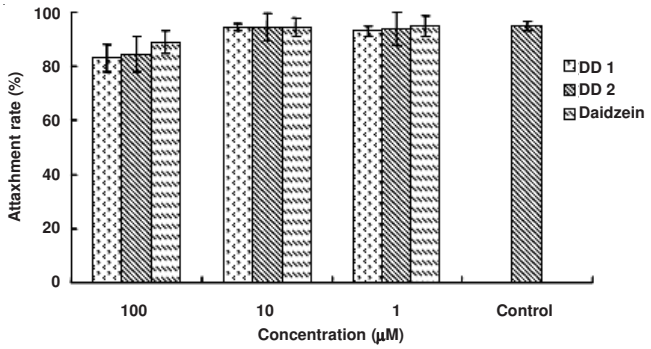
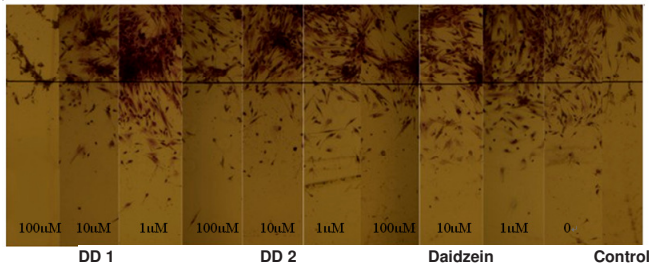


Fig. 6. Effect of DDs on attachment behaviour of HUVSMCs



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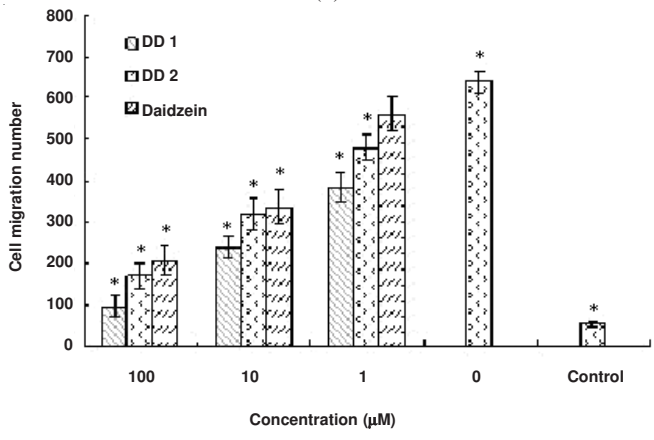

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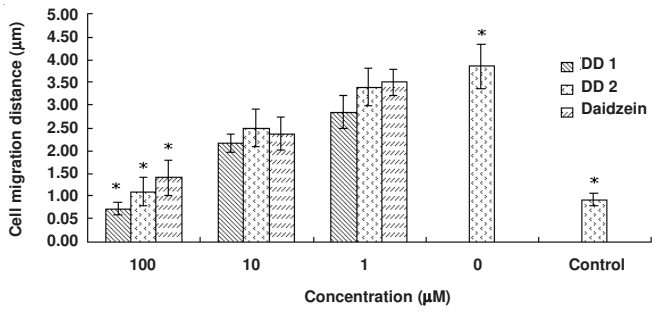

(c)

Fig. 7. Effect of DDs on migration ability of HUVSMCs. (a) The inhibition of DDs and daidzein on cell migration (b) effect of DDs and daidzein on migrated cell number; (c) effect of DDs and daidzein on cell migration distance. *Significantly difference *versus* untreated control

of cells treated with different concentrations of DD1, DD2 or daidzein were decreased compared with untreated control in dose-dependently manner. The migration number of cells treated with DD1, DD2 and daidzein were dose-dependently reduced compared with untreated control cells (Fig. 7b). The inhibitory effect of migrated cell number was at 85.00, 73.75

and 67.50 % of the untreated control at a concentration of 100 μM, respectively ($p < 0.05$). The migration distance of cells treated with DD1, DD2 and daidzein was inhibited compared with the untreated control cells (Fig. 7c). The inhibitory rate of cell migration distance was at 81.17, 71.43 and 63.64 % of the untreated control at a concentration of 100 μM, respectively ($p < 0.05$).

It has been reported that daidzein has a low bioavailability of 12.8 % due to its poor lipophilicity[16,28]. In the previous study, we designed and synthesized daidzein derivatives from daidzein and benzenesulphonic acid chloride (Fig. 1), which would be expected to enhance their cellular absorption. The octanol-water partition coefficients of DD1 (3.57) and DD2 (1.97) indicated that benzensulphonate modification of daidzein could significantly improve the lipophilicity of daidzein. The reason was probably due to the decrease of molecules lattice energy, while hydrogen of daidzein was replaced by the phenylsulphonyl moiety.

Results of recent studies have demonstrated that the bioavailability of compounds correlated with their degree of lipophilicity[24,29,30]. Leisen *et al*.[31] studied that ester derivatives of baclofen could improve the penetration through the blood-brain barrier compared with baclofen. And their affinity parameters correlated well with the lipophilicity parameters. Landowski *et al*.[32] synthesized amino acid ester derivatives of floxuridine and found that they effectively enhanced intestinal absorption and exhibited 8- and 11-fold uptake enhancement compared with floxuridine in HeLa/PEPT1cells. Therefore, ester derivation of weak bioavailability compounds could improve their absorption to exhibit their physiological function. Previously, it is found that the pharmacokinetics of daidzein derivatives, DD1 and DD2, were improved evidently compared with daidzein at the same molar dose in a Wistar rat model[23]. The relative bioavailability of DD1 and DD2 was calculated to be 42.93 and 21.56 %, respectively. In the present study, the absorption of these derivatives and their conversion were measured using an HPLC method (Fig. 3). The results showed that DD1 was transformed to DD2 (Fig. 3b) and then to daidzein (Fig. 3c) in cells. Finally absorptions of daidzein, DD1 and DD2, determined by HUVSMCs, were 54.584, 71.672 and 61.435 %, respectively. Diadzein derivatives were absorbed easier by HUVSMC probably due to their improved lipophilicity.

Moreover, daidzein is well known as a phytoestrogen. Both daidzein and its analogues have effects similar to estrogen. There are mounting evidence showing that estrogens might play an important role in anti-atherogenic effects possibly *via* suppressing cell proliferation and migration of VSMCs *in vitro*[33,34]. Results of the present study showed that DD1, DD2 or daidzein dose-dependently inhibited HUVSMCs proliferation (Fig. 4) and migration (Fig. 7) in response to high-concentration serum stimulation. Present results are consistent with the results of these reports mentioned above. DD1 and DD2 with their improved lipophilicity made themselves easier to be absorbed and metabolized by VSMCs and then they probably regulated cell proliferation through either antioxidant activity[11,35-37] or direct interaction with cellular signaling molecules[16,38,39]. The inhibitory effect of daidzein and its derivatives against VSMCs proliferation and migration might be directly through estrogen receptors in these cells as well as estrogen[39]. However, its precise cellular mechanism(s) was (were) incompletely understood and required further clarification.

To examine that DDs regulate cell proliferation, BrdU was incorporated into cells DNA to measure cell DNA synthesis (Fig. 5). We also found that DDs or daidzein inhibited serum-induced cell protein and DNA synthesis at the concentration of 10 and 100 μM. This is consistent with the results of MTT assay mentioned above. Furthermore, the inhibitions of DDs against protein and DNA synthesis were both in the order of DD1 > DD2 > daidzein. It was possible that the improved lipophilicity of DDs enhanced their physiological efficiency.

Activated VSMCs migrated to the intima of blood vessels and then proliferated there, thus resulting in forming of arterial plaques, narrowing of the vessel and organ dysfunction[8,9]. In the present study, the effect of DDs on serum-induced VSMCs migration was also investigated. Results showed that DDs inhibited HUVSMCs migration in a dose-dependent manner (Fig. 7). Diadzein derivatives decreased migrated cell number significantly at the concentration of 100 and 10 μM ($p < 0.05$). Meanwhile, the migration distance of cells treated with DDs was inhibited compared with untreated cells. The inhibitory intensities of DDs and daidzein on cell migration ability were as follows: DD1 > DD2 > daidzein. It was consistent with results of absorption assay mentioned above. This suggested that DDs effectively regulated cell migration probably due to their significant absorption and metabolism by VSMCs.

The results presented here provided experimental evidence that DDs were absorbed easier into HUVSMC due to their improved partition coefficients compared with daidzein and then effectively inhibited serum-induced VSMCs proliferation and migration. Diadzein derivatives might have the potential as functional food ingredients to contribute to the prevention of cardiovascular diseases through inhibiting VSMCs proliferation and migration.

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