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Mechanism of Action of Tetrandrine, a Natural Inhibitor of *Candida albicans* Drug Efflux Pumps

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Synergistic effects have previously been observed for a natural compound, tetrandrine (TET), with fluconazole (FLC) *in vitro* and in the treatment of *Candida albicans*-infected mice. To investigate the mechanisms of these synergistic effects, 16 strains of *C. albicans* from the same parent but with different FLC sensitivities were examined using flow cytometry and fluorescent spectrophotometry. Rhodamine 123 (Rh123)-positive cells and intracellular Rh123 fluorescence intensity were determined in accumulation/efflux experiments involving no or a noncytotoxic dose of TET. Total RNA extracted from each strain was used to compare the expressions of drug efflux pump genes in FLC-susceptible, -susceptible dose-dependent, and -resistant strains before and 24 h after TET administration. Accumulation experiments determined that mean percentages of Rh123-positive cells were 26.65% (TET-free) and 70.99% (TET 30 μg/ml), and mean respective intracellular Rh123 fluorescence intensities were 11.34 and 18.00. Efflux experiments showed that percentages of Rh123-positive cells were 1.79% (TET free) and 42.57% (TET 30 μg/ml), respectively, and respective mean intracellular Rh123 fluorescence intensities were 0.74 and 2.19. Differences in *MDR1*, *FLU1*, *CDR1*, and *CDR2* expression levels in the absence of TET were statistically significant ($p<0.05$) between FLC-susceptible, -susceptible dose-dependent, and -resistant strains. Compared with TET-free conditions, 24 h TET-treated strains showed statistically different ($p<0.05$) expression of *MDR1* (FLC-resistant strain), *FLU1* (FLC-susceptible dose-dependent and -resistant strains), and *CDR1* and *CDR2* (FLC-susceptible, -susceptible dose-dependent, and -resistant strains). Thus TET can inhibit the *C. albicans* drug efflux system and reduce drug efflux. Its mechanism of action is related to the inhibition of expression of the drug efflux pump genes *MDR1*, *FLU1*, *CDR1*, and *CDR2*.

Key words——*candida albicans*; tetrandrine; drug synergism; azoles; mechanism of action

INTRODUCTION

Candida albicans is a common opportunistic fungus seen in humans, which can cause cutaneous, mucosal, subcutaneous, and systemic infections. Fluconazole (FLC) is a first-line drug in the treatment of candidiasis,[1,2] but drug resistance is increasingly being observed.[3,4] Overexpression of the cell membrane drug efflux pump genes *MDR1*, *FLU1*, *CDR1*, and *CDR2*,[3–13] and alterations in the target gene *ERG11*[14] are the main mechanisms of azole drug resistance in *C. albicans*. Therefore drug efflux pump inhibitors are being sought as a method of reversing drug resistance in this organism.

In recent years, a number of studies have addressed this issue.[15–24] Drugs such as tacrolimus[22] and cyclosporine[18,19] can reverse *CDR*-mediated *C. albicans* FLC and ketoconazole resistance *in vitro* by inhibiting Cdr1p and Cdr2p, but because these drugs have significant cardio- and nephrotoxicity, their clinical applicability is low.

Tetrandrine (TET) is a low-toxicity drug extracted from the plant *Stephania tetrandra* S. Moore (or Fenfangji) of the Menispermaceae family. Our preliminary research demonstrated that TET can increase the sensitivity of *C. albicans* to FLC *in vitro* at noncytotoxic doses,[25] and that the compound acts synergistically with FLC in the treatment of *C. albicans*-induced vaginitis in mice.[26] The present study utilized flow cytometry and fluorescent spectrophotometry to evaluate the effects of TET on the *C. albicans* drug efflux system, while also using the semiquantitative

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reverse-transcriptase-polymerase chain reaction (RT-PCR) to examine *MDR1*, *FLU1*, *CDR1*, and *CDR2* mRNA levels to determine the molecular mechanism of action of TET.

MATERIALS AND METHODS

Strains A series of 16 clinical isolates of *C. albicans* (CA-1, CA-2, ……, CA-17, CA-10 was not available; generously provided by Theodore C. White, University of Washington, and the Seattle Biomedical Research Institute, USA) from a HIV-infected male who was treated with increasing doses of FLC. The MICs for this series of isolates from the patient increased at intervals, starting with an MIC of 0.25 μg/ml and eventually rising to an MIC greater than 64 μg/ml. They were susceptible (CA-1, CA-2, ……, CA-12), susceptible dose-dependent (CA-13, CA-14, CA-15), and resistant (CA-16, CA-17) to FLC. Several techniques were used to demonstrate that the isolates from this series were the same strain of *C. albicans*. The resistance phenotype of the final isolate (isolate CA-16, CA-17) is genetically stable, as it persists for more than 600 generations in the absence of azoles.

Culture Media YEPD agar slant, YEPD broth, and RPMI-1640 broth (with or without 10% bovine serum) were prepared as described in the CLSI M27-A protocol.[27]

Flow Cytometry Flow cytometry was utilized to determine the percentage of Rh123-positive cells in the 16 *C. albicans* strains before and after TET (Huico Plant Co. Ltd., Shanxi, China; purity 99.6 %) administration and after previously detailed accumulation/efflux experiments.[28,29] During the logarithmic growth phase, recovered *C. albicans* was suspended in 4 ml of sorbitol solution at a density of 5×10^6 cells/ml. Sixty units of lyticase (Sigma, St. Louis, MO, USA) and 50 μl of β-mercaptoethanol were added, and the suspension was incubated at 37℃ for 2 h. Precooled (4℃) phosphate-buffered saline (PBS) was used to wash the cells twice (cells and supernatant were separated by centrifugation at $744 \times g$ for 5 min). Processed cells were resuspended in 3 ml of RPMI-1640 broth and divided into three equal volumes (A, B, and C) in separate test tubes. Rh123 was added to tubes A and B at a final concentration of 2 μg/ml. TET was additionally added to tube B at a final concentration of 30 μg/ml. This dose has been shown to be noncytotoxic.[25] No drugs were added to tube C. The tubes were incubated at 37℃ for 45 min, washed twice with precooled PBS, and 500 μl was used for accumulation experiments. RPMI-1640 broth was added to the remaining suspensions in tubes A and B, and TET was again added to tube B at the same final concentration. The tubes were incubated at 37℃ for 45 min, washed twice with precooled PBS, and 500 μl was used for efflux experiments. Samples were examined using flow cytometry, and 10000 cells were counted in the fluorescein isothiocyanate (FITC) channel ($\lambda_{excitation}$=488 nm, $\lambda_{emission}$=530 nm) to construct single-parameter histograms. Data were processed with Cellques software, and the percentages of Rh123-positive cells were calculated.

Fluorescent Spectrophotometry Intracellular Rh123 fluorescence intensities before and after TET administration and after accumulation/efflux experiments[28] were determined using fluorescent spectrophtometry. Sample preparation was performed as described above. The samples were diluted with PBS to a final volume of 3 ml, and sample fluorescence intensities were measured using fluorescent spectrophotometry ($\lambda_{excitation}$=488 nm, $\lambda_{emission}$=530 nm). Ultraviolet spectrophotometry was used to determine sample intracellular OD_{600} values, and intracellular Rh123 florescence intensities were calculated.

Extraction of Total *C. albicans* RNA Extraction was performed as previously described.[5,6] Activated *C. albicans* was inoculated in RPMI-1640 broth containing no TET or TET 30 μg/ml, incubated at 30 ℃ for 24 h, and the cells were collected by centrifugation. One milliliter of Trizol RNA reagent (Invitrogen, CA, USA) was added and used to disperse each cell pellet thoroughly. After complete homogenization, the sample was incubated at room temperature for 5 min. Two hundred microliters of chloroform was added, vortexed vigorously for 15 s, and the sample was then incubated at room temperature for 2 min. The sample was centrifuged at $11913 \times g$ and 4℃ for 15 min. The supernatant was obtained, an equal volume of isopropanol was added, and the sample was incubated at room temperature for 10 min after mixing. The sample was centrifuged at $11913 \times g$ and 4℃ for 10 min. The supernatant was discarded, and ethanol 1 ml was added prior to a 30 s period of vortexing. The sample was centrifuged at $4653 \times g$ and 4 ℃ for 5 min. The supernatant was discarded, and the cells were dried before being resuspended in diethylpyrocarbonate (DEPC)-treated water and stored for

later use. RNA sample quality was determined with 1.2% agarose gel electrophoresis as described previously.[5)]

cDNA Synthesis cDNA was synthesized as described previously.[6)] The reaction volume of 20 μl consisted of Oligo (dt) 18 primer 1 μl (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China), total RNA 2 μl, RNasin (Toyobo, Osaka, Japan) 0.5 μl, dNTPs 1 μl, 10× reaction buffer 2 μl, and MMLV (Toyobo, Osaka, Japan) 1 μl, with the remaining volume composed of RNase-free water. The reaction temperatures were 37 ℃ for 90 min, 95℃ for 10 min, and reaction termination temperature of 4℃. Samples were stored at −70 ℃ until use.

PCR and Product Verification Analysis Target gene and internal reference gene (18S rRNA) primer sequences and annealing temperatures are shown in Table 1. The total reaction volume of 25 μl consisted of cDNA 2 μl from a single strain, target gene upstream primer 1 μl, target gene downstream primer 1 μl, dNTPs 1 μl, Taq DNA polymerase 0.5 μl (Shanghai Sangon Biological Engineering Technology & Services), 10×reaction buffer 2.5 μl, and sterile double-distilled water 16 μl. The reaction conditions were 94℃ for 3 min, 30 cycles of 94℃ for 40 s, the appropriate annealing temperature (see Table 1) for 30 s, and 72℃ for 30 s, followed by 72℃ for 10 min. Five microliters of PCR product was obtained for 2% agarose gel electrophoresis. After ethidium bromide staining, a gel electrophoresis imaging analysis system was used to perform gray value analysis for the amplified fragments. *MDR1*/18S rRNA, *FLU1*/18S rRNA, *CDR1*/18S rRNA, and *CDR2*/18S rRNA ratios were used to represent the relative expression levels of *MDR1*, *FLU1*, *CDR1*, and *CDR2*, respectively.

Statistical Analyses All experiments were performed three times, and the mean values were recorded as final results. SPSS 11.5 software was used for analysis, and the results are expressed as the mean± S.D. In the accumulation/efflux experiments, changes in the percentage of Rh123-positive *C. albicans* cells were analyzed using the χ^2 test. Changes in intracellular Rh123 fluorescence intensity and comparisons of drug efflux pump gene expression levels before and after TET administration were analyzed using the paired *t*-test. In the TET-free case, comparisons of drug efflux pump gene expression level between strains with different FLC sensitivities (susceptible, susceptible dose-dependent, and resistant) were performed using one-way analysis of variance (ANOVA). Statistical significance was defined as $p<0.05$.

RESULTS

Effects of TET on *C. albicans* Rh123 Accumulation and Efflux, as Determined by Flow Cytometry

For each of the 16 *C. albicans* strains, 10000 cells were counted using flow cytometry in the FITC channel to construct a single-parameter histogram. The results for strains CA−3 and CA−4 are shown in Fig. 1. The mean percentages of Rh123−positive cells 24 h after the accumulation experiments were 26.65% (TET free) and 70.99% (TET 30 μg/ml) ($\chi^2=62948$, $p=0.000$). In the efflux experiments, the percentages were 1.79% (TET free) and 42.57% (TET 30 μg/ml) (Fig. 2; $\chi^2=77078$, $p=0.000$).

Table 1. Target Gene and Internal Reference Gene (18S rRNA) Primer Sequences, Annealing Temperatures, and Amplified Fragment Lengths

Gene	Primer sequence (5′→3′)	Annealing temperature (℃)	Amplified fragment length (bp)
18S rRNA-F	CGAAAGCATTTACCAAGGAC	58	300
18S rRNA-R	TTATTGTGTCTGGACCTGG		
MDR1-F	TGAGATTCTTGGGTGGATTC	58	324
MDR1-R	CTTGTGATTCTGTCGTTACC		
FLU1-F	TGCTTCGGGTCCAGTTA	54	300
FLU1-R	CACAGTGAATGCTCCCA		
CDR1-F	GCTGGTGAAGGTTTGAATGT	60	213
CDR1-R	CGCTGATGGTTGATGGATAG		
CDR2-F	ATCTGGTGCTGGTAAGAC	54	500
CDR2-R	GCTGATGGTTGATGGATAG		

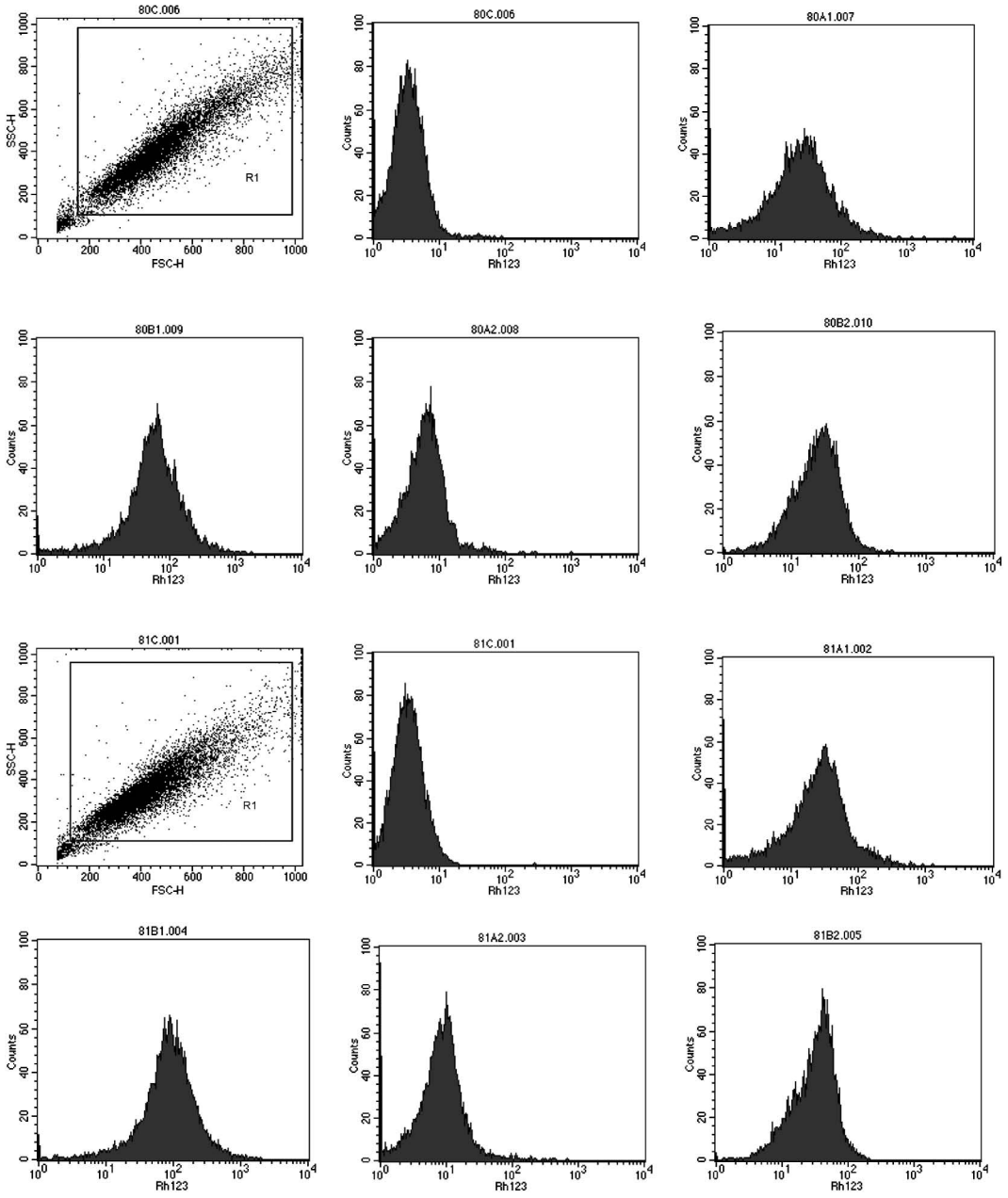

Fig. 1. Flow Cytometry Single-Parameter Histograms Showing the Effects of TET on *C. albicans* Rh123 Accumulation and Efflux

Numbers 80 and 81 represent strains CA-3 and CA-4 respectively. C is background control, A1 is the TET free strains after the accumulation experiments, B1 is the TET 30 μg/ml strains after the accumulation experiments, A2 is the TET free strains after the efflux experiments, and B2 is the TET 30 μg/ml strains after the efflux experiments.

Effects of TET on *C. albicans* Rh123 Accumulation and Efflux, as Determined by Fluorescent Spectrophotometry The mean intracellular Rh123 fluorescence intensities for the 16 *C. albicans* strains 24 h after the accumulation experiments were 11.34 (TET free) and 18.00 (TET 30 μg/ml), respectively. The difference was statistically significant ($t=-2.506$, $p=0.024$). After the efflux experiments, the intensities were 0.74 (TET free) and 2.19 (TET 30 μg/ml), which was also a statistical difference ($t=-5.428$, $p=0.000$). This indicates that TET can decrease *C. albicans* Rh123 efflux and increase intracellular Rh123 accumulation (Table 2).

Analysis of *MDR1*, *FLU1*, *CDR1*, and *CDR2* RT-PCR Products before and 24 h after TET Administration Extracted total *C. albicans* RNA was examined under ultraviolet light after undergoing 1.2% agarose gel electrophoresis. Three clear, complete electrophoresis bands corresponding to the 5.8S, 18S, and 28S subunits were evident.

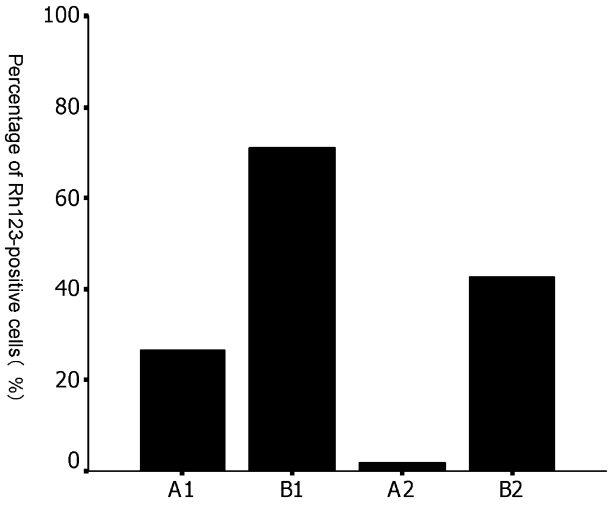

Fig. 2. Percentages of Rh123-Positive Cells in the 16 *C. albicans* Strains

A1 is the TET free strains after the accumulation experiments, B1 is the TET 30 μg/ml strains after the accumulation experiments, A2 is the TET free strains after the efflux experiments, and B2 is the TET 30 μg/ml strains after the efflux experiments.

Table 2. Intracellular Rh123 Fluorescence Intensities Values for the 16 *C. albicans Strains*

C. albicans	C	A1	A2	B1	B2
CA-1	0.7078	14.9995	2.1583	31.2118	2.2491
CA-2	1.2573	24.4552	1.6208	22.7383	4.9406
CA-3	0.4415	18.9154	0.9583	19.1826	3.7309
CA-4	0.8406	20.9867	2.761	33.7805	5.9666
CA-5	0.9048	19.6303	1.9093	33.9063	2.6935
CA-6	1.0914	16.3929	3.3265	28.6829	3.7093
CA-7	0.828	6.1636	0.9518	21.073	4.1949
CA-8	0.7072	20.7643	2.288	35.2586	3.3996
CA-9	0.4147	18.5223	3.1289	17.7162	4.0862
CA-11	0.7406	10.0528	2.9896	19.504	3.0543
CA-12	0.8039	13.2623	1.9174	27.7202	2.4253
CA-13	0.5193	20.969	1.9916	24.153	4.7182
CA-14	1.2572	16.5424	1.3476	35.9531	1.2534
CA-15	0.7298	17.4723	1.8923	33.1425	5.0238
CA-16	0.8122	28.3878	3.2281	28.1372	3.0955
CA-17	0.8263	19.6547	1.5819	17.7885	1.9713

C is background control, A1 is the TET free accumulation experimental result, A2 is the TET free efflux experimental result, B1 is the TET 30 μg/ml accumulation experimental result, and B2 is the TET 30 μg/ml efflux experimental result. Background was subtracted during calculations (OD_{600}=0.1).

RT-PCR product semiquantitative analysis was also conducted using the internal reference gene (18S rRNA) as a standard. Target gene (*MDR1*, *FLU1*, *CDR1*, and *CDR2*) mRNA levels were quantified in relation to the standard for each of the 16 *C. albicans* strains. The results are shown in Table 3. In the TET free case, expression level differences for the above target genes between the FLC-susceptible, -susceptible dose-dependent, and -resistant strains were statistically significant ($p<0.05$). In the susceptible dose-dependent and resistant strains, *MDR1*, *FLU1*, *CDR1*, and *CDR2* mRNA expression levels were higher than in the susceptible strains. In the TET free and TET 30 μg/ml cases, expression levels of each *C. albicans* target gene were compared 24 h after treatment. For *MDR1*, the difference in the FLC-resistant strain was statistically significant ($p<0.05$). Its expression level decreased after TET administration, but the differences in the susceptible strain and the susceptible dose-dependent strain were not statistically significant ($p>0.05$). For *FLU1*, the differences between the susceptible dose-dependent strain and the resistant strain were statistically significant ($p<0.05$). Its expression level decreased after TET administration, but the difference in the susceptible strain was not statistically significant ($p>0.05$). For *CDR1* and *CDR2*, the differences among the susceptible strain, susceptible dose-dependent strain, and resistant strain were all statistically significant ($p<0.05$), and their expression levels decreased after TET administration (Table 3).

DISCUSSION

We previously reported in that TET synergistically increases the candidacidal activity of TET *in vitro*[25] and *in vivo*.[26] Microdilution experiments showed that, with FLC alone, the MIC values for the 16 *C. albicans* strains were 0.250–64 μg/ml. With TET 40 μg/ml, FLC MIC values for the test organisms decreased to 0.125–16 μg/ml. Furthermore, the endpoints were clear, and no ''tailing'' phenomenon was observed.[25] In a mouse model of *C. albicans* vaginitis, TET and FLC in different dose combinations were administered vaginally once daily from day 3 onward for 7 days. The results showed that, up to day 11, the fungal load of the group given TET 26 mg/kg/day and FLC 26 mg/kg/day was significantly lower compared with the group given FLC 26 mg/kg/day alone. Compared with the other groups, microscopic examination of vaginal smears showed no pseudohyphae in the TET 26 mg/kg/day+FLC 26 mg/kg/day group mice, but varying amounts of pseudohyphae and pseudohyphae aggregates were seen in the other groups. Pathologic examination of vaginal tissues showed that in the TET 26 mg/kg/day+FLC 26 mg/

Table 3. Relative *C. albicans* Drug Efflux Pump Gene mRNA Expression Levels of the TET Free and TET 30 μg/ml cases, at 24 h

C. albicans	TET free				TET 30 μg/ml 24 h				*t*			
	MDR1	*FLU1*	*CDR1*	*CDR2*	*MDR1*	*FLU1*	*CDR1*	*CDR2*	*MDR1*	*FLU1*	*CDR1*	*CDR2*
FLC-susceptible	0.78±0.22	0.73±0.29	0.87±0.22	0.82±0.21	0.70±0.19	0.63±0.21	0.73±0.22	0.69±0.24	1.922	1.626	2.658*	2.785**
FLC-susceptible dose-dependent	0.96±0.15	1.19±0.26	1.28±0.19	1.13±0.21	0.83±0.30	0.55±0.18	0.67±0.25	0.71±0.32	1.062	6.357**	6.788**	3.215*
FLC-resistant	1.19±0.42	1.13±0.42	1.25±0.32	1.47±0.63	0.60±0.40	0.43±0.04	0.59±0.02	0.61±0.10	3.271*	4.190**	5.134**	3.246*
F	8.541**	11.156**	16.515**	14.611**								
p	0.001	0.000	0.000	0.000								

Results are expressed as the mean±S.D. * $p<0.05$, ** $p<0.01$.

kg/day group, mucosal edema was slight with minimal inflammatory cell infiltration, and no pseudohyphae were found within vaginal cavities and mucosal layers.[26]

The main molecular mechanisms of azole drug resistance in *C. albicans* are most often overexpression of efflux pumps[3,6,7] and mutation-mediated overexpression of genes that code for proteins targeted by azole drugs.[14] Two types of efflux pump system are active in the drug-resistance process. The first involves ATP-binding cassette transporters (ABC, coded by *CDR* genes), which are ATP-dependent active transporters.[10,11] In *C. albicans*, efflux pumps in the ABC superfamily include Cdr1p and Cdr2p, coded by *CDR1* and *CDR2*, respectively.[8,9,11] Efflux pumps belonging to this superfamily are ubiquitously present and structurally similar in organisms ranging from prokaryotic cells to mammals.[30] In mammalian cells, they are present mainly in the form of the transmembrane protein P-gp (coded by *MDR1*),[31,32] and in fungi they are mainly multidrug-resistance proteins (MRPs, mainly Cdrp).[11] In addition to their major function of extruding harmful material from the cell, upregulation of their activity is intimately related to antiparasitic resistance in parasites and chemotherapy resistance in tumors.[30] The second type of efflux pump is the major facilitator superfamily (MFS, coded by *MDR* genes), which are electrochemical gradient-dependent passive transporters. In *C. albicans*, they include CaMdr1p and Flu1p, coded by *CaMDR1*[16] and *FLU1*,[5] respectively. A molecular epidemiologic study of *C. albicans* FLC resistance showed that 83% of drug resistance is related to *CDR1* overexpression, and 67% is related to *CaMDR1* overexpression.[3]

Rh123 is a cationic dye with a high fluorescence quantum yield and no cytotoxicity. It is generally believed that Rh123 enters cells by diffusion before being pumped out of cells by efflux pumps of the ABC superfamily.[28] It is the substrate of many *MDR* efflux pumps including Cdr1p and Cdr2p and can be used to evaluate efflux function in tumor cells such as those possessing P-gp.[28,29] We compared the percentages of Rh123-postive cells and intracellular Rh123 fluorescence intensities in *C. albicans* strains before and after TET administration to evaluate the effects of TET on drug efflux pump function (such as Cdr1p and Cdr2p). Our results from accumulation/efflux experiments demonstrated that the mean percentages of Rh123-positive cells and mean intracellular Rh123 fluorescence intensities in the 16 *C. albicans* strains exposed to noncytotoxic doses of TET were significantly higher than in the unexposed strains. These observations indicate that TET can inhibit the drug efflux pump function in *C. albicans*, decreasing drug efflux activity and hence increasing intracellular Rh123 accumulation.

The RT-PCR results showed that prior to TET administration mRNA expression levels of the drug efflux pump genes *MDR1*, *FLU1*, *CDR1*, and *CDR2* in the susceptible dose-dependent strain and the resistant strain were significantly higher than in the susceptible strain. Twenty-four hours after the administration of noncytotoxic doses of TET, the expression levels of *MDR1* (resistant strain), *FLU1* (susceptible dose-dependent strain and resistant strain), *CDR1* (susceptible strain, susceptible dose-dependent strain, and resistant strain), and *CDR2* (susceptible strain, susceptible dose-dependent strain, and resistant strain) all significantly decreased compared with those before TET administration. This indicates that, in terms of mRNA levels, the

mechanism of the synergistic effect of TET on the candidacidal activity of FLC is related to the inhibition of the genes coding for the drug efflux pumps. It was also found that, among different strains (even strains with the same MIC), *MDR1*, *FLU1*, *CDR1*, and *CDR2* expression levels were not completely identical. After TET administration, the mean expression levels of each target gene mRNA were also downregulated to different degrees, suggesting the possible existence of other mechanisms in addition to the four efflux pumps described above, or a situation in which the mRNA expression level is not directly indicative of efflux pump function. It can be seen from the present results that the mechanism of TET in reversing *C. albicans* FLC resistance is similar to its mechanism in reversing chemotherapy resistance in human tumor cells.

RT-PCR has the advantages of simplicity, high sensitivity, and high efficacy, making it suitable for detecting multiple genes in large samples. Our study performed three parallel experiments under identical conditions and implemented corrections using the housekeeping gene (18S rRNA) as the internal reference gene, thereby increasing quantification accuracy.

TET is a bis-benzylisoquinoline alkaloid, which is the main active component in the root of *S. tetrandra* S. Moore (or Fenfangji) of the Menispermaceae *family*.[33] TET has been used in the treatment of hypertension, cardiac arrhythmia, and angina pectoris in China since the 1950s[33,34] and few side effects have been noted in clinical practice. TET has been shown to be a Ca^{2+} channel antagonist and to interact with the voltage-activated L-type and T-type Ca^{2+} channels and the slowly gating $K_{(Ca)}$ channel with varying degrees of specificity and affinity.[33] Recent studies have shown that TET has a reversal effect on P-gp-mediated MDR.[32,35] In preliminary screening, TET was shown to be more potent than other herbs in modulating *MDR in vitro*.[34] The *C. albicans* drug efflux pumps Cdr1p and Cdr2p are highly homologous to P-gp and have similar structures and functions.[30] Cdr1p and Cdr2p are also intimately related to *C. albicans* FLC drug resistance.[9,11] The present study showed that TET can increase the candidacidal activity of FLC by decreasing Cdr1p and Cdr2p FLC efflux.

In summary, tetrandrine, a Chinese herb extract, has no intrinsic antifungal activity against *C. albicans* at concentrations of less than 40 μg/ml but is a potent synergist in combination with FLC *in vitro* and *in vivo*. The main mechanism of action of TET may be to reverse *CDR1*, *CDR2*, *MDR1*, and *FLU1* associated with an increase in the intracellular azole accumulation by inhibiting the overexpression of MRP, CaMdr1p, and Flu1p and may interact directly with multidrug efflux transporters present in *C. albicans*. Thus, by inhibiting their activity, this drug could increase the susceptibility of *C. albicans* to azoles. Clearly, the effects of TET in combination with azoles needs to be more thoroughly investigated. This new concept of combining FLC, and perhaps other azoles, with TET or with its analogue compounds might open a new therapeutic approach for the management of fungal infections.

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