BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology, 2013, **112**, 83–89 Doi: 10.1111/j.1742-7843.2012.00929.x

The Role of Waixenicin A as Transient Receptor Potential Melastatin 7 Blocker

Byung J. Kim[1], Joo H. Nam[2], Young K. Kwon[1], Insuk So[3] and Seon J. Kim[4]

[1]Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Yangsan, Korea, [2]Department of Physiology, Dongguk University College of Medicine, Kyungju, Korea, [3]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea, and [4]Center for Bio-Artificial Muscle and Department of Biomedical Engineering, Hanyang University, Seoul, Korea

(Received 19 March 2012; Accepted 8 August 2012)

Abstract: Transient receptor potential melastatin 7 (TRPM7) plays a role in a number of physiological and pharmacological functions in variety of cells. The aim of this study was to clarify the role for TRPM7 channels and the effect of waixenicin A on the pacemaking activity of interstitial cells of Cajal (ICCs) and on the cell viability of the human gastric and breast adenocarcinoma cell lines, AGS and MCF-7, respectively. Waixenicin A decreased the amplitude of pacemaker potentials in cultured ICC clusters and inhibited TRPM7 currents, but had no effect on Ca^{2+}-activated Cl^- conductance (ANO1). Furthermore, waixenicin A was found to inhibit the growth and survival of AGS and MCF-7 cells. These findings indicate that TRPM7 channel modulates intestinal motility and regulates the pathophysiology of human gastric and breast adenocarcinoma cells. These findings suggest that TRPM7 channel be considered a potential target for the treatment of gut motor disorders and gastric and breast cancer.

Transient receptor potential (TRP) channels were first cloned from *Drosophila* species and constitute a superfamily of proteins that encode a diverse group of Ca^{2+}-permeable nonselective cation channels (NSCCs) [1]. Based on their amino acid sequences, the TRP superfamily can be divided into 7 subfamilies: the TRPC ('Canonical') family, the TRPM ('Melastatin') family, the TRPV ('Vanilloid') family, the TRPP ('Polycystin') family, the TRPML ('Mucolipin') family, the TRPA ('Ankyrin') family and the TRPN ('NOMPC') family [2]. TRPM7, a member of TRPM ion channel subfamily, is a widely expressed bifunctional protein with both ion channel and α-kinase domains [3,4].

Interstitial cells of Cajal (ICCs) are the pacemaking cells in gastrointestinal (GI) muscles that generate the rhythmic oscillations in membrane potential known as slow waves [5–7], and research into the biology of ICCs provides exciting new opportunities to understand the aetiologies of diseases that have long eluded comprehension. We previously suggested that TRPM7 is a primary molecular candidate for the NSCC responsible for ICC pacemaking activity in the murine small intestine [8], and thus, we view TRPM7 as a promising new target for the pharmacological treatment of GI motility disorders. Furthermore, others have suggested that human gastric and breast adenocarcinoma cells express TRPM7 channel and its expression is essential for cell survival, and thus, this TRPM7 channel is also a potential therapeutic target in gastric and breast cancer [9,10].

Recently, Zierler *et al.* [11] found that waixenicin A (a xenicane diterpenoid isolated from the Hawaiian soft coral *S. edmondsoni*) is a potent and relatively specific inhibitor of TRPM7 ion channels and suggested that it may have cancer-specific therapeutic potential. However, the effects of waixenicin A on ICC pacemaking activity and on human gastric and breast adenocarcinoma cells have not been investigated. Therefore, we undertook this investigation of the effects of waixenicin A on ICC pacemaking activity and on the cell viability of the human gastric and breast adenocarcinoma cells.

Materials and Methods

Materials. Waixenicin A was provided by the Huienna Chemical Export and Import Company (Tian Jin, China). All other agents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of cells and cell cultures. The mice used in this study were treated according to the Guidelines for the Care and Use of Animals issued by Pusan National University. Balb/c mice (8–13 days old) of either sex were anaesthetized with ether and euthanized by cervical dislocation. Small intestines from 1 cm below the pyloric ring to the caecum were removed and opened along the mesenteric border, and luminal contents were washed out with Krebs–Ringer bicarbonate solution. The tissues were then pinned to the base of a Sylgard dish, and mucosae were removed by sharp dissection. Small strips of intestinal muscle (consisting of both circular and longitudinal muscles) were then equilibrated in Ca^{2+}-free Hank's solution containing 5.36 mM KCl, 125 mM NaCl, 0.34 mM NaOH, 0.44 mM Na_2HCO_3, 10 mM glucose, 2.9 mM sucrose and 11 mM *N*-(2-hydroxyethyl) piperazine-*N*-2-ethanesulfonic acid (HEPES) for 30 min., and the cells so obtained were dispersed in a solution containing 1.3 mg/mL collagenase (Worthington Biochemical, Lakewood, NJ, USA), 2 mg/mL

Authors for correspondence: Insuk So, Department of Physiology, Seoul National University College of Medicine, 28 Yongon-dong, Chongro-gu, Seoul 110-799, Korea (fax +82 2 763 9667, e-mail insuk@plaza.snu.ac.kr).

Seon J. Kim, Center for BioArtificial Muscle and Department of Biomedical Engineering, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea (fax +82 2 2291 2320, e-mail sjk@hanyang.ac.kr).

bovine serum albumin (Sigma-Aldrich), 2 mg/mL trypsin inhibitor (Sigma-Aldrich) and 0.27 mg/mL ATP. Cells were then plated onto sterile glass coverslips coated with 2.5 mg/mL murine collagen (Falcon/BD Biosciences, San Jose, CA, USA) in 35-mm-diameter culture dishes and cultured at 37°C in a 95% O_2–5% CO_2 incubator in smooth muscle growth medium (SMGM; Clonetics, San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Franklin Lakes, NJ, USA) and 5 ng/mL murine stem cell factor (SCF; Sigma-Aldrich). ICCs were identified immunologically by incubation with anti-*c-kit* antibody [phycoerythrin (PE)-conjugated rat antimouse *c-kit* monoclonal antibody; eBioscience, San Diego, CA, USA] at a dilution of 1:50 for 20 min. As the morphology of ICCs differed from those of other cell types in the culture, it was possible to identify them by phase contrast microscopy after incubation with anti-*c-kit* antibody.

Cells. Human gastric (AGS) and breast (MCF-7) adenocarcinoma cell lines were used in this study. The AGS and MCF-7 cell lines were established at the Cancer Research Center, College of Medicine, Seoul National University, South Korea and propagated in RPMI-1640 medium (Gibco-BRL) supplemented with 10% heat-inactivated foetal bovine serum and 20 μg/mL penicillin and streptomycin in a 5% CO_2 atmosphere at 37°C.

Patch-clamp experiments. Whole-cell configuration patch-clamp experiments were performed at room temperature (22–25°C). AGS cells were transferred to a small chamber on an inverted microscope stage (IX70; Olympus, Japan), and constantly perfused with a solution containing (mM) KCl 2.8, NaCl 145, $CaCl_2$ 2, glucose 10, $MgCl_2$ 1.2 and HEPES 10 (adjusted to pH 7.4 with NaOH). The pipette solution contained (mM) Cs-glutamate 145, NaCl 8, Cs-2-bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid 10 and HEPES-CsOH 10 (adjusted to pH 7.2 with CsOH). An Axopatch I-D (Axon Instruments, Foster City, CA, USA) was used to amplify membrane currents and potentials. pCLAMP software v.9.2 and digidata 1322A (Axon Instruments) were used for data acquisition and to apply command pulses. Results were analysed using pClamp and Origin software (Microcal Origin version 6.0; Microcal Software, Northampton, MA, USA).

Ca^{2+}-activated Cl^- channel expression in HEK-293 cells. HEK-293 cells transfected with pEGFP-N1-mANO1 construct were grown on glass coverslips in Dulbecco's modified Eagle medium supplemented with 10% foetal bovine serum. Whole-cell patch-clamp experiments were performed at 21–25°C 24 hr after induction using cells grown on glass coverslips. The bath solution contained 146 mM N-methyl-D-glucamine (NMDG)-Cl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES at pH 7.4 (titrated with NMDG-OH). The pipette solution contained 134 mM NMDG-Cl, 1 mM $MgCl_2$, 3 mM MgATP, 10 mM EGTA, 7 mM $CaCl_2$ and 5 mM HEPES at pH 7.2 (titrated with NMDG-OH). The pipette solution had a free Ca^{2+} activity of 370 nM according to the web version of Winmax (C. Patton, Stanford University, http://www.stanford.edu/~cpatton/webmaxcS.htm).

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Cell viability was assessed using a MTT assay. AGS cells were seeded into each well of 12-well culture plates and then cultured in RPMI-1640 supplemented with other reagents for 72 hr. After incubation, 100 μL of MTT solution (5 mg/mL in phosphate-buffered saline [PBS]) was added to each well, and the plates were incubated at 37°C for 4 hr. After removing the supernatant and shaking with 200 μL of dimethyl sulfoxide (Jersey Lab Supply, Livingston, NJ, USA) for 30 min., absorbances were measured at 570 nm. All experiments were repeated at least three times.

Statistical analysis. Results are expressed as means ± S.E.M.s. The analysis was conducted using one-way analysis of variance (ANOVA) in GraphPad Prism version 5. Significance was determined using the Newman Keuls post hoc test. Statistical significance was accepted for p-values of < 0.05.

Results

Waixenicin A decreased the amplitudes of pacemaker potentials in cultured ICC clusters.

The patch-clamp technique was tested on ICCs that formed network-like structures in culture (2−4 days). Spontaneous rhythms were routinely recorded from cultured ICCs under current and voltage-clamp conditions, and ICC within networks displayed more robust electrical rhythms. Tissue-like spontaneous slow waves have also been recorded from these cells [12]. To investigate the relationship between waixenicin A and the modulation of pacemaker activity in ICCs, we examined the effects of waixenicin A on pacemaker potentials. In current clamp mode ($I = 0$), ICCs had a mean resting membrane potential of −52 ± 2 mV and produced electrical pacemaker potentials (n = 25) of frequency 16 ± 2 cycles/min and amplitude 30 ± 1 mV (n = 25). The addition of waixenicin A (1–10 μM) decreased the amplitudes of pacemaker potentials but had no effect on resting membrane potentials (fig. 1A). Amplitudes were 30.1 ± 3 mV at 1 μM of waixenicin A, 16.1 ± 2 mV at 5 μM and 10.0 ± 2 mV at 10 μM (fig. 1B, n = 4). Waixenicin A (0.1–10 μM) effectively inhibited the amplitudes of ICC pacemaker potentials with an IC_{50} on the pacemaker activity of ICC clusters of about 4.6 μM (fig. 2A). To investigate the effect of waixenicin A on pacemaking activity, we performed whole-cell voltage-clamp recordings on cultured single ICCs, which were identified using phycoerythrin-bound anti–c-kit antibody. Basal TRPM7 activity is regulated by millimolar levels of intracellular MgATP and Mg^{2+}, and hence, TRPM7 is activated by depletion of intracellular MgATP and Mg^{2+} and inhibited by high concentrations of MgATP and Mg^{2+} [3]. To provide evidence that inhibition by internal Mg^{2+} was involved in this current, various internal Mg^{2+} concentrations were included in the pipette solution and currents were recorded. TRPM7-like currents were elicited by voltage ramps ranging from −100 to +100 mV from a holding potential of −60 mV. After break-in, 2–4 min. was allowed for current amplitudes to reach the steady-state (fig. 2B,C). These currents in ICCs elicited a rapid large increase in outward whole-cell currents but only a slight increase in inward currents in the absence of intracellular Mg^{2+} (fig. 2C). The time course of outward current was plotted at + 100 mV and inward current at −100 mV for same cells (fig. 2C, n = 7). At a lower Mg^{2+} concentration, the amplitude of this current was indeed larger than at a higher concentration. Furthermore, the presence of 3 mM Mg^{2+} in the pipette solution significantly inhibited this current (n = 5; fig. 2B,C). Perfusing waixenicin A (10 μM) into the bath reduced both inward and outward TRPM7-like currents in single ICCs (n = 5) (fig. 2D). In addition, similar results were obtained in HEK293 cells expressing TRPM7 (fig. 2E). Recently, Zhu *et al.* [13] suggested that Ca^{2+}-activated Cl^- conductance is involved in the

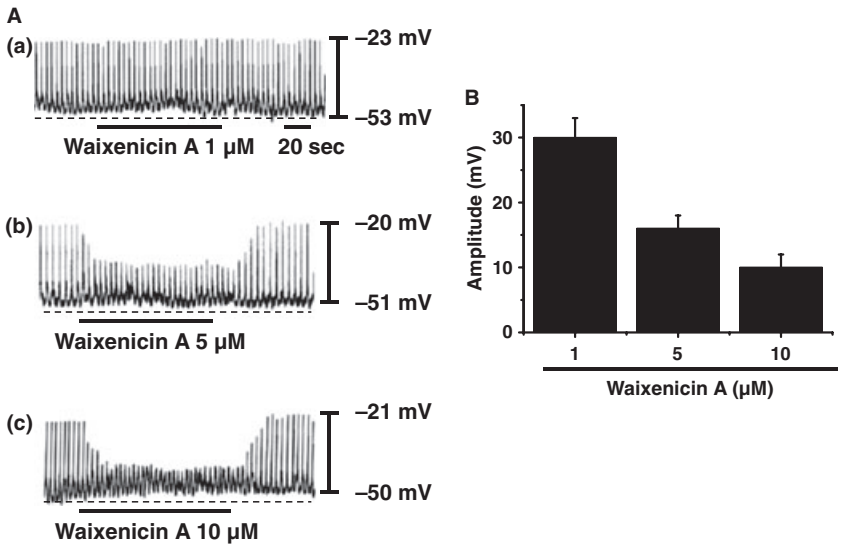

Fig. 1. Waixenicin A decreased the amplitude of pacemaker potentials in cultured ICCs. (A) In current clamp mode ($I = 0$), the addition of waixenicin A (1–10 μM) decreased the amplitude of pacemaker potentials. (B) The histograms summarize amplitude decreases realized on increasing waixenicin A concentrations.

pacemaking activity of ICCs, and another recent study confirmed that ICCs express ANO1 in the GI tract [14]. In addition, ANO1 was recently shown to function as a Ca^{2+}-activated Cl^- channel [15–17], and loss or block of ANO1 was found to block electrical slow waves in intact GI muscles [18]. Therefore, we investigated the effect of waixenicin A on Ca^{2+}-activated Cl^- conductance by over-expressing Ca^{2+}-activated Cl^- channels in HEK293 cells and recording whole-cell currents using patch-clamp techniques. To determine the current-voltage (*I-V*) relationship, we applied a ramp pulse from −100 mV to +100 mV for 2 sec. However, waixenicin A had no effect on Ca^{2+}-activated Cl^- conductance (fig. 2F).

Effects of waixenicin A on AGS cells.

In previous studies, we found that human gastric adenocarcinoma (AGS) cells express the transient receptor potential melastatin 7 (TRPM7) channel, which is known to be essential for cell survival and has been suggested as a pharmacological target for the treatment of gastric cancer [9]. Therefore, we investigated whether waixenicin A influences AGS cell survival. After incubation with waixenicin A at 10, 30 or 50 μM in the culture medium for 72 hr, AGS cell survival was inhibited by 0.1 ± 0.2%, 28.2 ± 5.2% or 40.3 ± 6.1%, respectively, according to MTT assay results (n = 5; fig. 3A). To confirm the involvement of TRPM7 channel, we investigated the effects of waixenicin A in AGS cells using patch-clamp techniques. Firstly, to provide evidence that inhibition by internal Mg^{2+} is involved in this TRPM7-like current, various internal Mg^{2+} concentrations were included in the pipette solution. Under the lower concentration, the amplitude of this current was indeed larger than at the higher concentration, and the presence of 2 mM Mg^{2+} in the pipette solution significantly inhibited this current (n = 5; fig. 3B). Then, we performed whole-cell voltage-clamp recordings to investigate the effect of waixenicin A in TRPM7-like current in AGS cell. A voltage ramp of +100 mV to −100 mV evoked small inward currents at negative potentials, whereas larger outward currents were evoked at positive potentials, showing that they were outward-rectifying cation currents (n = 5; fig. 3C). However, in the presence of 50 μM waixenicin A, the amplitudes of these currents were inhibited outwardly by 41.2 ± 2.1% and inwardly by 65.1 ± 2.4% (n = 5; fig. 3C).

Effects of waixenicin A on MCF-7 cells.

Guilbert *et al.* [10] suggested that TRPM7 contributes to the proliferative potential of breast cancer (MCF-7) cells. Therefore, we investigated the influence of waixenicin A on MCF-7 survival. After incubation for 72 hr with 10, 30 or 50 μM waixenicin A in culture medium, the number of surviving MCF-7 cells were reduced by 1.0 ± 1.2%, 20.3 ± 6.1% and 55.2 ± 5.2%, respectively, according to MTT assay results (n = 5; fig. 4A). To confirm the involvement of TRPM7 channels in these results, we investigated the effects of waixenicin A in MCF-7 cells using patch-clamp techniques. Firstly, to provide evidence that inhibition by internal Mg^{2+} is involved in the generation of this current, different internal Mg^{2+} concentrations were included in the pipette solution, and currents were recorded. At the lower concentration, the amplitude of this current was indeed larger than at the higher concentration, and the presence of 2 mM Mg^{2+} in the pipette solution significantly inhibited this current (n = 5; fig. 4B). We then performed whole-cell voltage-clamp recordings to investigate the effect of waixenicin A on TRPM7-like currents in MCF-7 cells. A voltage ramp ranging from +100 mV to −100 mV evoked small inward currents at

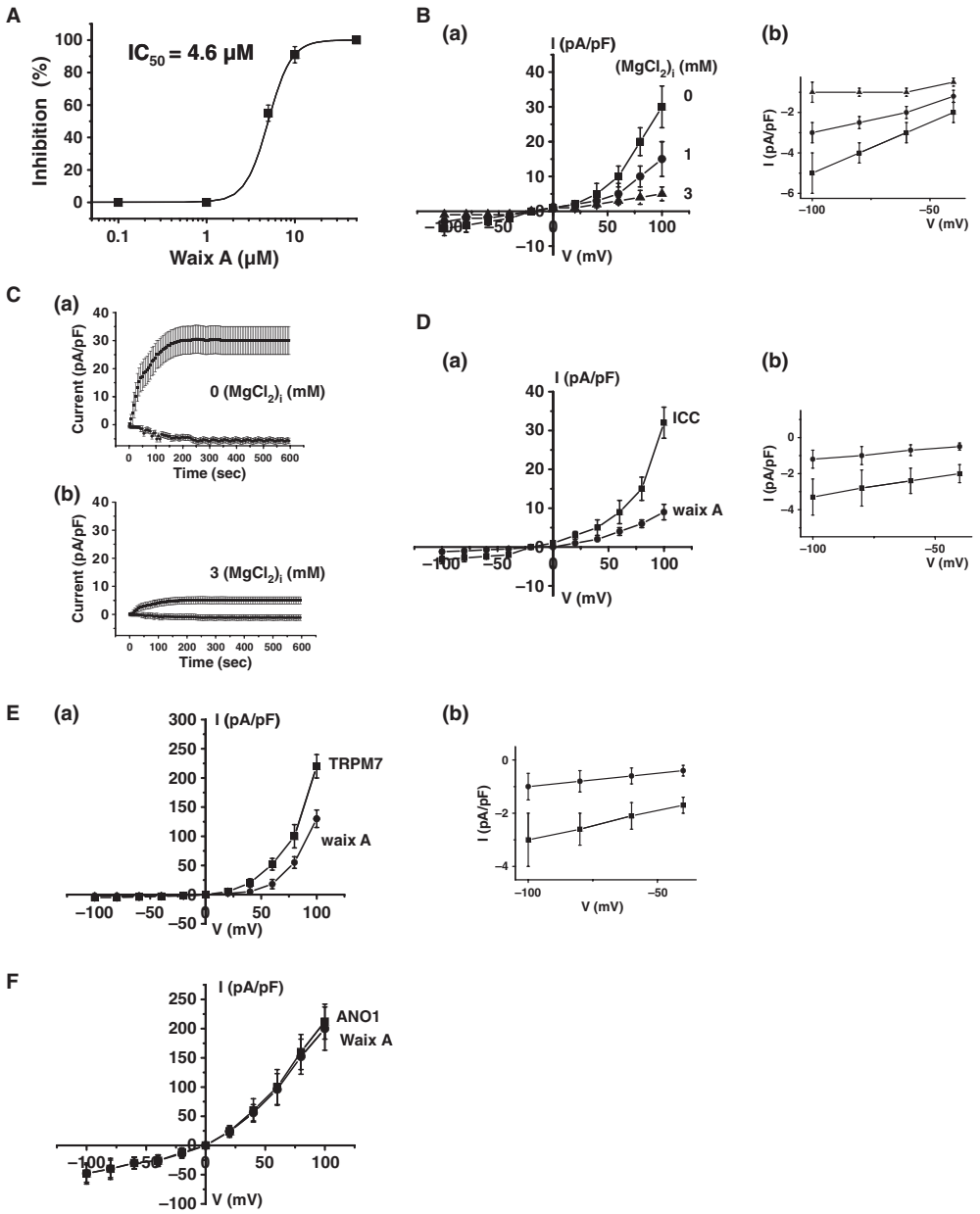

Fig. 2. Effects of waixenicin A on pacemaker potentials in ICC clusters, on TRPM7-like current in single ICCs and on over-expressed TRPM7 or Ca^{2+}-activated Cl^- channels in HEK293 cells. (A) Concentration-dependent inhibition of pacemaker potentials in ICC clusters. The estimated median inhibitory concentration value for waixenicin A was 4.6 μM. (B) (a) Representative TRPM7-like currents in ICCs at 0, 1 or 3 mM $[Mg^{2+}]_i$. A voltage ramp from +100 to −100 mV was applied from a holding potential of −60 mV. (b) Enlargement of the negative mV part of (a). (C) The time course of the experiment in fig. 1B plotted as an outward current at +100 mV and as an inward current at −100 mV in (a) 0 mM $[Mg^{2+}]_i$ and (b) 3 mM $[Mg^{2+}]_i$. (D) (a) Representative TRPM7-like currents in single ICCs at a waixenicin A concentration of 10 μM. (b) Enlargement of the negative mV part of a). (E) (a) Representative I–V relationships of the effect of waixenicin A on TRPM7 currents in HEK293 cells. (b) Enlargement of the negative mV part of (a). (F) Representative trace of the effect of waixenicin A on Ca^{2+}-activated Cl^- currents in HEK293 cells. Waixenicin A had no effect on these currents. A voltage ramp from +100 to −100 mV was applied from a holding potential of −60 mV.

negative potentials, whereas larger outward currents were evoked at positive potentials, showing that they were outward-rectifying cation currents (n = 5; fig. 4C). In the presence of 50 μM waixenicin A, current amplitudes were inhibited outwardly by 43.7 ± 1.5% and inwardly by 66.3 ± 3.1% (n = 5; fig. 4C).

Discussion

Ion channels play important roles in a wide variety of biological processes, but they are also associated with several diseases [19–21]. In particular, ion channels are crucial for tumour development and cancer growth. When epithelial cells

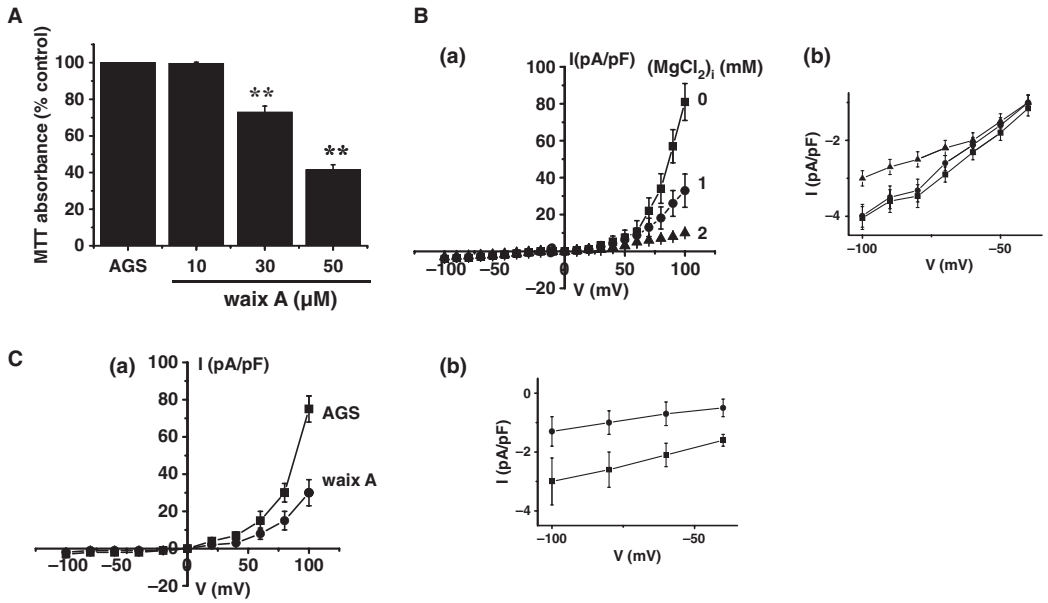

Fig. 3. Effect of waixenicin A on AGS cell viability and on cell death because of a transient receptor potential melastatin (TRPM7) blockade. (A) MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)–based viability assay. AGS cells were treated with increasing concentrations of waixenicin A for 72 hr. (B) (a) Representative TRPM7-like currents in AGS at 0, 1 or 2 mM $[Mg^{2+}]_i$. A voltage ramp from +100 to −100 mV was applied from a holding potential of −60 mV. (b) Enlargement of the negative mV part of a). (C) (a) Effect of waixenicin A on TRPM7-like current. I–V curves in the absence (■) or presence (●) of 50 μM waixenicin A. (b) Enlargement of the negative mV part of (a). $^{**}p < 0.01$.

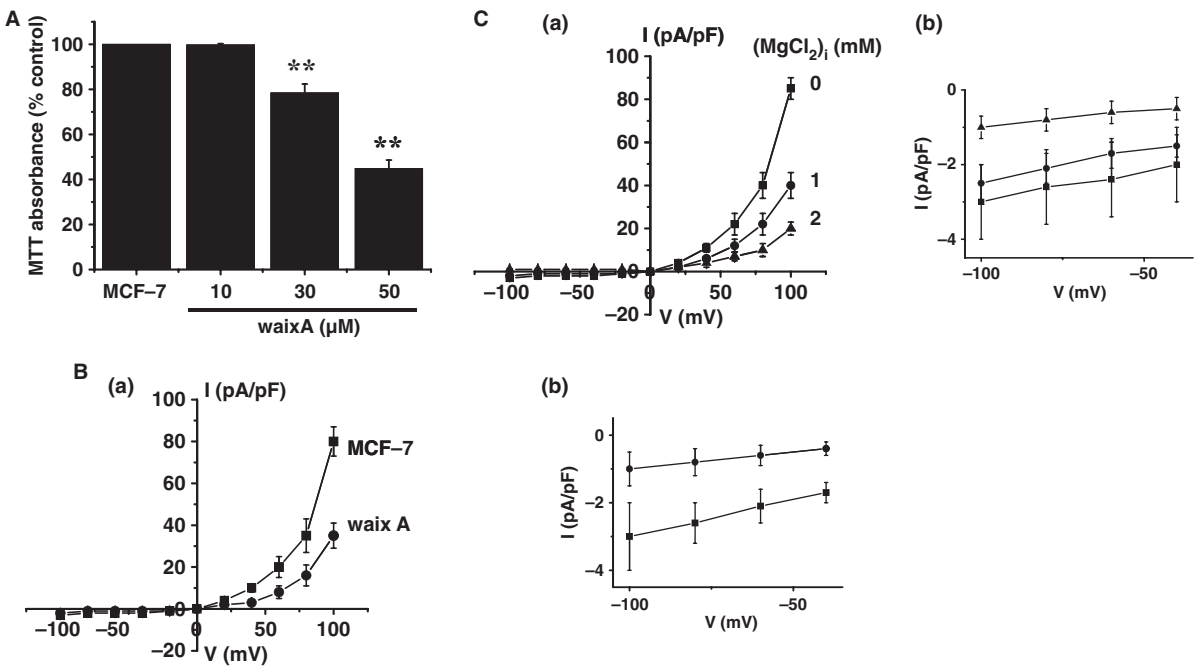

Fig. 4. Effect of waixenicin A on MCF-7 cell viability and on cell death because of a transient receptor potential melastatin (TRPM7) blockade. (A) MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)–based viability assay. MCF-7 cells were treated with increasing concentrations of waixenicin A for 72 hr. (B) (a) Representative TRPM7-like currents in MCF-7 cells at 0, 1 or 2 mM $[Mg^{2+}]_i$. A voltage ramp from +100 to −100 mV was applied from a holding potential of −60 mV. (b) Enlargement of the negative mV part of a). (C) (a) Effect of waixenicin A on TRPM7-like current. I–V curves in the absence (■) or presence (●) of 50 μM waixenicin A. (b) Enlargement of the negative mV part of (a). $^{**}p < 0.01$.

become cancerous, a series of genetic alterations occur that may also affect ion channel expressions and change ion channel activities [22]. Therefore, specific ion channel inhibitors fulfil important roles in studies on cell pathophysiology. The TRPM subgroup (named after its founding member melastatin) of the transient receptor potential (TRP) family of cation channels

consists of eight members that exhibit an interesting diversity of cation permeation characteristics and activation mechanisms. Within the TRPM subfamily, TRPM7 (ChaK1, TRPPLIK, LTRPC7) has the unique feature of possessing a kinase domain belonging to the atypical family of alpha-kinases fused to the ion channel domain. TRPM7 has been functionally characterized as a divalent cation permeable cation channel yielding outwardly rectifying currents when overexpressed in heterologous expression systems [3,4]. Furthermore, recent reports have documented that TRPM7 channel function is regulated by intracellular magnesium and magnesium nucleotides [23–25]. In addition, TRPM7 has been shown to be crucial for cellular magnesium homoeostasis, as targeted gene deletion of TRPM7 in cell lines led to intracellular magnesium deficiency and growth arrest [23].

Recently, Zierler *et al.* [11] suggested that waixenicin A is a potent and relatively specific inhibitor of TRPM7 ion channels. In this recent study, waixenicin A was found to be a selective inhibitor of the TRPM7 ion channel, and patch-clamp experiments confirmed waixenicin A as a TRPM7 antagonist. However, the inhibitory effects of waixenicin A on TRPM7 are strongly dependent on intracellular Mg^{2+} concentration [11]. Furthermore, it was also found that the pharmacological inhibition of TRPM7 by waixenicin A caused growth arrest in the G0/G1 phase [11].

Chen *et al.* [26] suggested 5-lipoxygenase (5-LOX) inhibitors are potent blockers of TRPM7 channel that are capable of attenuating the function of TRPM7, which makes them effective tools for the biophysical characterization and suppression of TRPM7 channel conductance. Furthermore, in a previous study, we found that the 5-LOX inhibitor AA861 (but not nordihydroguaiaretic acid (NDGA)) modulated the pacemaker activities of the ICCs.

In this study, we found that waixenicin A inhibited the pacemaking activity of ICCs (fig. 1) with an IC_{50} in ICC clusters of 4.6 μM (fig. 2A) and inhibited TRPM7-like currents in single ICCs (fig. 2B). TRPM7 or Ca^{2+}-activated Cl^- conductance is involved in pacemaking activity in ICCs [8,13]. To investigate the effects of waixenicin A on TRPM7 and Ca^{2+}-activated Cl^- conductances, we overexpressed TRPM7 or Ca^{2+}-activated Cl^- channels in HEK293 cells. Waixenicin A had no effects on Ca^{2+}-activated Cl^- conductances (fig. 2F), but inhibited TRPM7 currents (fig. 2E), and the inhibition effects of waixenicin A on TRPM7 channels were similar to its effects in ICCs. Therefore, we believe TRPM7 is involved in the pacemaking activity of ICCs and that waixenicin A modulates ICC pacemaking activity.

Kim *et al.* [9] reported that human gastric adenocarcinoma cells express TRPM7 channel and that this channel is essential for cell survival. In addition, they suggested that this channel be viewed as a potential target for the pharmacological treatment of gastric cancer. Guilbert *et al.* [10] also suggested that TRPM7 is required for breast cancer cell proliferation. In the present study, we found waixenicin A inhibited cell viability (fig. 3A) and TRPM7-like currents in AGS cells (fig. 3C). Furthermore, we investigated the effects of waixenicin A in MCF-7 cells (fig. 4) like AGS cells. These results suggested that TRPM7 plays an important pathophysiological role in AGS and MCF-7 cells.

TRPM7 has also been suggested to play a central role in cellular Mg^{2+} homoeostasis [23], in central nervous system ischaemic injury [27], in skeletogenesis in zebrafish [28], in defecation rhythm in *Caenorhabditis elegans* [29], in cholinergic vesicle fusion with the plasma membrane [30], in phosphoinositide-3-kinase signalling in lymphocytes [31] and in osteoblast proliferation [32]. However, the effects of waixenicin A on the physiological functions of TRPM7 have not been previously investigated. Accordingly, we suggest these effects be more comprehensively explored.

Summarizing, TRPM7 channel was found to modulate intestinal motility and to regulate the pathophysiology of human gastric and breast adenocarcinoma cells. Accordingly, we suggest that waixenicin A be viewed as a potential drug for the pharmacological treatment of gut motor disorders and gastric and breast cancer.

Acknowledgements

The Creative Research Initiative Center for Bio-Artificial Muscle of the Korea Ministry of Education, Science and Technology (MEST).

References

1 Clapham DE. TRP channels as cellular sensors. Nature 2003;**426**:517–24.
2 Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. Cell Calcium 2005;**38**:233–52.
3 Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ *et al.* LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. Nature 2001;**411**:590–5.
4 Runnels LW, Yue L, Clapham DE. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science 2001;**291**:1043–7.
5 Ward SM, Burns AJ, Torihashi S, Sanders KM. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. J Physiol 1994;**480**:91–102.
6 Huisinga JD, Thuneberg L, Kluppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 1995;**373**:347–52.
7 Sanders KM. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. Gastroenterology 1996;**111**:492–515.
8 Kim BJ, Lim HH, Yang DK, Jun JY, Chang IY, Park CS *et al.* Melastatin-type transient receptor potential channel 7 is required for intestinal pacemaking activity. Gastroenterology 2005;**129**:1504–17.
9 Kim BJ, Park EJ, Lee JH, Jeon JH, Kim SJ, So I. Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer. Cancer Sci 2008;**99**:2502–9.
10 Guilbert A, Gautier M, Dhennin-Duthille I, Haren N, Sevestre H, Ouadid-Ahidouch H. Evidence that TRPM7 is required for breast cancer cell proliferation. Am J Physiol Cell Physiol 2009;**297**:C493–502.
11 Zierler S, Yao G, Zhang Z, Kuo WC, Pörzgen P, Penner R *et al.* Waixenicin A inhibits cell proliferation through magnesium-dependent block of transient receptor potential melastatin 7 (TRPM7) channels. J Biol Chem 2011;**286**:39328–35.
12 Koh SD, Sanders KM, Ward SM. Spontaneous electrical rhythmicity in cultured interstitial cells of cajal from the murine small intestine. J Physiol 1998;**513**:203–13.
13 Zhu MH, Kim TW, Ro S, Yan W, Ward SM, Koh SD *et al.* A Ca (2 +)-activated Cl(-) conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. J Physiol 2009;**587**:4905–82.

14 Gomez-Pinilla PJ, Gibbons SJ, Bardsley MR, Lorincz A, Pozo MJ, Pasricha PJ *et al.* Ano1 is a selective marker of interstitial cells of Cajal in the human and mouse gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 2009;**296**:G1370–81.
15 Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E *et al.* TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science 2008;**322**:590–4.
16 Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell 2008;**134**:1019–29.
17 Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS *et al.* TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 2008;**455**:1210–15.
18 Hwang SJ, Blair PJA, Britton FC, O'Driscoll KE, Hennig G, Bayguinov YR *et al.* Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles. J Physiol 2009;**587**:4887–904.
19 Jiang J, Li MH, Inoue K, Chu XP, Seeds J, Xiong ZG. Transient receptor potential melastatin 7-like current in human head and neck carcinoma cells: role in cell proliferation. Cancer Res 2007;**67**:10929–38.
20 Pardo LA, Contreras-Jurado C, Zientkowska M, Alves F, Stühmer W. Role of voltage-gated potassium channels in cancer. J Membr Biol 2005;**205**:115–24.
21 Bodding M. TRP proteins and cancer. Cell Signal 2007;**19**: 617–24.
22 Kunzelmann K. Ion channels and cancer. J Membr Biol 2005;**205**: 159–73.
23 Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R *et al.* Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. Cell 2003;**114**:191–200.
24 Matsushita M, Kozak JA, Shimizu Y, McLachlin DT, Yamaguchi H, Wei FY *et al.* Channel function is dissociated from the intrinsic kinase activity and autophosphorylation of TRPM7/ChaK1. J Biol Chem 2005;**280**:20793–803.
25 Demeuse P, Penner R, Fleig A. TRPM7 channel is regulated by magnesium nucleotides via its kinase domain. J Gen Physiol 2006; **127**:421–34.
26 Chen HC, Xie J, Zhang Z, Su LT, Yue L, Runnels LW. Blockade of TRPM7 channel activity and cell death by inhibitors of 5-lipoxygenase. PLoS ONE 2010;**5**:e11161.
27 Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W *et al.* A key role for TRPM7 channels in anoxic neuronal death. Cell 2003;**115**:863–77.
28 Elizondo MR, Arduini BL, Paulsen J, MacDonald EL, Sabel JL, Henion PD *et al.* Defective skeletogenesis with kidney stone formation in dwarf zebrafish mutant for trpm7. Curr Biol 2005;**15**: 667–71.
29 Vriens J, Owsianik G, Voets T, Droogmans G, Nilius B. Invertebrate TRP proteins as functional models for mammalian channels. Pflugers Arch 2004;**449**:213–26.
30 Brauchi S, Krapivinsky G, Krapivinsky L, Clapham DE. TRPM7 facilitates cholinergic vesicle fusion with the plasma membrane. Proc Natl Acad Sci USA 2008;**105**:8304–8.
31 Sahni J, Scharenberg AM. TRPM7 ion channels are required for sustained phosphoinositide 3-kinase signaling in lymphocytes. Cell Metab 2008;**8**:84–93.
32 Abed E, Moreau R. Importance of melastatin-like transient receptor potential 7 and cations (magnesium, calcium) in human osteoblast-like cell proliferation. Cell Prolif 2007;**40**:849–65.