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Inhibition of intermediate-conductance Ca^{2+}-activated K^+ channel and cytoprotective properties of 4-piperidinomethyl-2-isopropyl-5-methylphenol

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Abstract

The ionic mechanisms and cytoprotective activities of 4-piperidinomethyl-2-isopropyl-5-methylphenol (THPI), an analogue of thymol, were investigated in HL-60 granulocytes and in human erythrocytes, respectively. THPI inhibited K^+ outward current (I_K) in a concentration-dependent manner in HL-60 leukocytes, with an IC50 value of 4 μM. Neither iberiotoxin (200 nM) nor paxilline (1 μM) suppressed the amplitude of I_K, whereas clotrimazole (5 μM) significantly inhibited it. In the inside-out configuration of single channel recordings, application of THPI (5 μM) into the bath medium did not alter the single-channel conductance of intermediate-conductance Ca^{2+}-activated K^+ (IK_{Ca}) channels (i.e $K_{Ca}3.1$ channels), but it suppressed the channel activity significantly. THPI-induced inhibition of IK_{Ca} channels was reversed by a further application of 1-ethyl-2-benzimidazolinone (10 μM). THPI-induced reduction in IK_{Ca}-channel activity in these cells was primarily due to a decrease in mean open time. These results provide direct evidence that THPI is capable of suppressing the activity of IK_{Ca} channels in HL-60 cells. The antioxidant action of THPI also revealed a beneficial cytoprotective effect against mitomycin C-mediated haemolytic effect in human erythrocytes. The results of this study suggest that blockade of IK_{Ca} channels and the membrane-protecting activity of THPI would combine to have beneficial effects in lessening the severity of haemolytic crisis and reducing anaemia in sickle cell disease.

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Introduction

Multiple types of Ca^{2+}-activated K^+ (K_{Ca}) channels with involvement in a variety of distinct physiological processes have been described. The channels of intermediate conductance, termed IK_{Ca} ($K_{Ca}3.1$) channels, which are encoded by the KCNN4 gene, are almost exclusively in non-excitable cells. These channels, which display a unitary conductance of 20–60 pS in symmetrical K^+ ions, play a pivotal role in the physiology of lymphocytes, erythrocytes and airway epithelial cells (Jensen et al 2001; Stocker 2004). Antagonists of the IK_{Ca} channel are known to have potential therapeutic uses as modulators of erythrocytes for the treatment of sickle cell disease (Jensen et al 2001).

Samples from patients with sickle cell anaemia were found to contain a significant population of abnormally dense, dehydrated, K^+-depleted cells (Hebbel 1991). It was reported that the formation of such dense cells was due to Ca^{2+}-dependent K^+ efflux and concomitant dehydration (Ohnishi et al 1989). The erythrocyte hydration state is controlled by monovalent cation content, while dehydration results primarily from K^+ depletion without equivalent Na^+ uptake (Brugnara 1997). Dehydration of erythrocytes can potentiate the polymerization of the haemoglobin S in patients with homozygous point mutations in the β-globin gene, thereby resulting in erythrocyte sickling (Joiner et al 1993; Brugnara 1997). Clotrimazole, an imidazole antimycotic P-450 inhibitor, has been utilized in clinical trials for the prevention of cell dehydration in sickle cell anaemia (Brugnara et al 1993; Jensen et al 2001), and has been proposed for the treatment of β-thalassaemia (de Franceschi et al 1996). Clotrimazole has been reported to suppress the activity of K_{Ca} channels in many types of cells (Jensen et al 2001).

Previous studies have also proposed that sickle erythrocytes and their membranes are susceptible to endogenous free radical mediated oxidative damage, which correlates with

the proportion of irreversibly sickle cells (Ohnishi & Ohnishi 2001). Protection of the red blood cells membrane from oxidative stress may improve the clinical manifestations of these patients (Schroder et al 2003). Because enhanced activity of IK_{Ca} channels plays an important role in the ion movements in red blood cells, it may be implicated in the observed free radical induced dehydration of sickle red cells (Ohnishi & Ohnishi 2001; Schroder et al 2003). Indeed, membrane disturbance caused by sickling may induce Ca^{2+} and K^+ efflux (Etzion et al 1993; Joiner et al 1993; Brugnara 1997).

THPI, a synthesized analogue of thymol, was previously reported to scavenge superoxide radicals in human neutrophils simulated with *N*-farmyl-Met-Leu-Phe (fMLP), to inhibit human platelet aggregation induced by arachidonic acid, and to inhibit L-type calcium current in NG108-15 cells (Huang et al 2005). Few natural non-peptide compounds have been described to possess IK_{Ca}-channel blocking activity. In this study, we report that THPI could be effective in suppressing the activity of IK_{Ca} channels in HL-60 cells. This compound also prevents mitomycin-induced haemolysis in human erythrocytes.

Materials and Methods

Cell culture

HL-60 cells, obtained from the Bioresource Collection and Research Center (Hsin-Chu, Taiwan), were maintained in RPMI 1640 medium (Grand Island, NY, USA) supplemented with 20% heat-inactivated fetal bovine serum, 2 mM glutamine, 50 U mL^{-1} penicillin and 50 U mL^{-1} streptomycin in a 5% CO_2 humidified incubator at 37°C. The cells were resuspended in fresh medium at a density of 2×10^5 mL^{-1} cells per 6-well plate.

Electrophysiological measurements

Immediately before each experiment, cells were dissociated by trypsin and an aliquot of cell suspension was transferred to a recording chamber positioned on the stage of a DM-IL inverted microscope (Leica, Wetzlar, Germany). The microscope was coupled to a video camera system with magnification up to 1500× to continually monitor cell size during the experiments. HL60 cells were bathed at room temperature (20–25°C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The recording pipettes were pulled on a PP-830 puller (Narishige, Tokyo, Japan) from Kimax-51 glass capillaries (Kimble Glass, Vineland, NJ, USA) and the tips were fire-polished with an MF-83 microforge (Narishige). When filled with pipette solution, their resistance ranged between 3 and 5 MΩ. Ion currents were recorded with glass pipettes in whole-cell, cell-attached and inside-out modes of the patch-clamp technique, using an RK-400 patch-clamp amplifier (Bio-Logic, Claix, France) (Wu et al 1999).

Data recording and analysis

The signals were displayed on an HM-507 oscilloscope (Hameg, East Meadow, NY, USA) and on a PJ550-2 liquid crystal device projector (ViewSonic, Walnut, CA, USA). The data were stored on-line in a Pentium III grade laptop computer (Slimnote VX_3; Lemel, Taipei, Taiwan) via a universal serial bus port at 10 kHz through an analog/digital interface (Digidata 1322A; Axon Instruments, Union City, CA, USA). This device was controlled by the pCLAMP 9.0 software (Axon Instruments). Cell membrane capacitance of 22–35 pF (25.4 ± 3.4 pF, $n=21$) was compensated. Series resistance, always over the range of 5–15 MΩ, was electronically compensated to 80–95%. Currents were low-pass filtered at 1 or 3 kHz before digitization.

To calculate the concentration-dependent inhibition of THPI on K^+ outward current (I_K), each cell was held at −40 mV and the ramp pulses from −120 to +50 mV with a duration of 1 s were applied. The amplitude of I_K during the exposure to different concentrations (0.5–200 μM) of this compound was measured at the level of 0 mV. The concentration of THPI required to reduce 50% of current amplitude was then determined using a Hill function:

$$\%\ \text{inhibition} = (E_{max}\times[C]^{n_h})/(IC50^{n_h}+[C]^{n_h}),$$

where [C] represents the THPI concentration, IC50 is the concentration that produced 50% of maximal inhibition, n_h is the Hill coefficient, and E_{max} is the THPI-induced maximal inhibition of I_K.

The single-channel amplitudes of IK_{Ca} channels were determined by fitting Gaussian distributions to the amplitude histograms of the closed and the open state. The channel open probability in a patch was expressed as NP_o, which was estimated using the following equation: $NP_o=(A_1+2A_2+3A_3+\ldots+nA_n)/(A_0+A_1+A_2+A_3+\ldots+A_n)$, where N is the number of active channels in the patch, A_0 is the area under the curve of an all-points histogram corresponding to the closed state, and $A_1\ldots.A_n$ represent the histogram areas reflecting the levels of distinct open state for 1 to n channels in the patch.

Haemolysis test

The method used in this study is a minor modification of that by Kok et al (2000). Human blood was collected into a heparinized tube. The experimental protocols were approved by our Institutional Review Board in accordance with international guidelines and informed consent was obtained from the subjects who participated in this study. Erythrocytes were separated from plasma by centrifugation at 1500 *g* for 20 min. The crude erythrocytes were washed and suspended in 5 volumes of phosphate-buffered saline solution. Red blood cell suspension (100 μL) was mixed with mitomycin C (100 μM) in the absence and presence of THPI. The mixture was placed in a rotary incubator at 37°C for 12 h. After incubation, 400 μL phosphate-buffered saline was added to each mixture. The diluted mixture was centrifuged at 1000 *g* for 10 min. The absorbance of each sample was read at 540 nm. The relative percentage inhibition of haemolysis (I%) of THPI was expressed as $I\% = [1 - (A_{\text{with THPI}}/A_{\text{without THPI}})]\times100\%$.

Drugs and solutions

THPI (4-piperidinomethyl-2-isopropyl-5-methylphenol) was synthesized by methods as described previously (Huang et al 2005). Mitomycin C was purchased from Sigma-Aldrich (St Louis, MO, USA). Clotrimazole and iberiotoxin were purchased from Sigma/RBI (St Louis, MO, USA). 1-Ethyl-2-benzimidazolinone (1-EBIO) was obtained from Tocris (Bristol, UK) and paxilline was from Biomol (Plymouth Meeting, PA, USA).

The composition of normal Tyrode's solution was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose and 5.5 mM HEPES-NaOH buffer, pH 7.4. To record K^+ currents or membrane potential, the recording pipette was backfilled with a solution consisting of 140 mM KCl, 1 mM $MgCl_2$, 3 mM Na_2ATP, 0.1 mM Na_2GTP, 0.1 mM EGTA, 0.1 μM $CaCl_2$ and 5 mM HEPES-KOH buffer, pH 7.2. For single-channel current recordings, the high-K^+ bathing solution contained 145 mM KCl, 0.53 mM $MgCl_2$ and 5 mM HEPES-KOH buffer, pH 7.4, and the pipette solution contained 145 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES-KOH buffer, pH 7.2.

Statistical analysis

All values are presented as the means ± s.e.m. Data were analysed by one-way analysis of variance followed by post-hoc Dunnett's test for pairwise comparison. Statistical significance was defined as $P < 0.05$.

Results

Inhibitory effect of THPI and other related compounds on I_K in HL-60 cells

In the initial set of experiments, ramp pulses from −120 to +50 mV with a duration of 1 s were applied to the cell. Figure 1A shows the inhibitory effect of THPI on I_K in HL-60 cells. When cells were exposed to THPI (5 μM), current amplitude, measured at the level of 0 mV, was significantly decreased from 57 ± 11 to 9 ± 8 pA (n = 8). After washout of the compound, current amplitude was returned to 45 ± 9 pA (n = 5). The effects of various K^+-channel blockers, including iberiotoxin, paxilline, clotrimazole and apamin, on I_K in these cells were further examined and compared. The results showed that neither iberiotoxin (200 nM) nor paxilline (1 μM) suppressed the amplitude of I_K significantly, while clotrimazole (5 μM) decreased it. Apamin (200 nM) was found not to have any effects on I_K in HL-60 cells. In addition, 1-ethyl-2-benzimidazolinone (10 μM), an opener of IK_{Ca} channels, could increase the amplitude of I_K and reverse THPI-induced reduction of I_K in HL-60 cells. Paxilline and iberiotoxin were reported to be the inhibitors of large-conductance Ca^{2+}-activated K^+ channels, while clotrimazole could block the activity of IK_{Ca} channels (Jensen et al 2001; Wu 2003; Ahluwalia et al 2004). In addition, thymol (30 μM) had no effect on I_K in these cells (data not shown). Thus, it is unlikely that the observed decrease in I_K by THPI is primarily due to its inhibition of I_K, which is sensitive to inhibition by paxilline or iberiotoxin. Figure 2C shows that THPI could reduce the amplitude of I_K in a concentration-dependent manner, with an IC50 value of

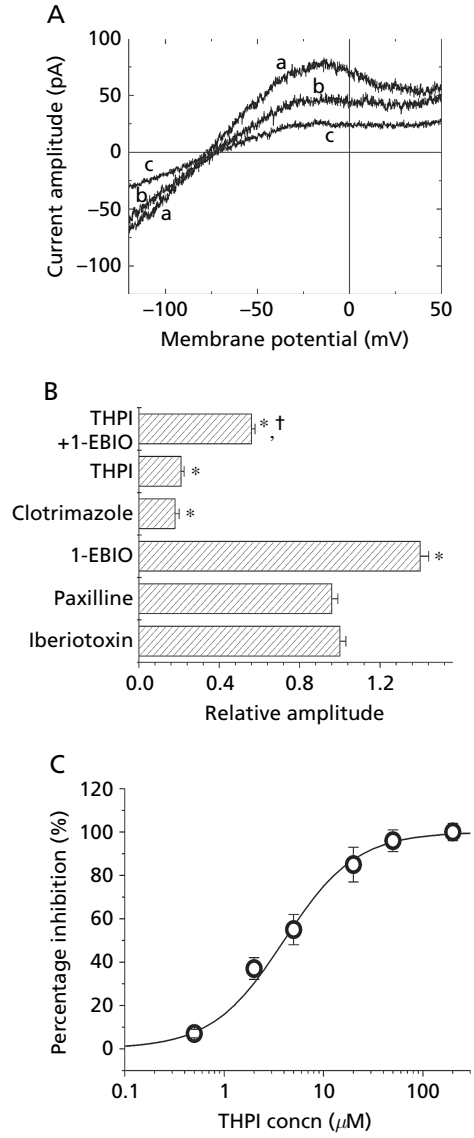

Figure 1 Effect of 4-piperidinomethyl-2-isopropyl-5-methylphenol (THPI) and other related compounds (100 μM 1-ethyl-2-benzimidazolinone (1-EBIO); 5 μM clotrimazole; 1 μM paxilline; 200 nM iberiotoxin) on K^+ outward current (I_K) recorded from HL-60 cells. HL-60 cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$ and ramp pulses from −120 to +50 mV with a duration of 1 s were applied. A. Original current traces obtained in the absence (a) and presence of 5 μM (b) and 20 μM (c) THPI. B. Effect of various related compounds on the amplitude of I_K measured. The amplitude of I_K measured at the level of 0 mV in the control was considered to be 1.0, and the relative amplitude of I_K in the presence of each agent was compared and plotted. Each point represents the mean ± s.e.m., n = 5–9. *$P < 0.05$, significantly different compared with the control. †$P < 0.05$, significantly different compared with the THPI alone group. C. Concentration–response relationship for THPI-induced inhibition of I_K. Cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Each cell was held at −40 mV and ramp pulses from −120 to +50 mV were applied. Current amplitudes at the level of 0 mV were obtained after addition of different concentrations (0.5–200 μM) of THPI. Each point represents the mean ± s.e.m., n = 6–9. The smooth line represents the best fit to a Hill function. The values for IC50, maximally inhibited percentage of I_K and the Hill coefficient were 4 μM, 100%, and 1.2, respectively.

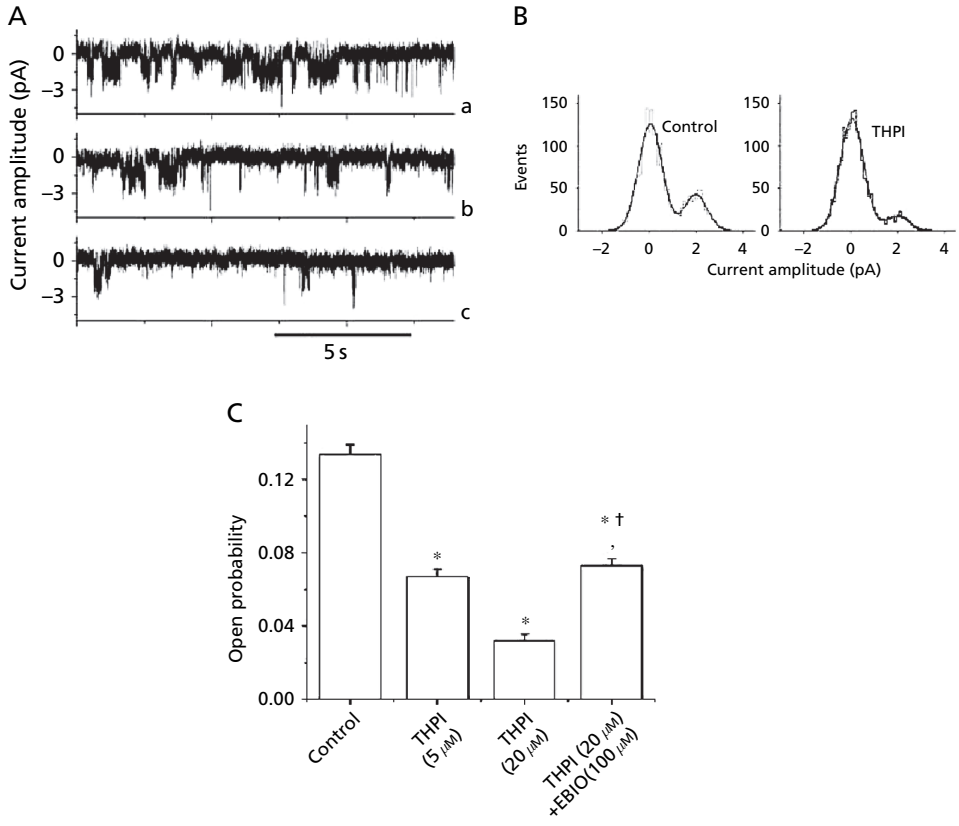

Figure 2 Inhibitory effect of 4-piperidinomethyl-2-isopropyl-5-methylphenol (THPI) on the activity of IK_{Ca} channels in HL-60 cells. The experiments were conducted with symmetrical K^+ concentration (145 mM). In the inside-out configuration, the hold potential was set at −80 mV and the bath medium contained 0.1 μM Ca^{2+}. A. The activity of IK_{Ca} channels recorded before (a) and during exposure to 5 μM (b) and 20 μM (c) THPI. Downward deflection indicates the opening events of the channel. Note that the channel exhibits only a few brief openings in the presence of THPI. B. Amplitude measured in the absence (control) and presence of 5 μM THPI. The closed state corresponds to the peak at 0 pA. C. Bar graph showing the effect of THPI and THPI plus 1-ethyl-2-benzimidazolinone (1-EBIO) on the open probability of IK_{Ca} channels. $*P < 0.05$, significantly different compared with the control. $\dagger P < 0.05$, significantly different compared with the THPI (20 μM) alone group.

4 μM. In addition, in the current-clamp configuration, the application of THPI (5 μM) could depolarize membrane potential from −41 ± 5 to −29 ± 3 mV (n = 5).

Inhibitory effect of THPI on IK_{Ca} channels in HL-60 cells

Previous studies have demonstrated the presence of IK_{Ca} channels in HL-60 cells (Varnai et al 1993; Fioretti et al 2004). The next series of experiments were designed to investigate whether THPI interacts with the IK_{Ca} channel to reduce I_K in HL-60 cells. Single-channel recordings performed in these experiments were conducted with a symmetrical K^+ concentration (145 mM), inside-out configuration was performed, and bath medium contained 0.1 μM Ca^{2+}. To provide 0.1 μM free Ca^{2+} in the bath solution, 0.5 mM $CaCl_2$ and 1 mM EGTA were added (Portzehl et al 1964). As shown in Figure 3, when the potential was held at −80 mV, application of THPI to the cytosolic surface of an inside-out patch reduced single-channel activity. When membrane patches were exposed to THPI (5 μM), the probability of channel openings was significantly reduced to 0.067 ± 0.004 from a control value of 0.134 ± 0.005 (n = 5). Channel activity was increased to 0.102 ± 0.004 (n = 4) after washout of this compound. However, no significant difference in the amplitude of single-channel current could be demonstrated between the absence and presence of THPI (Figure 2B). In addition, 1-EBIO at a concentration of 100 μM was able to partially reverse the reduced open probability caused by 20 μM THPI (Figure 2C).

Effect of THPI on kinetic behaviour of IK_{Ca} channels in HL-60 cells

The effect of THPI on mean open time of IK_{Ca} channels was examined and analysed during recordings from patches showing that there were only single-channel openings. As shown in Figure 3, in control cells, the open time histogram of IK_{Ca} channels at −80 mV can be fitted by a one-exponential curve with a mean open time of 2.68 ± 0.04 ms (n = 5). Interestingly, in the presence of THPI (5 μM), the mean open time of these channels was significantly reduced to 1.33 ± 0.03 ms (n = 5). However, no significant change in mean close time between the presence and absence of THPI (5 μM) was observed. Thus, despite the inability of THPI to alter single-channel conductance of IK_{Ca} channels, its changes in mean open time can primarily explain the inhibitory effect on the activity of these channels.

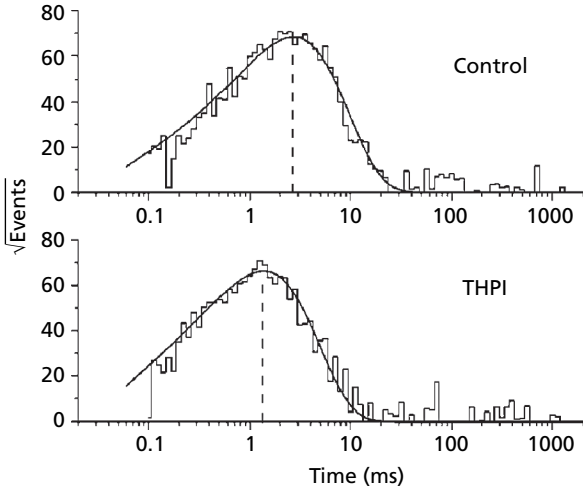

Figure 3 Effect of 4-piperidinomethyl-2-isopropyl-5-methylphenol (THPI) on mean open time of IK_{Ca} channels in HL-60 cells. Inside-out configuration with a holding potential of −80 mV was used in these experiments. Cells were bathed in symmetrical K^+ solution (145 mM) and bath medium contained 0.1 μM Ca^{2+}. The upper panel is the control and the lower panel was obtained after addition of 5 μM THPI to the bath. The abscissa and ordinate show the logarithm of open times (ms). Control data were obtained by measuring 641 channel openings with a total record time of 2 min; data obtained in the presence of 5 μM THPI were measured from 611 channel openings with a total recording time of 3 min. The dashed lines shown in each lifetime distribution are placed at the value of the time constant in open state.

Lack of effect of THPI on single-channel conductance of IK_{Ca} channels in HL-60 cells

The effect of THPI on IK_{Ca} channels at various membrane potentials was determined. In inside-out configuration, cells were bathed in symmetrical K^+ concentration (145 mM) and the bath solution contained 0.1 μM Ca^{2+}. The single-channel conductance of IK_{Ca} channels in the control was 27.4 ± 1.2 pS (n = 7), with a reversal potential of 0 ± 3 mV (n = 7). The value of single-channel conductance was not significantly different from that measured in the presence of 5 μM THPI (27.1 ± 1.3 pS, n = 6). The reversal potential of these channels in the presence of THPI was 0 ± 3 mV (n = 6). The results indicated that this compound produced no modification in single-channel conductance, but significantly reduced the activity of IK_{Ca} channels in these cells.

Effects of THPI on cytoprotective activity

Because the activity of IK_{Ca} channels in erythrocytes may affect their haemolysis, the effect of THPI on free radical mediated haemolysis of human erythrocytes was studied. In our preparations, we found that the maximum concentration required for mitomycin C to induce a haemolytic effect on human erythrocytes was 100 μM. We therefore used mitomycin C at a concentration of 100 μM in the experiments in this study. It was found that after erythrocytes were incubated with mitomycin C (100 μM) at 37°C for 12 h, in combination with various concentrations of THPI (3–30 μM), free radical mediated haemolysis on human erythrocytes could be suppressed. The data indicated that it could exert an inhibitory effect on free radical mediated haemolysis. A summary of the experimental results is shown in Table 1.

Table 1 Effect of 4-piperidinomethyl-2-isopropyl-5-methylphenol (THPI) on protection of erythrocytes co-incubated with mitomycin C

Drug	Co-incubation with THPI (μM)	Relative I%
Mitomycin C (100 μM)	0	0.00 ± 0.86
	3	16.18 ± 4.86*
	10	23.53 ± 6.12*
	30	30.88 ± 7.87*

Human erythrocytes were incubated with mitomycin C in the absence and presence of THPI at different concentrations. The relative percentage inhibition (I%) of haemolysis is expressed as the mean ± s.e.m., n = 3–5. *$P < 0.05$, significantly different compared with the control.

Discussion

The present results demonstrated that (i) THPI effectively suppressed the amplitude of I_K in a concentration-dependent manner in HL-60 cells; (ii) THPI reduced the open probability of IK_{Ca} channels, with a decrease in mean open time, although it did not alter single-channel conductance; and (iii) THPI could exert a cytoprotective effect on mitomycin C-mediated haemolysis in human erythrocytes.

Consistent with previous observations (Varnai et al 1993; Fioretti et al 2004), this study clearly demonstrated the presence of IK_{Ca} channels in HL-60 cells. The results also showed that clotrimazole suppressed the amplitude of I_K, and 1-EBIO increased it in these cells. In our study, the single-channel conductance of IK_{Ca} channels in HL-60 granulocytes was calculated to be 27.4 ± 1.2 pS (n = 7), a value similar to that of prototypical IK_{Ca} channels present in many types of cells (Varnai et al 1993; Fioretti et al 2004; Duffy et al 2005), but apparently less than that of large-conductance K_{Ca} channels (Ahluwalia et al 2004). In addition, the observed activity of these channels in HL-60 cells is sensitive to inhibition by clotrimazole and to stimulation by 1-EBIO. Taken together, it is anticipated that the activity of IK_{Ca} channels primarily constitutes the generation of I_K in HL-60 cells. Consistent with these findings, in current-clamp recordings of HL-60 cells, THPI could depolarize the cell membrane.

In this study, we found that THPI could inhibit the amplitude of I_K in HL-60 cells in a concentration-dependent manner, with an IC50 value of 4 μM. In inside-out patches, this compound applied to the intracellular surface was also observed to significantly decrease the open probability of IK_{Ca} channels in these cells. Although no change in single-channel conductance could be demonstrated in the presence of THPI, mean open times of these channels were significantly reduced during the exposure to this compound. Moreover, 1-EBIO, an activator of IK_{Ca} channels, could reverse THPI-induced reduction of channel open probability. The results suggest that the binding site of this compound was distinguishable from that of 1-EBIO and could be located in the

intracellular site of the channel. Because small-conductance K_{Ca} channels share ~40% amino acid sequence homology with IK_{Ca} channels (Jensen et al 2001; Stocker 2004), it remains to be determined to what extent THPI exerts an inhibitory effect on small-conductance K_{Ca} channels.

Previous studies have shown the ability of THPI to block L-type Ca^{2+} current in NG108-15 neuronal cells (Huang et al 2005). In this study, there was no evidence to show the presence of voltage-gated Ca^{2+} or Na^{+} channels in HL-60 cells. Erythrocytes were also believed to be non-excitable. Thus, the cytoprotective effect of THPI against mitomycin C could be unrelated to its blockade on the L-type Ca^{2+} channel.

Reactive oxygen species have been implicated in damage of erythrocytes in patients with β-thalassaemia and sickle cell anaemia (Rice-Evans et al 1986; Vives-Corrons et al 1995). Erythrocytes are highly susceptible to oxidative damage as a result of the high polyunsaturated fatty acid content of their membranes and the high cellular concentrations of oxygen and haemoglobin, a potentially powerful promoter of oxidative process (Scott et al 1993). Thus, membrane-protecting compounds would have beneficial effects in reducing anaemia in sickle cell disease. The use of aged garlic extract and other antioxidants to improve sickle cell disease has been proposed (Ohnishi & Ohnishi 2001). Sickle cell patients have decreased levels of vitamin E and glutathione peroxides (Chiu & Lubin 1979), all of which are important compounds in the defence against oxygen free radical attack. THPI could function as an inhibitor of the oxidation induced by fMLP in human neutrophils in our previous study (Huang et al 2005). Thus, antioxidant activity of THPI might occur in human erythrocytes. Many quinone-containing antitumour drugs such as mitomycin C have been reported to inhibit proliferation of tumour cells mainly by intercalating into DNA and, less importantly, via free radical mediated reactions (Abdella & Fisher 1985). The side-effects of quinone-containing antitumour drugs are the result of electron-transferring activity catalysed by NADPH dehydrogenase, NADPH cytochrome P450 reductase or xanthine oxidase, leading to the formation of reactive oxygen species (Pawlowska et al 2003). This is the major obstacle at higher doses of the quinone moiety drug, mitomycin C, which causes haemolytic–uraemic syndrome during chemotherapy. In the treatment of breast cancer using mitomycin C, the majority of patients were reported to show haematological and cardiac toxicities, which lead to anaemia during multiple cycles of chemotherapy (Meadowcroft et al 1998). In our study, we examined the protective effect of THPI on human erythrocytes under treatment with mitomycin C. The assay for free radical mediated haemolysis showed that THPI (3–30 μM) could minimize the free radical mediated damage generated by mitomycin C in human erythrocytes. Taken together, the experimental results presented here suggest that the studies on THPI with antioxidant and IK_{Ca} channel inhibition properties may be of interest and have therapeutic relevance.

In summary, we report that THPI, a derivative of thymol, can effectively block the activity of IK_{Ca} channels in HL-60 cells. It also prevented mitomycin-induced haemolysis in human erythrocytes. The effect of this compound on erythrocytes appears to be associated with its blockade of IK_{Ca} channels.

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