



The exploration of effect of terfenadine on Ca^{2+} signaling in renal tubular cells

He-Hsiung Cheng, Wei-Zhe Liang, Chun-Chi Kuo, Lyh-Jyh Hao, Chiang-Ting Chou & Chung-Ren Jan

To cite this article: He-Hsiung Cheng, Wei-Zhe Liang, Chun-Chi Kuo, Lyh-Jyh Hao, Chiang-Ting Chou & Chung-Ren Jan (2019): The exploration of effect of terfenadine on Ca^{2+} signaling in renal tubular cells, Journal of Receptors and Signal Transduction, DOI: [10.1080/10799893.2019.1620777](https://doi.org/10.1080/10799893.2019.1620777)

To link to this article: <https://doi.org/10.1080/10799893.2019.1620777>



Published online: 11 Jun 2019.



Submit your article to this journal [↗](#)



Article views: 6



View Crossmark data [↗](#)

RESEARCH ARTICLE



The exploration of effect of terfenadine on Ca^{2+} signaling in renal tubular cells

He-Hsiung Cheng^{a*}, Wei-Zhe Liang^{b,c*}, Chun-Chi Kuo^d, Lyh-Jyh Hao^e, Chiang-Ting Chou^f and Chung-Ren Jan^b

^aDepartment of Medicine, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan; ^bDepartment of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; ^cDepartment of Pharmacy, Tajen University, Pingtung, Taiwan; ^dDepartment of Nursing, Tzu Hui Institute of Technology, Pingtung, Taiwan; ^eDepartment of Metabolism, Kaohsiung Veterans General Hospital Tainan Branch, Tainan, Taiwan; ^fDepartment of Nursing, Division of Basic Medical Sciences, Chang Gung University of Science and Technology, Chia-Yi, Taiwan

ABSTRACT

Terfenadine, an antihistamine used for the treatment of allergic conditions, affected Ca^{2+} -related physiological responses in various models. However, the effect of terfenadine on cytosolic free Ca^{2+} levels ($[\text{Ca}^{2+}]_i$) and its related physiology in renal tubular cells is unknown. This study examined whether terfenadine altered Ca^{2+} signaling and caused cytotoxicity in Madin–Darby canine kidney (MDCK) renal tubular cells. The Ca^{2+} -sensitive fluorescent dye fura-2 was used to measure $[\text{Ca}^{2+}]_i$. Cell viability was measured by the fluorescent reagent 4-[3-[4-iodophenyl]-2-4(4-nitrophenyl)-2H-5-tetrazolium-1,3-benzene disulfonate] water soluble tetrazolium-1 (WST-1) assay. Terfenadine at concentrations of 100–1000 μM induced $[\text{Ca}^{2+}]_i$ rises concentration dependently. The response was reduced by approximately 35% by removing extracellular Ca^{2+} . In Ca^{2+} -free medium, treatment with the endoplasmic reticulum Ca^{2+} pump inhibitor 2,5-di-tert-butylhydroquinone (BHQ) partly inhibited terfenadine-evoked $[\text{Ca}^{2+}]_i$ rises. Conversely, treatment with terfenadine abolished BHQ-evoked $[\text{Ca}^{2+}]_i$ rises. Inhibition of phospholipase C (PLC) with U73122 inhibited 95% of terfenadine-induced Ca^{2+} release. Terfenadine-induced Ca^{2+} entry was supported by Mn^{2+} -caused quenching of fura-2 fluorescence. Terfenadine-induced Ca^{2+} entry was partly inhibited by an activator of protein kinase C (PKC), phorbol 12-myristate 13 acetate (PMA) and by three modulators of store-operated Ca^{2+} channels (nifedipine, econazole, and SKF96365). Terfenadine at 200–300 μM decreased cell viability, which was not reversed by pretreatment with the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-acetoxymethyl ester (BAPTA/AM). Together, in MDCK cells, terfenadine induced $[\text{Ca}^{2+}]_i$ rises by evoking PLC-dependent Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry via PKC-sensitive store-operated Ca^{2+} entry. Furthermore, terfenadine caused cell death that was not triggered by preceding $[\text{Ca}^{2+}]_i$ rises.

ARTICLE HISTORY

Received 27 February 2019
Revised 15 April 2019
Accepted 24 April 2019

KEYWORDS

Ca^{2+} ; cytotoxicity; endoplasmic reticulum; terfenadine; MDCK renal tubular cells

Introduction

Terfenadine acts as an antihistamine for treatment of allergic conditions [1]. Although terfenadine has been used by over 100 million patients worldwide as of 1990 [2], it was superseded by fexofenadine in the 1990s due to the risk of a particular type of disruption of the electrical rhythms of the heart [3]. *In vitro*, terfenadine also altered Ca^{2+} signaling in different models. It has been shown that terfenadine inhibited cytosolic Ca^{2+} dynamics in cultured guinea pig cardiomyocytes [4], and reduced Ca^{2+} influx, cyclic guanosine monophosphate (cGMP) formation, and N-methyl-D-aspartate (NMDA) receptor-dependent neurotoxicity following activation of L-type voltage sensitive Ca^{2+} channels in neurons [5]. Furthermore, terfenadine prevented L-type Ca^{2+} channel current in rat ventricular myocytes [6] and blocked time-dependent Ca^{2+} , Na^+ , and K^+ channels in guinea pig ventricular myocytes [7]. On the contrary, terfenadine-induced apoptosis in A375 human melanoma cells was mediated

through elevation in cytosolic free Ca^{2+} levels ($[\text{Ca}^{2+}]_i$) [8]. Furthermore, terfenadine was reported to increase Ca^{2+} release in human atrial myocytes [9] and rat mast cells [10]. However, the effects of terfenadine on Ca^{2+} homeostasis in renal tubular cells have not been explored.

Ca^{2+} is a pivotal ion that plays a key role in numerous cellular responses. Transient and controlled rises in $[\text{Ca}^{2+}]_i$ have been established to induce diverse pathophysiological cellular responses [11], including fluid secretion, protein regulation, proliferation, etc. [12]. $[\text{Ca}^{2+}]_i$ can rise via Ca^{2+} entry through various channels on the plasma membrane and/or Ca^{2+} release from the endoplasmic reticulum and other organelles. G protein-coupled phospholipase C (PLC) can trigger formation of inositol 1,4,5-trisphosphate (IP_3) which is a pivotal messenger for the endoplasmic reticulum Ca^{2+} release [13]. In Madin–Darby canine kidney (MDCK) cells, literature shows that the main Ca^{2+} entry pathway is the store-operated Ca^{2+} entry [14]. Thus, the exploration of the mechanisms of a chemical-induced $[\text{Ca}^{2+}]_i$ rises is very

important for knowing the chemical's pharmacological effect on the cell.

A previous study has shown that terfenadine inhibited autoregulation of renal blood flow and glomerular filtration rate in mammals such as the dog [15]. Therefore, the risk of exposure to terfenadine in human kidney should be cautioned. The goal of this study was to examine the effect of terfenadine on $[Ca^{2+}]_i$ in renal tubular cells. The MDCK cell line is a useful model for renal cell research. In this cell, $[Ca^{2+}]_i$ rises and death can be induced by stimulation with several agents including allyl sulfides [16], phenethyl isothiocyanate [17], and sertraline [18]. The Ca^{2+} -sensitive fluorescent dye fura-2 was applied as a dye to detect $[Ca^{2+}]_i$. The pathways underlying terfenadine-induced $[Ca^{2+}]_i$ rises were explored. The cytotoxicity of terfenadine was examined by enzyme-linked immunosorbent assay (ELISA) using the fluorescent probe 4-(3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio-1,3-benzene disulfonate) tetrazolium-1 (WST-1).

Methods

Chemicals

The chemicals for cell culture were purchased from Gibco® (Gaithersburg, MD, USA). Terfenadine (Figure 1(A)) and other

reagents were purchased from Sigma-Aldrich® (St. Louis, MO, USA) unless otherwise indicated. 1,2-Bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid/acetoxymethyl (BAPTA/AM) and aminopolycarboxylic acid/acetoxymethyl (fura-2/AM) were purchased from Molecular Probes® (Eugene, OR, USA).

Cell culture

After purchasing from Bioresource Collection and Research Center (Taiwan), MDCK cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were kept in an incubator at 37 °C containing 5% CO₂ atmosphere.

$[Ca^{2+}]_i$ analyses and experimental solutions

$[Ca^{2+}]_i$ was measured as previously described [17]. Ca^{2+} -free medium (pH 7.4) had 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mM $CaCl_2$, 140 mM NaCl, 3 mM $MgCl_2$, 5 mM KCl, 0.3 mM ethylene glycol tetraacetic acid (EGTA), and 5 mM glucose. Ca^{2+} -containing medium contained similar chemicals as Ca^{2+} -free medium except that 0.3 mM EGTA and 2 mM $MgCl_2$ were replaced with 2 mM

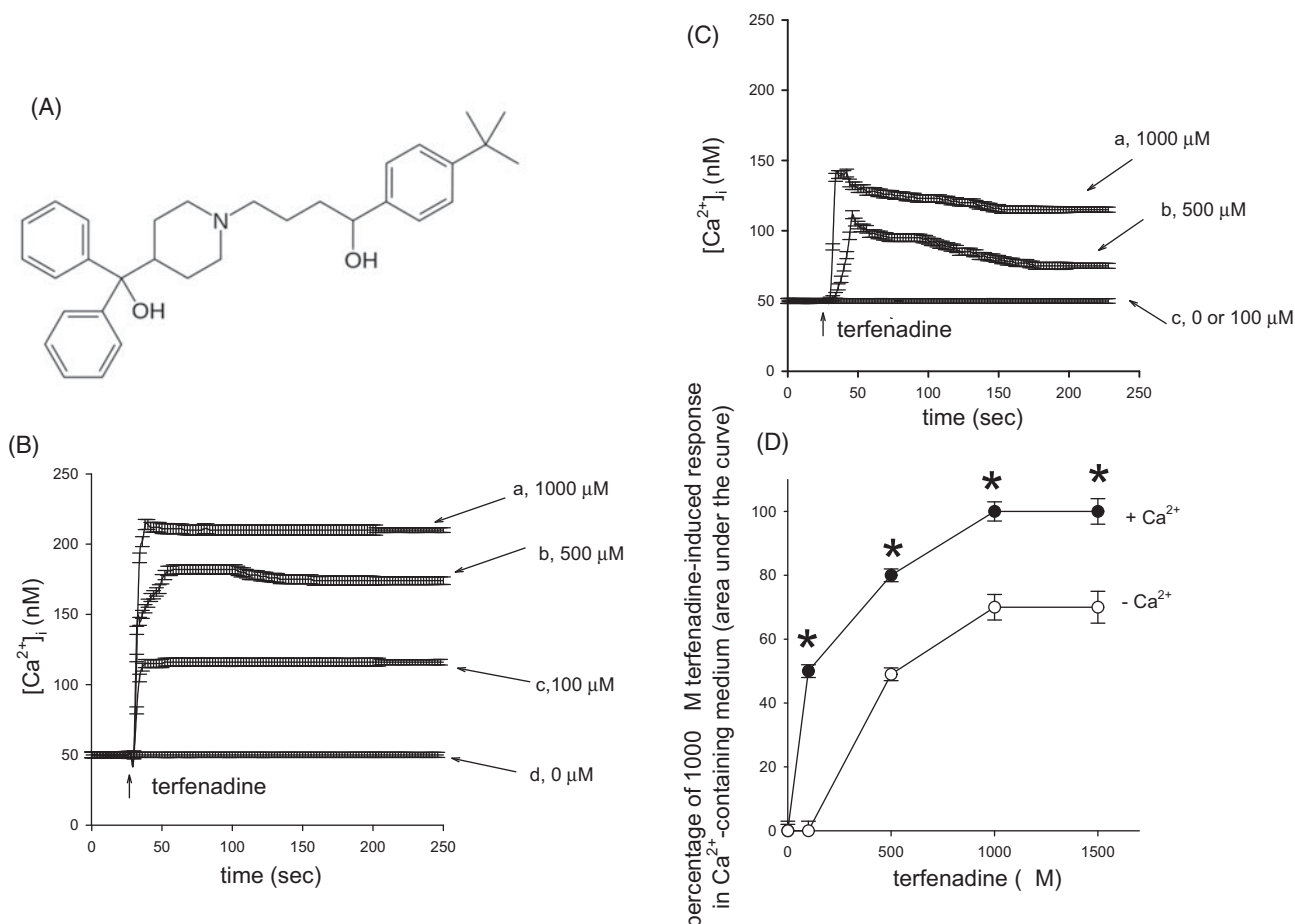


Figure 1. Effect of terfenadine on $[Ca^{2+}]_i$ in MDCK cells. (A) The chemical structure of terfenadine (B) The experiments were conducted in Ca^{2+} -containing medium. Terfenadine was added to cells at 25 s. Y axis is the $[Ca^{2+}]_i$ evoked by terfenadine. (C) Effect of terfenadine on $[Ca^{2+}]_i$ in Ca^{2+} -free medium. Terfenadine was added at 25 s in Ca^{2+} -free medium. Y axis is the $[Ca^{2+}]_i$ rises evoked by terfenadine. (D) Concentration-response plots of terfenadine-evoked $[Ca^{2+}]_i$ rises in the presence or absence of extracellular Ca^{2+} . Y axis is the percentage of the net area under the curve (25–250 s) of the $[Ca^{2+}]_i$ rises induced by 1000 µM terfenadine in Ca^{2+} -containing medium. Data are mean $\pm$ SEM of three independent experiments. * $p < .05$ compared to open circles.

CaCl₂. Terfenadine was dissolved in absolute ethanol. The reagents used in experiments were dissolved in dimethyl sulfoxide (DMSO), water, or ethanol. The concentration of solvents in the experimental solutions was <0.1%, and did not alter viability or basal [Ca²⁺]_i.

Cells were trypsinized and suspended in medium at a concentration of 1×10^6 cells/ml. Cell viability was detected by trypan blue exclusion by adding 0.2% trypan blue to 0.1 ml cell suspension, and the viability was routinely greater than 95%. Subsequently, cells were incubated with 2 μ M fura-2/AM for 30 min at 25 °C in Ca²⁺-containing medium. Then, cells were washed with Ca²⁺-containing medium twice and were resuspended in Ca²⁺-containing medium at a concentration of 1×10^7 cells/ml. Fura-2 fluorescence measurements were conducted in a water-jacketed cuvette (25 °C) with stirring. The cuvette had 1 ml of medium and 0.5 million cells. After 0.1 ml cell suspension was added to 0.9 ml Ca²⁺-containing or Ca²⁺-free medium, a Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu, Tokyo, Japan) was applied to detect the fluorescence by recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1 s intervals. Chemicals were added to the cuvette by pausing the recording for 2 s to open and close the cuvette-containing chamber. After the completion of the experiments, Triton X-100 (0.1%) and CaCl₂ (5 mM) were added to obtain the maximal fura-2 fluorescence. The Ca²⁺ chelator EGTA (10 mM) was subsequently added to chelate Ca²⁺ in the cuvette to obtain the minimal fura-2 fluorescence. Control experiments suggested that cells suspended in a cuvette had 95% viability after 20 min of fluorescence recording. [Ca²⁺]_i was calculated as described previously [19].

Mn²⁺ analyses

Quenching of fura-2 fluorescence by manganese ion (Mn²⁺) was performed in Ca²⁺-containing medium. MnCl₂ (50 μ M) was added to cell suspension in the cuvette 30 s before the fluorescence recording started. Data were recorded at excitation signal at 360 nm and emission signal at 510 nm at 1 s intervals [20].

Water soluble tetrazolium-1 (WST-1) analyses

Cells were seeded in 96-well plates at a concentration of 1×10^4 cells/well in culture medium for 24 h in the presence of 0–300 μ M terfenadine. WST-1 (10 μ l) was added to samples after terfenadine treatment, and cells were further incubated for 30 min in the incubator. When BAPTA/AM was used to chelate cytosolic Ca²⁺, cells were treated with 5 μ M BAPTA/AM for 1 h prior to incubation with terfenadine. The cells were washed once with Ca²⁺-containing medium and incubated with/without terfenadine for 24 h. An ELISA reader was used to detect the absorbance of samples (A₄₅₀). Absolute optical density was normalized to the absorbance of control cells in each plate and expressed as a percentage of control.

Statistic analyses

Measurement data were expressed as mean $\pm$ SEM of three independent experiments. All data were analyzed by one-way analysis of variances (ANOVA) using the Statistical Analysis System (SAS®; SAS Institute Inc., Cary, NC, USA). Multiple comparisons between group means were performed by *post-hoc* analysis using the Tukey's honestly significantly difference (HSD) procedure. **p* < .05 represents significant differences.

Results

Terfenadine increased [Ca²⁺]_i in MDCK cells

Figure 1(B) shows that the basal [Ca²⁺]_i was 50 ± 1 nM. Terfenadine in Ca²⁺-containing medium induced [Ca²⁺]_i rises between 100 and 1000 μ M. At a concentration of 1000 μ M, terfenadine evoked [Ca²⁺]_i rises that reached a net increase of 172 ± 2 nM (*n* = 3). The Ca²⁺ response saturated at 1000 μ M terfenadine because 1500 μ M terfenadine did not evoke a greater response (not shown). Figure 1(C) shows that 500–1000 μ M terfenadine induced concentration-dependent rises in [Ca²⁺]_i in Ca²⁺-free medium. Figure 1(D) depicts the concentration-response plots of terfenadine-induced [Ca²⁺]_i rises. The half maximal effective concentration (EC₅₀) value was 100 ± 1 μ M in Ca²⁺-containing medium or 375 ± 5 μ M in Ca²⁺-free medium, respectively, by fitting to a Hill equation. Filled circles stand for the different concentrations of terfenadine used in Ca²⁺-containing medium. Open circles stand for the different concentrations of terfenadine used in Ca²⁺-free medium. +Ca means the condition of Ca²⁺-containing medium; –Ca means the condition of Ca²⁺-free medium.

Terfenadine dominantly induced Ca²⁺ release from the endoplasmic reticulum

Because terfenadine-induced Ca²⁺ response saturated at 1000 μ M (Figure 1), in the following experiments the response induced by 1000 μ M terfenadine was used as control. Evidence shows that the endoplasmic reticulum plays a dominant role in depleting Ca²⁺ store and enhancing a Ca²⁺ signal in most cells [21]. Thus the following experiments explored the role of the endoplasmic reticulum in terfenadine-induced Ca²⁺ release in MDCK cells. The experiments were conducted in Ca²⁺-free medium to exclude the involvement of Ca²⁺ influx. Figure 2 shows that addition of 50 μ M tert-butylhydroquinone (BHQ), an endoplasmic reticulum Ca²⁺ pump inhibitor [22], induced [Ca²⁺]_i rises of 70 ± 3 nM (*n* = 3). Terfenadine (1000 μ M) added afterwards induced a Ca²⁺ signal of 25 ± 2 nM. Figure 3 shows that after terfenadine induced [Ca²⁺]_i rises of 90 ± 2 nM (*n* = 3), BHQ failed to induce [Ca²⁺]_i rises. BHQ incubation decreased terfenadine-induced [Ca²⁺]_i rises by 72%. It appears that the endoplasmic reticulum played a pivotal role in terfenadine-evoked Ca²⁺ release from organelles.

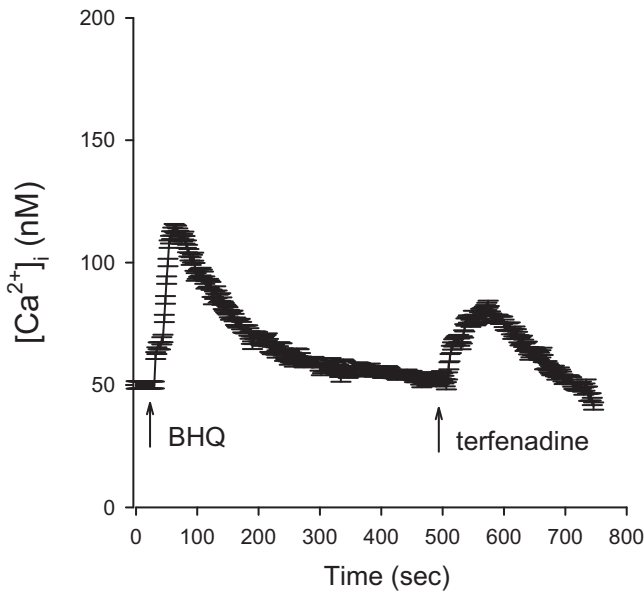


Figure 2. Effect of BHQ on terfenadine-induced Ca^{2+} release. BHQ (50 μM) and terfenadine (1000 μM) were added at time points indicated. Experiments were performed in Ca^{2+} -free medium. Data are mean $\pm$ SEM of three independent experiments.

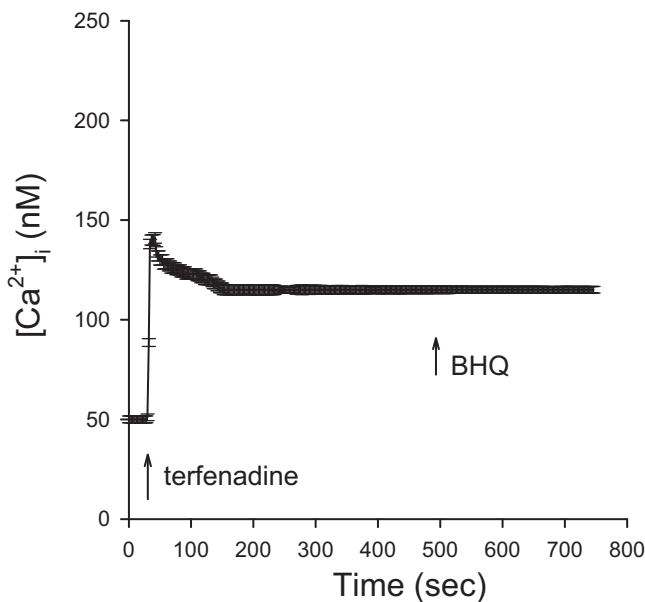


Figure 3. Effect of terfenadine on BHQ-induced Ca^{2+} release. Terfenadine (1000 μM) and BHQ (50 μM) were added at time points indicated. Experiments were performed in Ca^{2+} -free medium. Data are mean $\pm$ SEM of three independent experiments.

PLC activation played a key role in terfenadine-induced Ca^{2+} release

The PLC inhibitor U73122 [23] was used to test whether PLC activation participated in terfenadine-evoked Ca^{2+} release. First, efforts were made to examine the activity of PLC. Figure 4(a) shows that adenosine triphosphate (ATP) (10 μM) induced $[\text{Ca}^{2+}]_i$ rises of 99 ± 2 nM ($n=3$). Literature shows that ATP releases internal Ca^{2+} mostly in a PLC-dependent manner in most cell types [24]. Figure 4(b) shows that

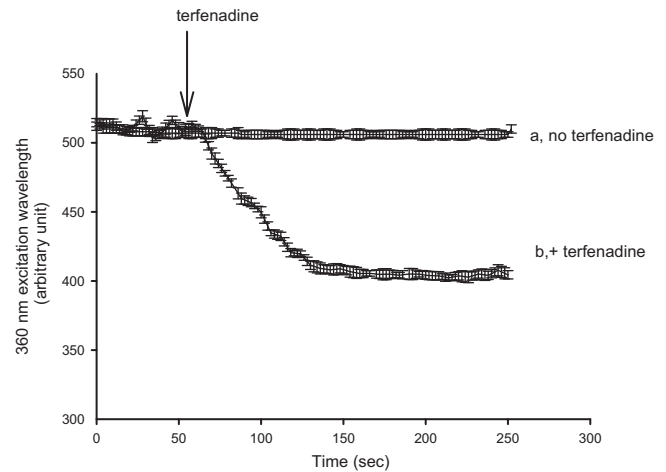


Figure 4. Measuring Mn^{2+} quenching of fura-2 fluorescence to explore the effect of terfenadine on Ca^{2+} influx. In Ca^{2+} -containing medium, 1 min before fluorescence recordings, MnCl_2 (50 μM) was added to cells. The y axis is fluorescence intensity (in arbitrary units) detected at the Ca^{2+} -insensitive excitation wavelength of 360 nm and the emission wavelength of 510 nm. Trace a: without terfenadine. Trace b: terfenadine (1000 μM) was present. Data are mean $\pm$ SEM of three independent experiments.

incubation with 2 μM U73122 did not change basal $[\text{Ca}^{2+}]_i$, but abolished ATP-induced $[\text{Ca}^{2+}]_i$ rises. This suggests that U73122 effectively suppressed PLC activity. The data further show that incubation with 2 μM U73122 did not alter basal $[\text{Ca}^{2+}]_i$ but inhibited 95% of 1000 μM terfenadine-induced $[\text{Ca}^{2+}]_i$ rises. U73343 is a U73122 analog lacking inhibition on PLC. Thus it was used to examine if it could abolish ATP-evoked $[\text{Ca}^{2+}]_i$ rises. Our data show that U73343 (2 μM) did not alter ATP-evoked Ca^{2+} signal (not shown). It suggests that terfenadine-induced Ca^{2+} release from the endoplasmic reticulum required PLC activation.

Terfenadine induced Mn^{2+} influx-caused fluorescence quenching

Experiments were performed to exclude the possibility that the smaller terfenadine-induced response in Ca^{2+} -free medium was caused by 0.3 mM EGTA-induced depletion of intracellular Ca^{2+} . Mn^{2+} enters cells through similar pathways as Ca^{2+} but quenches fura-2 fluorescence at all excitation wavelengths [20]. Figure 5 shows that 1000 μM terfenadine induced an immediate decrease in the 360 nm excitation signal (compared to control) by 112 ± 2 arbitrary units at 220 s ($n=3$). Therefore, terfenadine-induced $[\text{Ca}^{2+}]_i$ rises involved Ca^{2+} influx from extracellular space.

Regulation of terfenadine-induced $[\text{Ca}^{2+}]_i$ rises

Phorbol 12-myristate 13 acetate (PMA; 1 nM; a protein kinase C (PKC) activator), GF109203X (2 μM ; a PKC inhibitor) or three store-operated Ca^{2+} channel modulators (econazole, 0.5 μM ; nifedipine, 1 μM ; SKF96365, 5 μM) was applied 1 min before terfenadine (1000 μM), in Ca^{2+} -containing medium. GF109203X failed to alter terfenadine-induced $[\text{Ca}^{2+}]_i$ rises. In contrast, PMA, econazole, nifedipine, and SKF96365 partly inhibited terfenadine-induced $[\text{Ca}^{2+}]_i$ rises ($n=3$) ($p < .05$)

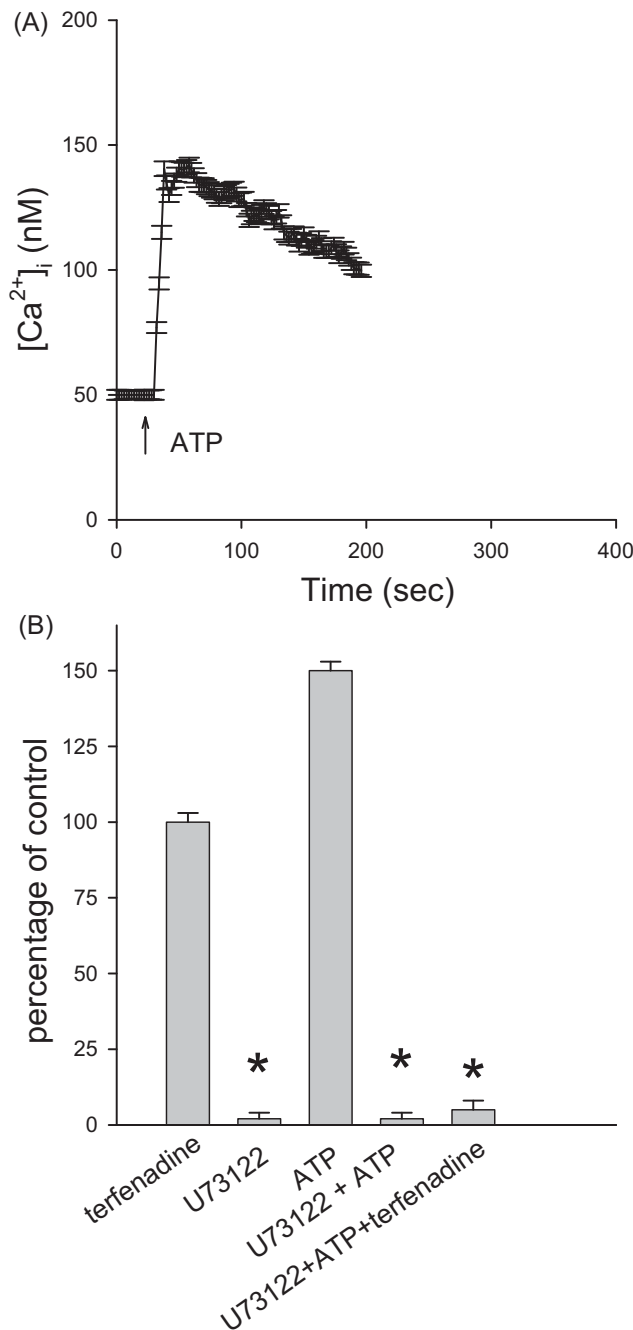


Figure 5. Effect of U73122 on terfenadine-induced Ca^{2+} release. Experiments were performed in Ca^{2+} -free medium. (A) ATP (10 μM) was added at 25 s. (B) First column is 1000 μM terfenadine-induced $[\text{Ca}^{2+}]_i$ rises. Second column shows that 2 μM U73122 had no effect on basal $[\text{Ca}^{2+}]_i$. Third column depicts ATP-evoked $[\text{Ca}^{2+}]_i$ rises. Fourth column demonstrates that U73122 pretreatment for 200 s abolished ATP-evoked $[\text{Ca}^{2+}]_i$ rises (* $p < .05$ compared to third column). Fifth column depicts that U73122 incubation for 200 s plus ATP incubation for 50 s abolished 1000 μM terfenadine-induced $[\text{Ca}^{2+}]_i$ rises. Data are mean $\pm$ SEM of three independent experiments.

(Table 1). Thus it appears that PKC-regulated store-operated Ca^{2+} entry participated in terfenadine-induced $[\text{Ca}^{2+}]_i$ rises.

Terfenadine killed MDCK cells in a Ca^{2+} -independent manner

Literature shows that uncontrolled $[\text{Ca}^{2+}]_i$ rises may alter cell viability [25]. Since terfenadine induced substantial $[\text{Ca}^{2+}]_i$

Table 1. Effect of Ca^{2+} channel regulators on terfenadine-evoked $[\text{Ca}^{2+}]_i$ rises.

Treatment	
Control	100 $\pm$ 5
+ PMA	79 $\pm$ 3*
+ GF109203X	99 $\pm$ 2
+ econazole	70 $\pm$ 4*
+ nifedipine	53 $\pm$ 5*
+ SKF96365	69 $\pm$ 3*

In regulator-treated group, the regulator was added 1 min before terfenadine (1000 μM). The concentration was 2 μM for GF109203X, 10 nM for PMA, 0.5 μM for econazole, 1 μM for nifedipine, and 5 μM for SKF96365.

Data are presented as the percentage of control that is the area under the curve (25–200 s) of 1000 μM terfenadine-induced $[\text{Ca}^{2+}]_i$ rises in Ca^{2+} -containing medium, and are mean $\pm$ SEM of three independent experiments.

* $p < .05$ compared to control.

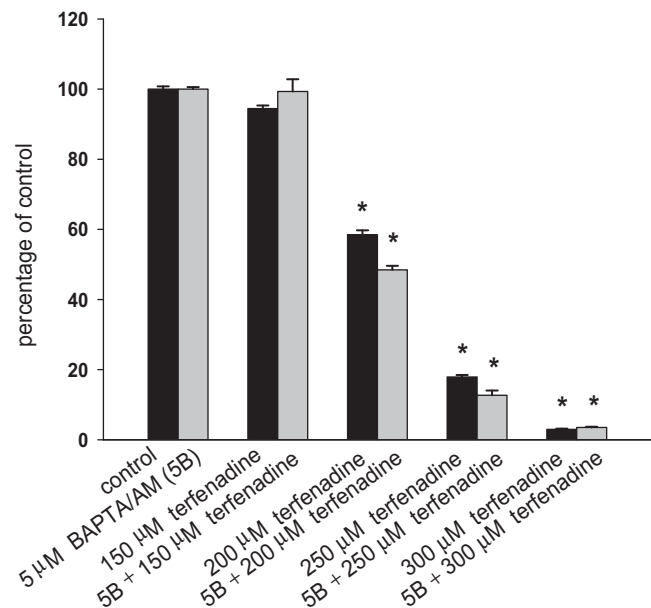


Figure 6. Action of terfenadine on cell viability. Cells were treated with 0–300 μM terfenadine for 24 h and the cell viability analysis was performed. Data are mean $\pm$ SEM of three independent experiments. Each treatment represented six replicates. Data are presented as percentage of control that is the increase in cell numbers in terfenadine-free groups. Control had 10,656 $\pm$ 155 cells/well before experiments, and had 13,555 $\pm$ 389 cells/well after incubation for 24 h. * $p < .05$ compared to control. In each group, the Ca^{2+} chelator BAPTA/AM (5 μM) was added to cells followed by treatment with terfenadine in Ca^{2+} -containing medium.

rises, experiments were conducted to explore the action of terfenadine on viability of MDCK cells. Cells were treated with 0–300 μM terfenadine for 24 h, and the tetrazolium assay was performed. In the presence of 200–300 μM terfenadine, cell viability decreased in a concentration-dependent manner (Figure 6). The intracellular Ca^{2+} chelator BAPTA/AM [26] was applied to prevent $[\text{Ca}^{2+}]_i$ rises during terfenadine pretreatment, in order to explore the role of Ca^{2+} in terfenadine-induced cell death. Figure 6 also shows that 5 μM BAPTA/AM loading did not change the control value of cell viability. Terfenadine (1000 μM) did not evoke $[\text{Ca}^{2+}]_i$ rises in BAPTA/AM-treated cells in both Ca^{2+} -containing and Ca^{2+} -free solutions (not shown). This suggests that BAPTA loading for 25 h still chelated cytosolic Ca^{2+} . BAPTA/AM incubation did not prevent terfenadine from inducing cell death.

Discussion

The effect of terfenadine on Ca^{2+} mobilization is demonstrated in various models. Previous studies showed that terfenadine inhibited $[\text{Ca}^{2+}]_i$ rises in cultured guinea pig cardiomyocytes [4] and rat ventricular myocytes [6] but increased $[\text{Ca}^{2+}]_i$ in A375 human melanoma cells [8] and human atrial myocytes [9]; however, whether terfenadine affects Ca^{2+} homeostasis in MDCK cells is unclear. Because so far the effect of terfenadine on Ca^{2+} signaling in different cell types is controversial, more research is needed to have a better knowledge of how terfenadine alters $[\text{Ca}^{2+}]_i$. Our study shows that terfenadine increased $[\text{Ca}^{2+}]_i$ in MDCK cells. The Ca^{2+} signal was composed of Ca^{2+} release and Ca^{2+} entry because the signal was reduced by approximately 35% by removing extracellular Ca^{2+} .

In terms of the Ca^{2+} stores that contributed to terfenadine-induced Ca^{2+} release, the BHQ-sensitive endoplasmic reticulum store appears to play a main role. A mitochondrial Ca^{2+} channel known as the uniporter drives the rapid and massive entry of Ca^{2+} ions into mitochondria. The uniporter operates at high μM cytosolic Ca^{2+} concentrations that are only reached transiently in cells, near Ca^{2+} release channels [27]. The results show that incubation with the endoplasmic reticulum Ca^{2+} pump inhibitor BHQ inhibited 72% of terfenadine-induced $[\text{Ca}^{2+}]_i$ rises. In contrast, incubation with terfenadine abolished BHQ-induced $[\text{Ca}^{2+}]_i$ rises. The residual 28% might be contributed by other stores such as mitochondria, cytoskeleton, nucleus, etc. [28].

Many pathways regulate the release of Ca^{2+} from intracellular stores. Among them, PLC is one of the pivotal proteins [28]. Furthermore, PLC has been shown to regulate the release of Ca^{2+} from the endoplasmic reticulum [28]. The role of PLC in terfenadine-induced Ca^{2+} release was explored because terfenadine released Ca^{2+} from the endoplasmic reticulum. Our results suggest that PLC activity was required for terfenadine-induced Ca^{2+} release because the release was decreased by 95% under the condition that PLC activity was fully suppressed.

In terms of Ca^{2+} influx, quenching of fura-2 fluorescence excited at the Ca^{2+} -insensitive excitation wavelength of 360 nm by Mn^{2+} implies Ca^{2+} influx. The Mn^{2+} quenching data further support that Ca^{2+} influx occurred during terfenadine incubation. Previous researches have shown that in MDCK cells the dominant Ca^{2+} entry pathway is the store-operated Ca^{2+} channels [17]. Terfenadine-evoked $[\text{Ca}^{2+}]_i$ rises were inhibited by 30–50% by econazole, nifedipine, and SKF96365. These three compounds have been used to inhibit store-operated Ca^{2+} entry [29–31]. Store-operated Ca^{2+} channels have been shown to play a role in $[\text{Ca}^{2+}]_i$ rises stimulated with several compounds such as allyl sulfides [16], phenethyl isothiocyanate [17], and sertraline [18] in MDCK cells. Therefore, it appears that terfenadine induced store-operated Ca^{2+} entry in MDCK cells.

It has been established that activity of many protein kinases is closely linked to Ca^{2+} homeostasis [32,33]. Our data show that terfenadine-evoked $[\text{Ca}^{2+}]_i$ rises were inhibited by 20% by PKC activation (a PKC activator, PMA) but was unaltered by inhibition of PKC (a PKC inhibitor, GF109203X).

This suggests that a normal PKC level is needed for terfenadine to induce full Ca^{2+} responses. The interactive relationship between PKC and Ca^{2+} signaling has been well established. Human cytomegalovirus pUL37x1-caused Ca^{2+} entry was shown to activate PKC, alter cell shape and accumulate cytosolic vesicles [34]. On the contrary, Ca^{2+} -unrelated activation of endocannabinoid/endovanilloid signaling by PKC was reported [35]. Because Ca^{2+} influx contributed to 35% of terfenadine-induced $[\text{Ca}^{2+}]_i$ rises, this influx appears to be partially contributed by PKC-regulated store-operated Ca^{2+} entry.

Cell viability may or may not be associated with rises in $[\text{Ca}^{2+}]_i$. Literature shows that sensitization to autoimmune hepatitis in Ca^{2+} -independent phospholipase A_2 -null mice resulted in leaked bile acids, goblet cell hyperplasia, and duodenal villous atrophy [36]. In contrast, paclitaxel was shown to suppress Bcl-2 resistance to apoptosis *via* an endoplasmic reticulum-mediated Ca^{2+} release in a concentration-dependent fashion in breast cancer cells [37]. Our data show that BAPTA/AM pretreatment for 25 h did not overcome terfenadine-evoked cell death. Thus, terfenadine-induced cell death did not appear to be caused by $[\text{Ca}^{2+}]_i$ rises.

Previous studies have shown that the plasma level of terfenadine in experimental animals and healthy volunteers may reach 20 μM [38]. In patients with kidney malfunction this level may go higher [39]. Evidence suggests that because terfenadine in the blood requires active transport into the liver, it is less metabolized by the cytochrome P450 family but shows more renal excretion in human [40]. Our findings suggest that terfenadine at a concentration of 200 μM caused death of 45% of MDCK cells. Thus, the clinical relevance of our data may not be excluded.

Collectively, the results show that terfenadine induced Ca^{2+} release from the endoplasmic reticulum in a PLC-dependent manner and Ca^{2+} influx *via* PKC-sensitive store-operated Ca^{2+} entry. Terfenadine also evoked Ca^{2+} -independent cell death. Since $[\text{Ca}^{2+}]_i$ rises play a triggering or modulatory role in numerous cellular phenomena, the effect of terfenadine on $[\text{Ca}^{2+}]_i$ and cell viability should be taken into account in other *in vitro* studies.

Disclosure statement

The authors report no declarations of interest.

Funding

This work was supported by RD-107001, RD-106001, RD-105001, RD-105002, RD-104002 from Chang Bing Show Chwan Memorial Hospital to He-Hsiung Cheng.

References

- [1] Reichmuth D, Lockey RF. Present and potential therapy for allergic rhinitis: a review. *Biodrugs*. 2000;14:371–387.
- [2] Thompson D, Oster G. Use of terfenadine and contraindicated drugs. *JAMA*. 1996;275:1339–1341.

- [3] Fossa AA. Assessing QT prolongation in conscious dogs: validation of a beat-to-beat method. *Pharmacol Ther.* 2008;118:231–238.
- [4] Qian JY, Guo L. Altered cytosolic Ca^{2+} dynamics in cultured Guinea pig cardiomyocytes as an in vitro model to identify potential cardiotoxicants. *Toxicol in Vitro.* 2010;24:960–972.
- [5] Díaz-Trelles R, Fernández-Sánchez MT, Marini AM, et al. Antihistamine terfenadine inhibits calcium influx, cGMP formation, and NMDA receptor-dependent neurotoxicity following activation of L-type voltage sensitive calcium channels. *Neurotox Res.* 2002;4:15–24.
- [6] Liu S, Melchert RB, Kennedy RH. Inhibition of L-type Ca^{2+} channel current in rat ventricular myocytes by terfenadine. *Circ Res.* 1997;81:202–210.
- [7] Ming Z, Nordin C. Terfenadine blocks time-dependent Ca^{2+} , Na^{+} , and K^{+} channels in guinea pig ventricular myocytes. *J Cardiovasc Pharmacol.* 1995;26:761–769.
- [8] Jangi SM, Ruiz-Larrea MB, Nicolau GF, et al. Terfenadine-induced apoptosis in human melanoma cells is mediated through Ca^{2+} homeostasis modulation and tyrosine kinase activity, independently of H1 histamine receptors. *Carcinogenesis.* 2007;29:500–509.
- [9] Hove-Madsen L, Llach A, Molina CE, et al. The proarrhythmic antihistaminic drug terfenadine increases spontaneous calcium release in human atrial myocytes. *Eur J Pharmacol.* 2006;553:215–221.
- [10] Tasaka K, Mio M, Okamoto M. Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. *Ann Allergy.* 1986;56:464–469.
- [11] Morad M, Soldatov N. Calcium channel inactivation: possible role in signal transduction and Ca^{2+} signaling. *Cell Calcium.* 2005;38:223–231.
- [12] Wacquier B, Voorsluijs V, Combettes L, et al. Coding and decoding of oscillatory Ca^{2+} signals. *Semin Cell Dev Biol.* 2019. DOI:10.1016/j.semcdb.2019.01.008.
- [13] Yang J, Zhao Z, Gu M, et al. Release and uptake mechanisms of vesicular Ca^{2+} stores. *Protein Cell.* 2019;10:8–19.
- [14] Jardín I, Albarran L, Salido GM, et al. Fine-tuning of store-operated calcium entry by fast and slow Ca^{2+} -dependent inactivation: involvement of SARAF. *Biochim Biophys Acta Mol Cell Res.* 2018;1865:463–469.
- [15] Banks RO, Inscho EW, Jacobson ED. Histamine H1 receptor antagonists inhibit autoregulation of renal blood flow in the dog. *Circ Res.* 1984;54:527–535.
- [16] Jan CR, Lo HR, Chen CY, et al. Effect of allyl sulfides from garlic essential oil on intracellular Ca^{2+} levels in renal tubular cells. *J Nat Prod.* 2012;75:2101–2107.
- [17] Chen IS, Mok KT, Chou CT, et al. Effect of phenethyl isothiocyanate on Ca^{2+} movement and viability in MDCK canine renal tubular cells. *Hum Exp Toxicol.* 2012;31:1251–1261.
- [18] Huang CJ, Kuo DH, Chang KH, et al. Effect of the antidepressant sertraline on Ca^{2+} fluxes in Madin-Darby canine renal tubular cells. *J Recept Signal Transduct Res.* 2009;29:342–348.
- [19] Ratto GM, Payne R, Owen WG, et al. The concentration of cytosolic free calcium in vertebrate rod outer segments measured with fura-2. *J Neurosci.* 1988;8:3240–3246.
- [20] Sage SO, Merritt JE, Hallam TJ, et al. Receptor-mediated calcium entry in fura-2-loaded human platelets stimulated with ADP and thrombin. Dual-wavelength studies with Mn^{2+} . *Biochem J.* 1989;258:923–926.
- [21] Ong HL, Subedi KP, Son GY, et al. Tuning store-operated calcium entry to modulate Ca^{2+} -dependent physiological processes. *Biochim Biophys Acta Mol Cell Res.* 1866:1037–1045.
- [22] García-Casas P, Arias-Del-Val J, Alvarez-Illera P, et al. Inhibition of sarco-endoplasmic reticulum Ca^{2+} ATPase extends the lifespan in *C. elegans* worms. *Front Pharmacol.* 2018;9:669.
- [23] Bleasdale JE, Bundy GL, Bunting S, et al. Inhibition of phospholipase C dependent processes by U-73, 122. *Adv Prostaglandin Thromboxane Leukot Res.* 1989;19:590–593.
- [24] Song S, Jacobson KN, McDermott KM, et al. ATP promotes cell survival via regulation of cytosolic $[\text{Ca}^{2+}]$ and Bcl-2/Bax ratio in lung cancer cells. *Am J Physiol Cell Physiol.* 2016;310:C99–114.
- [25] Friedrich O, Head SI. Quantitative ratiometric Ca^{2+} imaging to assess cell viability. *Methods Mol Biol.* 2017;1601:171–193.
- [26] Dormer RL. Introduction of calcium chelators into isolated rat pancreatic acini inhibits amylase release in response to carbamylcholine. *Biochem Biophys Res Commun.* 1984;119:876–883.
- [27] Losi G, Mariotti L, Sessolo M, et al. New tools to study astrocyte Ca^{2+} signal dynamics in brain networks *in vivo*. *Front Cell Neurosci.* 2017;11:134.
- [28] Clapham DE. Calcium signaling. *Cell.* 2007;131:1047–1058.
- [29] Dago CD, Maux PL, Roisnel T, et al. Preliminary structure-activity relationship (SAR) of a novel series of pyrazole SKF-96365 analogues as potential store-operated calcium entry (SOCE) inhibitors. *Int J Mol Sci.* 2018;19:pii: E856.
- [30] Nilius B, Prenen J, Vennekens R, et al. Pharmacological modulation of monovalent cation currents through the epithelial Ca^{2+} channel ECaC1. *Br J Pharmacol.* 2001;134:453–462.
- [31] Zhang J, Wier WG, Blaustein MP. Mg^{2+} blocks myogenic tone but not K^{+} -induced constriction: role for SOCs in small arteries. *Am J Physiol Heart Circ Physiol.* 2002;283:H2692–705.
- [32] Wang Z, Cole PA. Catalytic mechanisms and regulation of protein kinases. *Meth Enzymol.* 2014;548:1–21.
- [33] Yamashita M, Sueyoshi N, Yamada H, et al. Characterization of CoPK02, a Ca^{2+} /calmodulin-dependent protein kinase in mushroom *Coprinopsis cinerea*. *Biosci Biotechnol Biochem.* 2018;82:1335–1343.
- [34] Sharon-Friling R, Shenk T. Human cytomegalovirus pUL37x1-induced calcium flux activates PKC α , inducing altered cell shape and accumulation of cytoplasmic vesicles. *Proc Natl Acad Sci USA.* 2014;111:E1140–E1148.
- [35] Vellani V, Petrosino S, De Petrocellis L, et al. Functional lipodomics. Calcium-independent activation of endocannabinoid/endovanilloid lipid signalling in sensory neurons by protein kinases C and A and thrombin. *Neuropharmacology.* 2008;55:1274–1279.
- [36] Jiao L, Gan-Schreier H, Tuma-Kellner S, et al. Sensitization to autoimmune hepatitis in group VIA calcium-independent phospholipase A2-null mice led to duodenal villous atrophy with apoptosis, goblet cell hyperplasia and leaked bile acids. *Biochim Biophys Acta.* 2015;1852:1646–1657.
- [37] Pan Z, Gollahon L. Paclitaxel attenuates Bcl-2 resistance to apoptosis in breast cancer cells through an endoplasmic reticulum-mediated calcium release in a dosage dependent manner. *Biochem Biophys Res Commun.* 2013;432:431–437.
- [38] Leeson GA, Chan KY, Knapp WC, et al. Metabolic disposition of terfenadine in laboratory animals. *Arzneimittelforschung.* 1982;32:1173–1178.
- [39] Eikel D, Vavrek M, Smith S, et al. Liquid extraction surface analysis mass spectrometry (LESA-MS) as a novel profiling tool for drug distribution and metabolism analysis: the terfenadine example. *Rapid Commun Mass Spectrom.* 2011;25:3587–3596.
- [40] Furuta S, Kamada E, Omata T, et al. Drug-drug interactions of Z-338, a novel gastroprokinetic agent, with terfenadine, comparison with cisapride, and involvement of UGT1A9 and 1A8 in the human metabolism of Z-338. *Eur J Pharmacol.* 2004;497:223–231.