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Inhibitory effects of α-Mangostin on T cell cytokine secretion *via* ORAI1 calcium channel and K^+ channels inhibition

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Background. As one of the main components of mangosteen (*Garcinia mangostana*), α-mangostin has been reported to have numerous pharmacological benefits such as anti-cancer, anti-inflammatory, and anti-allergic effects through various mechanisms of action. However, few studies have been conducted on the effects of α-mangostin on ion channels. Immune cell activation begins with an increase in intracellular calcium concentration and calcium influx via ORAI1. In addition, for sufficient calcium influx, potassium ion channels, which repolarize the cell membrane potential depolarized by calcium influx to regulate cell membrane voltage, required.

Methods. This study analyzed the inhibitory effect of α-mangostin on immune cell activity *via* inhibition of calcium and potassium ion channels expressed in immune cells.

Results. α-mangostin inhibited ORAI1 in a concentration-dependent manner, and the IC_{50} value was 1.29 ± 1.078 μM. $K_v1.3$ was suppressed by 41.38 ± 6.191% at 3 μM, and $K_{Ca}3.1$ was suppressed by 51.16 ± 5.385% at 3 μM. To measure the inhibition of cytokine secretion by immune cells, Jurkat T cells were co-stimulated with anti-CD3/CD28 antibodies to induce IL-2 secretion, and α-mangostin was found to inhibit it. This study demonstrated the anti-inflammatory effect of α-mangostin, the main component of mangosteen, through the regulation of calcium signals.

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Abstract

Background. As one of the main components of mangosteen (*Garcinia mangostana*), a tropical fruit, α-mangostin has been reported to have numerous pharmacological benefits such as anti-cancer, anti-inflammatory, and anti-allergic effects through various mechanisms of action. However, few studies have been conducted on the effects of α-mangostin on ion channels. Immune cell activation begins with an increase in intracellular calcium concentration and calcium influx via ORAI1. In addition, for sufficient calcium influx, potassium ion channels, which repolarize the cell membrane potential depolarized by calcium influx to regulate cell membrane voltage, required.

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Introduction

Calcium acts as a secondary messenger in most cells, and is important for immune responses such as immune cell activation and differentiation, cytokine production, and phagocytosis [1]. When the T cell receptor (TCR) is stimulated, phosphatidyl inositol 4,5-biphosphate (PIP_2) is hydrolyzed into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). At this time, IP_3 binds to the IP_3 receptor present in the endoplasmic reticulum (ER), and calcium stored in the ER is depleted [2,3]. Thereafter, calcium influx occurs via Ca^{2+} released activated channels (CRAC), and it was found that the gene constituting this is ORAI1 [4]. Stromal interaction molecule (STIM) was known to act as a calcium sensor in the ER calcium reservoir in 2005, and STIM, which recognizes ER calcium depletion, form puncta and binds directly to ORAI to regulate the opening and closing of channels [2,5,6]. ORAI and STIM are highly expressed in lymphocytes or mast cells, and play a crucial role in the immune response via Ca^{2+} influx. In fact, patients with mutations or deficiencies in ORAI or STIM are known to develop severe immunodeficiency [4,7,8]. Another ion channel, which regulates the calcium signal besides ORAI in immune cells, is the K^+ channel. Potassium channels common to immune cells include the voltage-gated potassium channel shaker-related subfamily, member 1.3 (also known as KCNA3 or $K_v1.3$), potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 (also known as KCNN4 or $K_{Ca}3.1$) [7,9]. These are important in maintaining or enhancing calcium influx via ORAI in immune cells. When ORAI1 is activated and the cell membrane voltage is depolarized due to Ca^{2+} influx, this influx is limited as the driving force is weakened by the electrochemical gradient. At this time, $K_{Ca}3.1$ is activated by the depolarized cell

membrane voltage. Moreover, $K_{Ca}3.1$ is activated by increased calcium in the cell, and the cell membrane voltage is repolarized to maintain continuous calcium influx for immune cell activation [7,9,10].

Mangosteen (*Garcinia mangostana*) is a tropical fruit grown in Southeast Asia. Mangosteen juice is used as a folk remedy to relieve dehydration, dysentery, and diarrhea [11]. The skin of mangosteen contains several xanthone series. Thus far, 68 xanthone-type compounds have been found in mangosteen, including α, β, and γ-mangostin [12]. Among them, α-mangostin is known to be the most important component. Since α-mangostin was first identified by Schmid in 1855, various pharmacological effects such as anticancer, antiviral, and antioxidant activity have been proven by several researchers worldwide over the past decades [13-15]. Several reports have also demonstrated the anti-inflammatory effect of α-mangostin [16-19]. As described above, intracellular calcium signals are important for immune cell activity; however, there are no studies related to ion channels, except for that on α-mangostin conducted by Tomohiro in 2008 [20]. Therefore, this study aimed to confirm the pharmacological effect of α-mangostin on Ca^{2+} and K^+ channels associated with calcium signaling in immune cells.

Materials & Methods

Cell culture

Human embryonic kidney 293 T (HEK293T) and Jurkat T cells were purchased from the American Type Culture Collection (Manassas, VA, USA). HEK293T cells were cultured in a 10% CO_2 incubator at 37 °C in Dulbecco's modified Eagle's medium (DMEM, Welgene, Gyeongsan, Korea). The culture medium contained 10% fetal bovine serum (FBS, Welgene) and 1% penicillin/streptomycin (P/S, Hyclone). Jurkat T cells were cultured in a 5% CO_2 incubator at 37 °C, in RPMI1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10% FBS and 1% P/S.

Transient transfection

To measure the ORAI1 current, HEK293T cells were co-transfected with the human ORAI1 (hORAI1) and human STIM1 (hSTIM1) vector, which were purchased from Origene Technologies (Rockville, MD, USA). Transfection was performed using Turbofect (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol, and green fluorescence protein (pEGFP-N1, Life Technologies) was injected at a 10:1 ratio for labeling of transfected cells.

Cell cytotoxicity

Cell viability was determined using the Cell Counting Kit 8 (CCK-8). Sample preparation and analysis were performed according to the manufacturer’s protocol. Jurkat T cells

were prepared and 2 × 10^4 cells/well were seeded in a 96-well microtiter plate, treated with cape jasmine (*Gardenia jasminoides*; GJ) 70% ethanol extract (GJ_{EtOH}) 0.001 mg/mL to 1 mg/mL, GJ_{H2O}: 0.001 mg/mL to 0.1 mg/mL, GJ_{BuOH}: 0.001 mg/mL to 0.1 mg/mL, GJ_{H2O}: 0.001 mg/mL to 0.1 mg/mL; and incubated for 72 h. Following treatment, 10 μL of CCK-8 per 100 μL of culture medium was added into each well, incubated for 3 h at 37 °C, and the absorbance measured at 450 nm.

Electrophysiology

ORAI1 was measured using transiently transfected HEK293T cells, and the $K_V1.3$ current in Jurkat T cells was measured directly. A stable cell line, in which the corresponding ion channels were overexpressed, was used for $K_{Ca}3.1$. Recording and analysis of the whole-cell patch clamp for I_{ORAI1} has been previously reported [21]. The composition of the extracellular fluid for recording $K_V1.3$ current (I_{KV}) and $K_{Ca}3.1$ current (I_{KCa}) was 145 mM NaCl, 3.6 mM KCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 mM glucose, 1.3 mM $CaCl_2$, and 1 mM $MgCl_2$; pH 7.4 adjusted with NaOH. The composition of the internal solution was 5 mM NaCl, 140 mM KCl, 10 mM HEPES, 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 2 mM Mg-ATP, 4.37 mM $CaCl_2$, and 0.5 mM $MgCl_2$; pH 7.2, adjusted with KOH. The intracellular calcium concentration for recording I_{KCa} was titrated to 1 μM and calculated using WEBMAXC (Stanford University, https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcS.htm). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA. Stock solutions and were prepared in dimethyl sulfoxide (DMSO). All stock solutions were

131 stored at -20˚C.

132

133 **Cytokine assay**

134 Jurkat T cells were stimulated with anti-CD3 (Peprotech, Rocky Hill, NJ) and anti-CD28
135 (Peprotech) to induce the secretion of interleukin-2 (IL-2). Briefly, 50 μL/well of anti-CD3
136 at a concentration of 5 μg/mL was added to a 96-well plate, incubated at 37 °C for 3 h,
137 and washed three times with Dulbecco's phosphate-buffered saline (DPBS). Jurkat T
138 cells were seeded at a density of 5×10^5 cells/well. Thereafter 2 μg/mL anti-CD28 was
139 added into each well and cultured in a 5% CO_2 incubator at 37 °C for 72 h. The culture
140 solution was subsequently collected and diluted 1:3 with DMEM. The total amount of IL-
141 2 secreted by Jurkat T cells was measured using the IL-2 ELISA kit (Peprotech)
142 according to the manufacturer's protocol.

143

144 **Fura-2 Ca^{2+} imaging**

145 $[Ca^{2+}]_i$ was measured using the fluorescent Ca^{2+} indicator fura-2 acetoxymethyl ester
146 (Fura-2 AM; Thermo Fisher Scientific). Jurkat T cells were incubated with normal
147 Tyrode (NT) solution (145 mM NaCl, 10 mM HEPES, 5 mM glucose, 3.6 mM KCl, 2 mM
148 $CaCl_2$, and 1 mM $MgCl_2$; pH 7.4, adjusted with NaOH) containing 2 μM Fura-2 AM for
149 30 min at 37 °C and subsequently washed twice with NT.

150

151 **Statistical analysis**

Data analysis was carried out using GraphPad prism 6.0 (GraphPad) and Origin 8.0 (Microcal). Data were expressed as the mean ± standard error of the mean (SEM). Bonferroni multiple comparison analysis was used following a one-way analysis of variance (ANOVA) multiple concentrations of components and IL-2 analysis. A p-value was <0.05 was considered statistically significant.

Results

Inhibitory effect of α-mangostin on ORAI1

We measured I_{ORAI1} using the whole-cell patch clamp technique following hORAI1 overexpression in HEK293T cells by transient transfection to determine whether α-mangostin inhibits I_{ORAI1}. Calcium stored in the ER to induce I_{ORAI1} activity was depleted in IP_3 contained in the pipette solution. When the induced current was stable, it was treated with α-mangostin to confirm its inhibitory effect. α-Mangostin inhibited I_{ORAI1} in a concentration-dependent manner (Fig. 1A-B). Fig. 1A shows the chart trace of I_{ORAI1}, and Fig. 1B shows the inhibition by α-mangostin as a current-voltage relationship curve. Fig. 1C shows the I_{ORAI1} half-maximal inhibitory concentrations (IC_{50}) of α-mangostin, with an IC_{50} of 1.27 ± 1.144 μM. To confirm that ORAI1 inhibits intracellular calcium signaling, we measured the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Jurkat T cells using the fluorescent dye Fura-2. To activate ORAI1, the ER was depleted using thapsigargin, an SERCA pump inhibitor. Upon changing from 0 Ca^{2+} to 2 mM Ca^{2+} following thapsigargin treatment, calcium influx by ORAI1 occurs. When calcium influx was maintained at a constant level, it was treated with 1 μM and 3 μM α-mangostin (Fig. 2A); $[Ca^{2+}]_i$ was inhibited by α-mangostin by 23.90 ± 12.501% and 77.14 ± 6.600%, respectively (Fig. 2B).

Inhibitory effect of α-mangostin on KV1.3 and KCa3.1, the regulators of calcium signaling in immune cells

We measured the activity of potassium channels, which is necessary for maintaining the

membrane driving force for sufficient calcium influx via ORAI1, and examined whether α-mangostin could inhibit it. It has been reported that $K_V1.3$ expression increases when the T-cell receptors (TCRs) of T-lymphocytes are stimulated [22]. Therefore, Jurkat T cells were treated with 3 μg/mL anti-CD3 (Peprotech) for 24 h, and I_{KV} subsequently measured using the whole-cell patch clamp technique. When the cell membrane voltage was changed from -120 mV to +60 mV for 500 ms, I_{KV} increased from -60 mV (Fig. 3A). It was confirmed that I_{KV} decreased by 41.38 ± 6.191% at 3 μM (Fig. 3B) upon α-mangostin treatment when the magnitude of the current remained stable. Finally, it was confirmed that the current was completely reduced by treatment with PAP-1, an inhibitor of $K_V1.3$ (Fig. 3B). In addition, we examined the effect of α-mangostin on $K_{Ca}3.1$, which is activated when calcium signals are generated in T cells [23]. However, in Jurkat T cells, $K_{Ca}3.1$ expression was extremely low and the measured current was extremely small, which is not suitable for analyzing the inhibitory effect of α-mangostin. Therefore, whole-cell patch clamp was performed using the stable cell line overexpressing $K_{Ca}3.1$. As I_{KCa} is activated by an increase in intracellular calcium concentration, the intracellular calcium concentration was fixed at 1 μM. When I_{KCa}, activated by a fixed calcium concentration, was maintained at a constant level, it was inhibited by α-mangostin treatment in a concentration-dependent manner. As shown in Fig. 4A, α-mangostin inhibited I_{KCa} by 28.28 ± 5.412 % and 51.16 ± 5.385% at concentrations of 1 μM and 3 μM, respectively. Normalized data are summarized in Fig. 4B.

Inhibitory effect of α-mangostin on IL-2 production in Jurkat T cells stimulated by anti-CD3/anti-CD28

Finally, we investigated whether α-mangostin inhibits cytokine secretion. First, the cytotoxicity of α-mangostin was evaluated. Following α-mangostin treatment of Jurkat T lymphocytes, cell viability was measured. Most cells died When treated with α-mangostin at a concentration of 10 μM, most cells died; however, >80% survived when treated with α-mangostin at a concentration of 3 μM (Fig. 5A). Thus, we subsequently analyzed whether the cytokine secretion of Jurkat T cells was inhibited at a maximum concentration of 3 μM or less. Consequently, α-mangostin inhibited IL-2 production in a concentration-dependent manner, and the highest inhibition rate (80.14 ± 3.987%) was observed at 3 μM (Fig. 5B).

Discussion

In this study, we investigated whether α-mangostin inhibits the calcium ion channels that cause intracellular calcium increase in immune cells and the potassium channels that play a crucial role in regulating the cell membrane voltage, and whether cytokine production can be suppressed by it. Several studies have reported the anti-inflammatory effects of α-mangostin. Inducible nitrogen oxide synthase (iNOS) is an enzyme that causes inflammation, and α-mangostin and γ-mangostin have been reported to inhibit the production of NO and PEG2 in LPS-induced RAW264.7 cells through inhibition of iNOS expression [16,17]. In 2013, Falbiola reported that α-mangostin inhibited the secretion of inflammatory mediators in various human cell lines, thereby exhibiting anti-inflammatory effects [18]. Immune cells generate calcium signals due to antigen stimulation and trigger various immune responses. In 2012, Chin reported that α-mangostin inhibited degranulation induced by A23187 and PMA in bone marrow mast cells, inhibited the production of IL-6, prostaglandin D_2 (PGD_2), and leukotriene, and reduced the expression of COX-2 mRNA, thereby having anti-allergic effects [19]. As such, the anti-inflammatory benefits of α-mangostin by various mechanisms have been reported; however, only a few studies have focused on the ion channels related to the generation of calcium signals.

Effects of α-mangostin on the calcium channel (ORAI1)

TCR stimulation by antigens activates T cells and triggers calcium signaling. This increased calcium is known to be involved in T cell proliferation and the production and

237 secretion of cytokines [7,24]. TCR stimulation activates ORAI1, and the intracellular
238 calcium increase by this process binds to Ca^{2+}-modulated protein (calmodulin) to
239 generate various sub-signals. Calcium-calmodulin activates the nuclear factor for
240 activated T cells (NFAT), thereby regulating cytokine production and immune cell
241 proliferation [25,26]. Ion channels that are known to generate calcium signals in immune
242 cells include ORAI, transient receptor potential (TRP) channels, and voltage-dependent
243 Ca^{2+} channels (TRP); however, the role of ion channels other than ORAI1 and STIM1 is
244 controversial among researchers [7,27-29]. Functional impairment of ORAI1 or STIM1
245 deficiency is known to cause severe combined immunodeficiency (SCID) in mouse
246 models and human patients, and various functional problems have been
247 reported [2,7,28].

248 Therefore, I_{ORAI1} was measured using HEK293T cells overexpressing hORAI1 and
249 hSTIM1 to observe the pharmacological effects of α-mangostin. α-Mangostin
250 suppressed most ORAI1 currents at a concentration of 10 μM, and demonstrated
251 extremely high potency with an IC_{50} of 1.27 ±1.144 μM (Fig 1). In Jurkat T cells, 3 μM α-
252 mangostin inhibited thapsigargin-induced store operated Ca^{2+} entry (SOCE) by
253 approximately 77.14 ± 6.600% (Fig 2). The same concentration of α-mangostin inhibited
254 ORAI1 by approximately 70.51 ± 6.185%. Considering that this is quite similar to the
255 inhibition rate of calcium signaling, it appears that α-mangostin inhibits intracellular
256 calcium signaling by inhibiting ORAI1. Degranulation in mast cells is also a response to
257 intracellular calcium signals. A previous study reported that calcium signaling by
258 antigens in RBL-2H3 cells was inhibited by α-mangostin; this appears to be due to the
259 inhibitory effect of α-mangostin on ORAI1 [20].

Effects of α-mangostin on K^+ channels ($K_V1.3$ and $K_{Ca}3.1$)

When Ca^{2+}, which is a divalent cation, continuously flows into the cell, the cell membrane voltage is depolarized, and the driving force to introduce calcium is weakened. During this time, $K_V1.3$ and $K_{Ca}3.1$ maintain the driving force for calcium influx by maintaining a negative cell membrane potential in T cells [9]. They are known to be regulated by immunological synapses after TCR activation [7]. Inhibition of $K_V1.3$ and $K_{Ca}3.1$ has been reported to attenuate calcium, and consequently, are involved in the activity and proliferation of T cells as well as the production and proliferation of cytokines [23,30,31]. KV1.3 and KCa3.1 are typically mentioned as ion channels that regulate calcium influx in T cells, and research is being conducted to develop new immunosuppressive agents using them [23,32]. In addition to the inhibition of ORAI1, whether α-mangostin can inhibit $K_V1.3$ and $K_{Ca}3.1$, which controls calcium influx, were observed. As a result, 3 μM α-mangostin, that could inhibit 70% ORAI1 by approximately 70%, inhibited $K_V1.3$ by $41.38 \pm 6.191\%$, and $K_{Ca}3.1$ by $51.16 \pm 5.385\%$. Therefore, the inhibitory effect of α-mangostin on calcium influx appears to contribute to the inhibition of ORAI1,as well as the inhibition of the potassium channels that regulate it.

Inhibitory effects of α-mangostin on cytokine production

Anti-CD3/anti-CD28 stimulation generates calcium signals and activates NFAT to promote T cell proliferation and production of IL-2. Thus, we investigated whether α-mangostin, with its inhibitory effects on ORAI1 and calcium signaling, could inhibit IL-2 production in Jurkat T cells. Prior to confirming the inhibition of IL-2 secretion,

cytotoxicity of α-mangostin was first evaluated, and most of the cells died at a concentration of 10 μM. Therefore, we examined the inhibitory effects of α-mangostin on IL-2 production using concentrations up to 3 μM at which cells survived. At a concentration of 3 μM α-mangostin inhibited IL-2 secretion of Jurkat T cells stimulated with anti-CD3 and anti-CD28 by 80.14 ± 3.987% at a concentration of 3 μM. The results obtained earlier confirmed that 3 μM α-mangostin inhibited ORAI1 by approximately 70% and SOCE calcium signaling by approximately 77%, which is similar to the inhibition of IL-2 production observed with the same concentration of α-mangostin. Moreover, according to the results of a previous study on the inhibitory effects of α-mangostin on the secretion of cytokines in human peripheral blood mononuclear cells, α-mangostin was reported to inhibit IL-2 secretion by concanavalin A (ConA) stimulation. ConA is known to play a role in producing IL-2 by stimulating TCRs, leading to Ca^{2+} signaling and NFAT activation [33,34]. This evidence indirectly supports the inhibitory effect on calcium signaling identified in this study.

Conclusions

Most of the existing studies of α-mangostin have been molecular investigations, focusing on the sub-signaling mechanisms. Only a few studies related to the underlying mechanisms such as ion channels and calcium signal regulation exist. We investigated whether α-mangostin could regulate the activity of ion channels related to calcium signaling, an underlying mechanism. It was possible to obtain similar results for the inhibition of ion channels and calcium signaling as well as the inhibition of cytokine production. Therefore, this study revealed the effects of inhibiting the activity of immune cells by inhibiting the calcium ion channels, which play an important role in T cell

activity.

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Figure Legends

Figure 1 Inhibitory effects of α-mangostin on ORAI1 current (I_{ORAI}) in HEK293T cells co-expressed with ORAI1 and STIM1.

A. Representative chart trace documenting I_{ORAI1} inhibition of α-mangostin. Inhibitory effects of 0.1 to 10 μM α-mangostin and BTP2

B. The current (I)-voltage (V) relationship curve of I_{ORAI1} suppressed by each concentration of α-mangostin. (1) control, (2) α-mangostin 0.1 μM, (3) 0.3 μM, (4) 0.6 μM, (5) 1 μM, (6) 3 μM, (7) 10 μM ($n \leq 8$)

C. Concentration-dependent I_{ORAI1} inhibition by α-mangostin at -120 mV, and fitted dose-response curves. Data are expressed as the mean ± SEM.

Figure 2 Store operated Ca^{2+} entry (SOCE) induced by thapsigargin in Jurkat T cells and the inhibitory effects of α-Mangostin on SOCE.

SOCE was induced with thapsigargin, and the inhibitory effects were confirmed by treatment with 1 μM and 3 μM α-mangostin. BTP2 was used as a positive control.

Trace (n = 18) shows changes in the intracellular calcium signal by α-mangostin in Jurkat T cells stimulated with thapsigargin.

B. Average value at the stabilization point of the calcium signal. The average value of points marked with †. Data are expressed as the mean ± SEM. **** $p < 0.0001$

Figure 3 Inhibitory effects of α-mangostin on $K_V1.3$ current (I_{KV}) measured in Jurkat T cells.

A. The representative current (I)-voltage (V) relationship curve showing the inhibition of I_{KV} by α-mangostin ($n \leq 7$)

B. Average value of current measured at +50 mV. Current without α-mangostin treatment and reduced current with α-mangostin treatment were compared. Data are expressed as the mean ± SEM. **** $p < 0.0001$

Figure 4 Inhibitory effects of α-mangostin on $K_{Ca}3.1$ current (I_{KCa}) measured in HEK293T cells overexpressed with $K_{Ca}3.1$.

A. The representative current (I)-voltage (V) relationship curve showing the inhibition of I_{KCa} by α-mangostin ($n \leq 7$)

B. Average value of current measured at +50 mV. Current without α-mangostin treatment and reduced current with α-mangostin treatment were compared. Data are expressed as the mean ± SEM. **** $p < 0.0001$

Figure 5 Inhibitory effects of α-mangostin on IL-2 secretion in Jurkat T cells co-stimulated by CD3 and CD28.

A. Cell viability analyzed after 72 h of treatment with α-mangostin in Jurkat T cells. An equal volume of DMSO was used to compare the effect of the solvent (n = 3).

B. Concentration-dependent inhibitory effects of α-mangostin in Jurkat T cells co-stimulated with anti-CD3/anti-CD28. BTP2 was used as a positive control. Data are expressed as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$

Figure 1

Inhibitory effects of α-mangostin on ORAI1 current (I_{ORAI}) in HEK293T cells co-expressed with ORAI1 and STIM1.

A. Representative chart trace documenting I_{ORAI1} inhibition of α-mangostin. Inhibitory effects of 0.1 to 10 μM α-mangostin and BTP2 B. The current (I)-voltage (V) relationship curve of I_{ORAI1} suppressed by each concentration of α-mangostin. (1) control, (2) α-mangostin 0.1 mM, (3) 0.3 mM, (4) 0.6 mM, (5) 1 mM, (6) 3 mM, (7) 10 mM ($n \leq 8$) C. Concentration-dependent I_{ORAI1} inhibition by α-mangostin at -120 mV, and fitted dose-response curves. Data are expressed as the mean ± SEM.

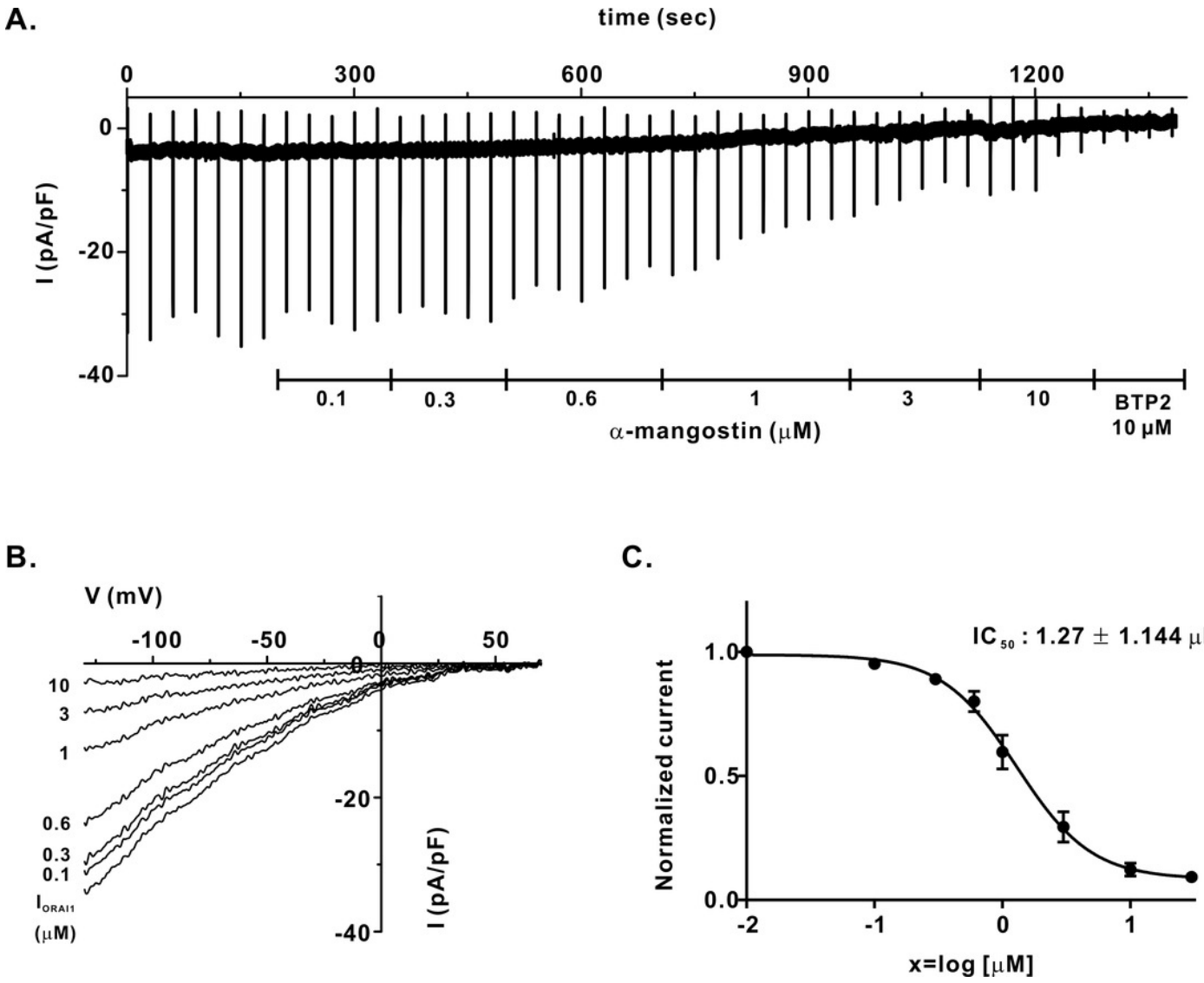
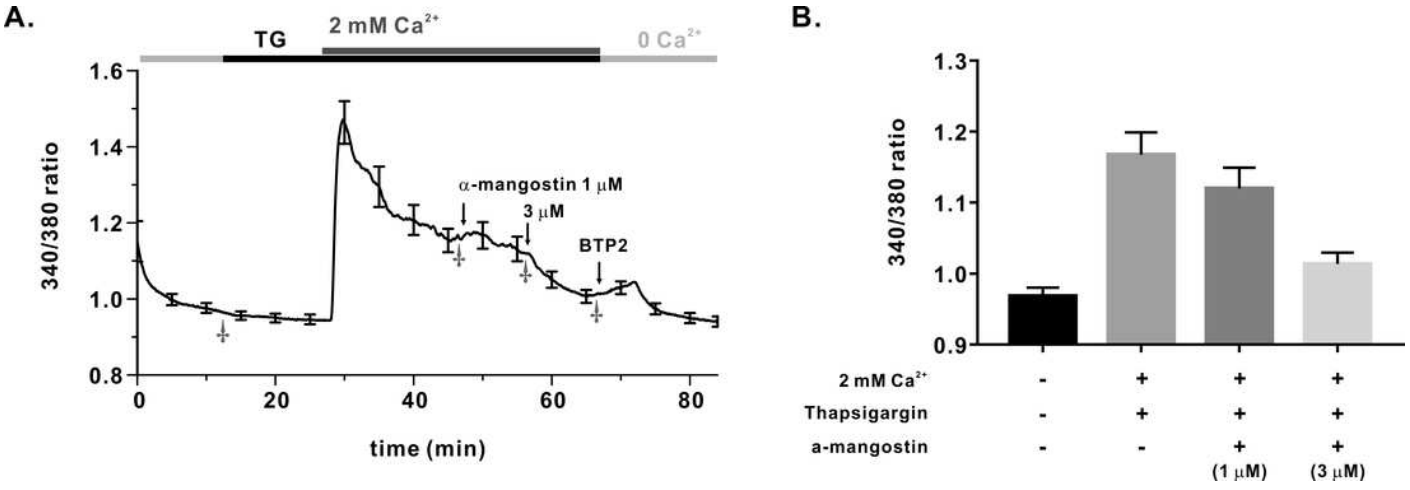

A.
time (sec)
0
300
600
900
1200
0
-20
-40
I (pA/pF)
0.1
0.3
0.6
1
3
10
BTP2
10 μM
α-mangostin (μM)
B.
V (mV)
-100
-50
0
50
0
-20
-40
10
3
1
0.6
0.3
0.1
I ORAI1
(μM)
I (pA/pF)
C.
IC 50 : 1.27 ± 1.144 μM
1.0
0.5
0.0
Normalized current
-2
-1
0
1
x=log [μM]

Figure 2

Store operated Ca^{2+} entry (SOCE) induced by thapsigargin in Jurkat T cells and the inhibitory effects of α-Mangostin on SOCE.

SOCE was induced with thapsigargin, and the inhibitory effects were confirmed by treatment with 1 μM and 3 mM α-mangostin. BTP2 was used as a positive control. A. Average trace (n = 18) shows changes in the intracellular calcium signal by α-mangostin in Jurkat T cells stimulated with thapsigargin. B. Average value at the stabilization point of the calcium signal. The average value of points marked with †. Data are expressed as the mean ± SEM. **** $p <$ 0.0001



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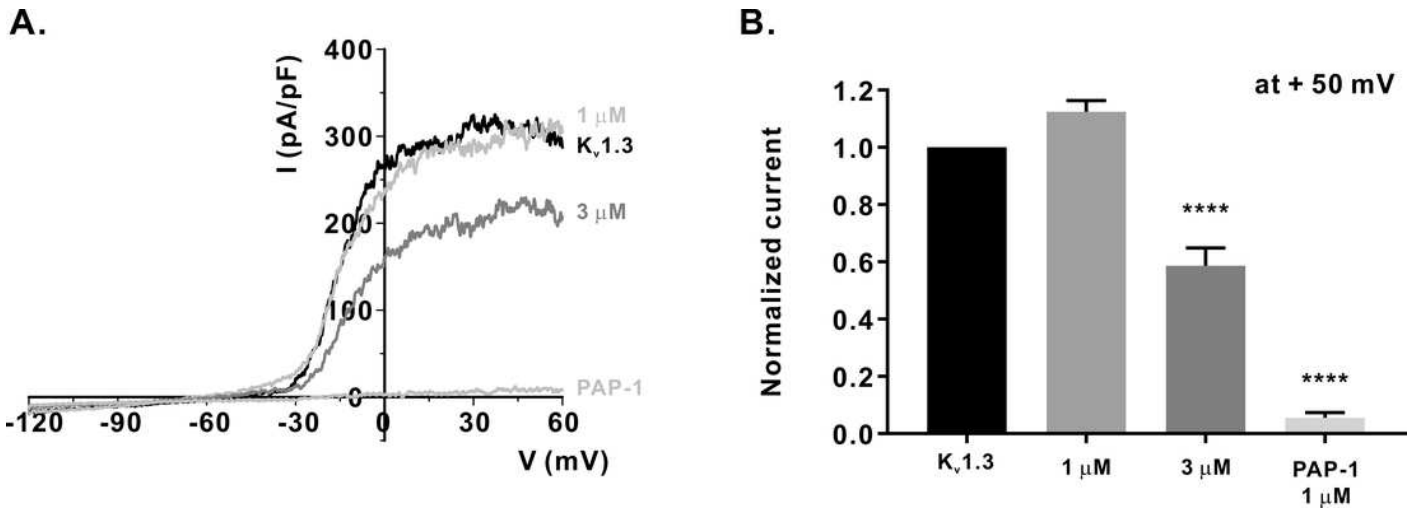

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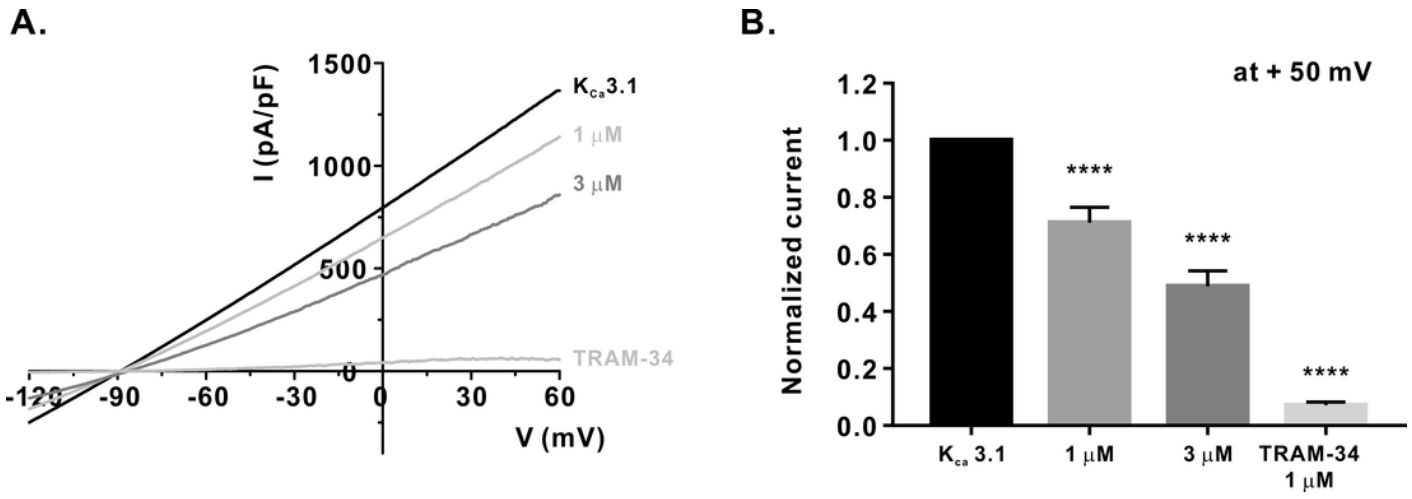
A. The representative current (I)-voltage (V) relationship curve showing the inhibition of I_{KCa} by α-mangostin (n £7) B. Average value of current measured at +50 mV. Current without α-mangostin treatment and reduced current with α-mangostin treatment were compared. Data are expressed as the mean ± SEM. **** $p < 0.0001$



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