IK1 channel activity contributes to cisplatin sensitivity of human epidermoid cancer cells

Elbert L. Lee, Yuichi Hasegawa, Takahiro Shimizu, and Yasunobu Okada

Department of Cell Physiology, National Institute for Physiological Sciences, Okazaki, Japan

Submitted 17 September 2007; accepted in final form 25 March 2008

CISPLATIN (cis-diaminedichloroplatinum II) is a drug widely used in cancer chemotherapy. It has been used clinically for about 30 years and is effective against a variety of cancers, including testicular, ovarian, head and neck, cervical, and non-small-cell lung cancers. Cisplatin forms adducts with DNA, activating signaling pathways (which are still poorly understood) that trigger cell cycle arrest and apoptosis. However, cancer cells can have intrinsic or acquired resistance to cisplatin; despite increasing insight into the signaling pathways activated by cisplatin and the mechanisms contributing to resistance (18, 37, 40), resistance remains a major problem that severely limits the usefulness of cisplatin as a chemotherapeutic agent. To identify new ways to combat resistance and improve the effectiveness of cisplatin, it is crucial to gain a more complete understanding of the mechanisms by which cisplatin induces apoptosis.

Ion fluxes have been reported to modulate the response of cancer cells to cisplatin. Mounting evidence indicates the importance of cation flux: amphotericin B, a K+ ionophore, caused changes in the cisplatin resistance of ovarian carcinoma cells with reduced cisplatin uptake (35), and, in combination with bumetanide, an Na+-K+-2Cl– cotransporter inhibitor, it enhanced cisplatin-induced apoptosis in mesothelioma cells (29, 30). Cisplatin-induced apoptosis of spiral-ligament fibrocytes of the cochlea was reduced by inhibition of Ca2+-activated, large-conductance K+ (BK) channels (24). Anion flux has also been found important: it has been reported that blockers of Cl– transport induced cisplatin resistance in mouse mammary tumor cells (22) and canine osteosarcoma cells (44).

We previously found that the volume-sensitive, outwardly rectifying Cl– channel in cisplatin-induced apoptosis. To investigate the possibility that cation channels also have a role in the cellular response to cisplatin, we examined the activity of cation channels in cisplatin-sensitive KB-3-1 (KB) epidermoid cancer cells by the whole cell patch-clamp method. A cation channel in KB cells, activated by hypotonic stress, was identified as the Ca2+-activated, intermediate-conductance K+ (IK1) channel on the basis of its requirement for intracellular Ca2+2, its blockage by the blockers clotrimazole and triarylmethane-34, and its suppression by a dominant-negative construct. Activity of this channel was not observed in KCP-4 cells, a cisplatin-resistant cell line derived from KB cells, and its molecular expression, observed by semiquantitative RT-PCR and immunostaining, appeared much reduced. Cell volume measurements confirmed a physiological role for the IK1 channel as a component of the volume-regulatory machinery in KB cells. A possible role of the IK1 channel in cisplatin-induced apoptosis was investigated. It was found that clotrimazole and triarylmethane-34 inhibited a cisplatin-induced decrease in cell viability and increase in caspase-3/7 activity, whereas 1-ethyl-2-benzimidazolinone, an activator of the channel, had the opposite effect. Thus IK1 channel activity appears to mediate, at least in part, the response of KB cells to cisplatin treatment.

Address for reprint requests and other correspondence: Y. Okada, Dept. of Cell Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan (e-mail: okada@nips.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We have investigated whether the IK1 channel, like the VSOR Cl\(^{-}\) channel, might contribute to the sensitivity of KB cancer cells to cisplatin. We show that although the IK1 channel is expressed in KB cells, where it functions to regulate cell volume, it is not expressed in cisplatin-resistant KCP-4 cells. Furthermore, cell viability and caspase activity assays indicated that inhibition of the IK1 channel did, in fact, reduce sensitivity of KB cells to cisplatin, whereas activation of the IK1 channel increased sensitivity to cisplatin.

**MATERIALS AND METHODS**

*Cell culture.* KB human epidermoid cancer cells were maintained at 37°C in a humidified 5% CO\(_2\)-95% air incubator in Eagle’s minimum essential medium (Sigma, St. Louis, MO) containing 10% fetal bovine serum, 40 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin. The cisplatin-resistant KCP-4 cells, derived from the KB cell line (10), were maintained in the same conditions but in medium that also contained 23.3 \(\mu\)M cisplatin (Sigma). HEK-293T human embryonic kidney cells were maintained in Dulbecco’s modified Eagle’s medium (Nissui, Tokyo, Japan), instead of Eagle’s minimum essential medium, but were otherwise cultured in the same way as KB cells.

For most patch-clamp experiments with KB cells, cells were plated at a density of \(~1.5 \times 10^5\) cells per well in six-well plates 2 days before recordings were made. In experiments with transfected KB cells, cells were transfected 1 day after they were plated and used in patch experiments 36–48 h later. KCP-4 cells, which proliferate more slowly, were plated at a density of \(~2.5 \times 10^5\) cells per well in six-well plates 2 days before recordings were made. After cells were detached from the plastic substrate with a cell scraper and dissociated by pipetting, they were placed in a glass-bottomed chamber (0.3 ml volume) on an inverted microscope (model IX71, Olympus, Tokyo, Japan) with filters for red fluorescent protein.

For cell viability and caspase-3/7 activity assays, cells were plated at \(~3 \times 10^4\) cells per well in 96-well plates; in the case of cell viability assays, the medium was replaced with drug-containing or drug-free medium 1 day after the cells were plated; after 24 h of treatment, the medium was renewed for a total treatment time of 48 h. In the case of caspase-3/7 activity assays, the medium was replaced with drug-containing or drug-free medium 1 day after the cells were plated; the total treatment time was 24 h for experiments with inhibitors and 18 h for experiments with 1-ethyl-2-benzimidazolinone (1-EBIO; Tocris Bioscience, Bristol, UK) to limit toxicity associated with longer incubations. For the annexin V binding assay, cells were plated at \(~3 \times 10^4\) cells per well in 24-well plates; drug treatment was initiated on the following day and continued for 15 h before the

<table>
<thead>
<tr>
<th>Target</th>
<th>GenBank Accession No.</th>
<th>Primer Sequences</th>
<th>Expected Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK1</td>
<td>NM_002248</td>
<td>5'-CTTCTCCTGGTGACCTGTC-3' (forward)</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-ACACGGTCAGTGTCATCGA-3' (reverse)</td>
<td>320</td>
</tr>
<tr>
<td>SK2</td>
<td>NM_002247</td>
<td>5'-GGCTCAAGATGGCAGACTCA-3' (forward)</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-AAAGCTTGCAAGCTGGTCG-3' (reverse)</td>
<td>390</td>
</tr>
<tr>
<td>SK3</td>
<td>NM_002249</td>
<td>5'-GACGACACCTGCTGGGACCTC-3' (forward)</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-CTTCTGCTGGTCTGAGATT-3' (reverse)</td>
<td>387</td>
</tr>
<tr>
<td>IK1</td>
<td>NM_002250</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pair 1</td>
<td>5'-GGAGAGCGAGGCTCATTAGGC-3' (forward)</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GAGCTCTGCTGTTTCTCAG-3' (reverse)</td>
<td>390</td>
</tr>
<tr>
<td>BK</td>
<td>NM_001014797</td>
<td>5'-AGCCTCTGAGGCGGTAAA-3' (forward)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-TTGACCAGAGACGAGATGA-3' (reverse)</td>
<td>320</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM_002046</td>
<td>5'-GAGCTTCTGGGCTGATGGA-3' (forward)</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-AGATGGTGATGTGATGGTTG-3' (reverse)</td>
<td>367</td>
</tr>
</tbody>
</table>

SK1, SK2, and SK3, small-conductance K\(^{+}\) channels; IK1, Ca\(^{2+}\)-activated intermediate-conductance K\(^{+}\) channel; BK, large-conductance K\(^{+}\) channel.

![Fig. 1. Ca2\(^{+}\) dependence of hypotonicity-induced whole cell cation current in KB cells. A: current responses to step pulses from -100 to +100 mV in 20-mV increments. Step pulses were applied after steady-state activation of currents during isotonic (ISO) and hypotonic (HYPO) stimulation. Top: control currents elicited by step pulses during isotonic and hypotonic stimulation. Bottom: inclusion of BAPTA in the pipette solution prevented activation of hypotonicity-induced currents. Arrowheads indicate zero-current level. Traces shown are those of unsubtracted currents. B: current (I)-voltage (V) relationships for steady-state whole cell currents (unsubtracted) in KB cells. Values are means ± SE of n samples.](http://ajpcell.physiology.org/Downloadedfrom)
assay. For semiquantitative RT-PCR, KB cells were plated at a density of \(1.5 \times 10^5\) cells per well and KCP-4 cells at \(2.5 \times 10^5\) cells per well in six-well plates 2 days before RNA extraction.

For expression of the dominant-negative IK1 construct in KB cells or wild-type IK1 in HEK-293T cells, cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol.

Electrophysiology. Hypotonicity-induced \(K^+\) currents were recorded by the whole cell patch-clamp method. The patch electrodes were fabricated from borosilicate glass capillaries (1.4 mm OD, 1.0 mm ID; Asahi Rika-Glass Industry, Nagoya, Japan) using a micro-pipette puller (model P-2000, Sutter Instruments, Novato, CA). The wide-tipped electrodes had a resistance of \(\sim 2 \, \text{M}\Omega\) when filled with pipette solution. Series resistance (\(<5\, \text{M}\Omega\)) was compensated (to 70–80%) to minimize voltage errors. An amplifier (model EPC9, HEKA Elektronik, Lambrecht/Pfalz, Germany) was used to record currents; Pulse software (version 8.76, HEKA Elektronik) was used for command pulse control, data acquisition, and analysis. Current signals were sampled at 5 kHz and filtered at 1 kHz. The time course of current activation was monitored by repetitive application (every 15 s) of alternating 2-s pulses from a holding potential of \(-40\) to \(+100\) mV. For observation of voltage dependence of the current profile, 1-s step pulses were applied from \(-100\) to \(+100\) mV in 20-mV increments before and during activation of the swelling-induced current. The amplitude of steady-state current was measured \(\sim 20\) ms before the end of each step pulse. The isotonic bath solution contained (in mM) 4.2 potassium gluconate, 125 sodium gluconate, 2 MgCl2, 2 CaCl2, 10 HEPES, and 65 mannitol (with pH adjusted to 7.4 with NaOH, 320 mosmol/kgH2O). The composition of the hypotonic bath solution (270 mosmol/kgH2O) was the same as the composition of the isotonic solution, except the hypotonic solution contained 15 mM mannitol. The pipette solution contained (in mM) 130 potassium gluconate, 2 MgCl2, 0.03 CaCl2, 0.08 EGTA, 10 HEPES, and 43 mannitol (with pH adjusted to 7.3 with KOH, 300 mosmol/kgH2O, pCa 7). The Ca\(^{2+}\)-free pipette solution contained (in mM) 122 potassium gluconate, 2 MgCl2, 2 K\(_4\)-BAPTA, 8 gluconic acid, 10 HEPES, and 43 mannitol (with pH adjusted to 7.3 with KOH, 300 mosmol/kgH2O). ATP was not included in the pipette solutions to prevent activation of volume-sensitive anion channels. The osmolality of solutions was measured using a freezing-point-depression osmometer (model OMB02, Vogel, Giessen, Germany). The effects of clotrimazole (Sigma) and triarylmethane-34 (TRAM-34; Sigma) were tested by perfusion of the cells with hypotonic bath solutions containing the blockers.

Expression constructs. An expression plasmid containing the full-length human IK1 cDNA (KCNN4 pCMV-XL4) was purchased from Origene (Rockville, MD). The gene-encoding region between the EcoR I and Sac II restriction sites was inserted between the identical sites in the bicistronic pIRES2-EGFP and pIRES2-DsRed Express vectors (Clontech, Palo Alto, CA). A dominant-negative IK1 con-

---

**Fig. 2.** Specific inhibition of hypotonicity-induced Ca\(^{2+}\)-activated intermediate-conductance \(K^+\) (IK1) current in KB cells. **A:** representative recording (left) shows time course of current activation by hypotonic stimulation and block by 10 \(\mu\)M clotrimazole (CLT). Currents were monitored with alternating pulses from \(-100\) to \(+100\) mV from a holding potential of \(-40\) mV. Step pulses in 20-mV increments were applied at times indicated by arrows (a, b, and c). Current responses to 20-mV incremental step pulses from \(-100\) to \(+100\) mV are shown at right. Perfusion of hypotonic bath solution containing CLT inhibited hypotonicity-activated current. Subtracted currents [currents in isotonic conditions were subtracted from currents in hypotonic (b – a) or hypotonic-CLT (c – a) condition] are shown. Arrowheads indicate zero-current level. **B:** I–V relationships showing block of IK1 current by 10 \(\mu\)M CLT. Subtracted currents are shown. **C:** I–V relationships showing block of IK1 current by 10 \(\mu\)M triarylmethane-34 (TRAM-34). Subtracted currents are shown. Values are means ± SE of \(n\) samples.
IK1 CHANNEL INVOLVED IN CISPLATIN SENSITIVITY

Fig. 3. Molecular expression of IK1 channel in KB cells and inhibition of conductance by a dominant-negative construct. A: RT-PCR results showing IK1 transcript expression in KB cells. IK1 primer pair (Table 1) was used. Ca2+-activated, small-conductance K+ channel 3 (SK3) transcript also appears to be expressed. GAPDH was used as a control. SK1 and SK2, Ca2+-activated, small-conductance K+ channels 1 and 2, respectively; BK, Ca2+-activated, large-conductance K+ channel. +, Reverse transcriptase included in cDNA synthesis reaction; −, negative control with no reverse transcriptase. M, 100-bp DNA ladder. B: I-V relationships showing that expression of a dominant-negative construct (IK1dn) in KB cells suppressed IK1 current. Subtracted currents are shown. Mock, cells transfected with empty vector. Values are means ± SE of n samples.

Fig. 4. Absence of IK1 channel activity in cisplatin-resistant KCP-4 cells. A: representative recordings showing time course of hypotonicity-induced current activation in KB cells (top) and lack of current activation in KCP-4 cells (bottom). Currents were monitored with alternating pulses from −100 to +100 mV from a holding potential of −40 mV. Step pulses in 20-mV increments were applied at times indicated by arrows. B: I-V relationships comparing currents activated in KB and KCP-4 cells. Subtracted currents are shown. Values are means ± SE of n samples.
Kobe, Japan), as reported previously (14). The mean volume of the cell population was calculated from the cell volume distribution after the machine was calibrated with latex beads of known volume. Isotonic or hypotonic solution consisted of (in mM) 95 NaCl, 4.5 KCl, 1 MgCl₂, 1 CaCl₂, 110 or 0 mannitol, and 5 HEPES (with pH adjusted to 7.3 with NaOH, 310 or 200 mosmol/kgH₂O).

Cell viability and caspase-3/7 activity assays. Cell viability of drug-treated KB cells was assessed by measurement of mitochondrial succinate dehydrogenase activity using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer’s instructions. Caspase-3/7 activity in drug-treated KB cells was measured using the Apo-ONE Homogeneous Caspase-3/7 Assay (Promega) according to the manufacturer’s instructions.

Annexin V binding assay. Annexin V binding to apoptotic cells was assessed with a procedure similar to that described previously (45) using the Annexin V-FITC Apoptosis Detection Kit (Sigma). Briefly, cells were visualized with an Olympus IX70 fluorescence microscope (×20 objective), and digital images of 438 x 330 μm fields containing ~100 cells were taken. Images were visually inspected to identify and count annexin V-positive/propidium iodide (PI)-negative (corresponding to apoptotic) and annexin V-positive/PI-positive (corresponding to necrotic) cells. Overlaid images were generated with Photoshop 7.0 software (Adobe Systems, San Jose, CA).

Chemicals. Stocks of clotrimazole (5 mM), TRAM-34 (10 mM), and 1-EBIO (200 mM) were prepared in DMSO and stored in a −30°C freezer; immediately before use, they were diluted to the required concentrations.

Statistical analysis. Values are means ± SE of n observations. Data were evaluated by unpaired Student’s t-test, and P < 0.05 was taken to indicate statistical significance.

Fig. 5. IK1 molecular expression in KB and KCP-4 cells. A: semiquantitative RT-PCR comparison of level of IK1 transcript expression in KB and KCP-4 cells. Top and middle: IK1 mRNA expression was checked with 2 different primer pairs. Cycle numbers at which reaction aliquots were taken are shown below each image, and PCR product sizes in base pairs (bp) are indicated. −RT, negative controls in which reverse transcriptase was excluded from cDNA synthesis reaction. Markers, 100-bp DNA ladder. Bottom: GAPDH expression was checked as a control. B: immunostaining of KB and KCP-4 cells with an anti-IK1 polyclonal antibody shown by light (left) and confocal fluorescence (right) microscopy images. Strong immunoreactivity was seen in peripheral plasma membrane regions of KB, but not KCP-4, cells. HEK-293T cells transiently transfected with the wild-type IK1 pIRES2-EGFP expression construct also showed strong immunoreactivity (HEK-IK1) and served as a positive control for antibody specificity. Enhanced green fluorescence protein (EGFP) fluorescence was quenched by acetone fixation of the cells and did not interfere with imaging. HEK-Neg, HEK-293T cells transiently transfected with negative control HcRed pIRES2-EGFP. Scale bar, 20 μm.

Fig. 6. IK1 channel activity mediates regulatory volume decrease in KB cells. Time course of cell volume changes in response to a 64.5% hypotonic stimulation and effects of IK1 blockers clotrimazole and TRAM-34 are shown. Cells were added to drug-containing isotonic or hypotonic solutions. Hypotonic stimulation started from time 0. Values were normalized to values in isotonic solution. *Significantly different from control (P < 0.05).
RESULTS

A hypotonicity-activated, Ca\(^{2+}\)-dependent K\(^{+}\) channel in KB cells. We observed cation currents in KB cells by the whole cell patch-clamp method. Whole cell currents, monitored with alternating voltage pulses to \pm 100 mV from a holding potential of \(-40\) mV, were activated in response to hypotonic exposure (Fig. 1; also, Fig. 4A). The hypotonicity-activated currents were inwardly rectifying and had a reversal potential (determined after subtraction of currents in isotonic conditions) of \(-93.8 \pm 6.6\) mV, which is close to the theoretical value for K\(^{+}\) conductance calculated by the Nernst equation (\(-88.2\) mV). Activation of the current required Ca\(^{2+}\); no current activation was seen when 2 mM BAPTA was included in the pipette solution (Fig. 1). Pharmacological studies provided evidence that the channel was the IK1 channel encoded by the KCNN4 gene. The currents were blocked by 10 \mu M clotrimazole, a nonspecific blocker of the IK1 channel (1), and 10 \mu M TRAM-34, a more specific blocker of the IK1 channel (43) (Fig. 2). Expression of IK1 transcript in KB cells was confirmed by RT-PCR (Fig. 3A). Heterologous expression in these cells...
cells of a dominant-negative IK1 construct previously shown to suppress IK1 currents (2) prevented activation of the currents (Fig. 3B), serving as further evidence that the currents observed were, in fact, IK1 currents.

**Lack of the IK1 conductance in KCP-4 cells.** Previously, we found functional expression of VSOR Cl\(^-\) channels in KB cells, but not in the closely related cisplatin-resistant KCP-4 cell line (23). Hypothesizing that KCP-4 cells might have a similar deficiency in functional IK1 channels, we used the whole cell patch-clamp method to examine whether IK1 currents could be activated by hypotonicity in these cells (Fig. 4). IK1 channel activity was not observed when KCP-4 cells were exposed to isotonic or hypotonic bath solutions.

To verify that the channel gene was expressed at a higher level in KB than KCP-4 cells, transcript expression was compared. Semi-quantitative RT-PCR showed a much higher level of IK1 transcript expression in KB than KCP-4 cells (Fig. 5A). For comparison of IK1 protein expression, KB and KCP-4 cells were immunostained with a polyclonal antibody to IK1 and imaged by confocal fluorescence microscopy (Fig. 5B). In KB cells, immunoreactivity was observed in the peripheral regions near or in the plasma membrane (Fig. 5B), whereas in KCP-4 cells, there was little, if any, observable immunoreactivity (Fig. 5B); these results provide evidence that IK1 protein was expressed in KB, but not KCP-4, cells. As a control for antibody specificity, HEK-293T cells were transfected with wild-type IK1; these cells also showed strong immunoreactivity in their peripheral regions (Fig. 5B). HEK-293T cells do not express endogenous IK1, judging from an RT-PCR examination of IK1 mRNA expression (data not shown).

**Role of IK1 in KB cell volume regulation.** On the basis of its previously reported volume-regulatory function in other cell types (2, 3, 21, 41), IK1 channels might be expected to play a role in KB cell volume regulation. Using a Coulter counter to monitor changes in the volume of KB cells over a 15-min period of hypotonic stimulation, we checked the volume-regulatory response of these cells (Fig. 6). We observed a robust RVD immediately after cell swelling; however, RVD was inhibited when clotrimazole or TRAM-34 was included in the solution, indicating involvement of IK1 channels in the response.

**Role of IK1 channels in KB cell cisplatin sensitivity.** The much higher level of IK1 expression in KB cells than in cisplatin-resistant KCP-4 cells suggests that the presence of IK1 channels might contribute to the cisplatin sensitivity of KB cells. A cell viability assay showed that cisplatin treatment resulted in a reduction in the viability of KB cells measured at 48 h and that this reduction was significantly inhibited by application of the IK1 channel blocker clotrimazole or TRAM-34 (Fig. 7A). In addition, an increase in caspase-3/7 activity after 24 h of cisplatin treatment was inhibited to a significant degree by the IK1 channel blockers (Fig. 7B).

The effect of 1-EBIO, an activator of the IK1 channel (8), was also examined. We first confirmed, by the whole cell patch-clamp method, that 1-EBIO induced IK1 currents in KB cells: currents were elicited immediately on perfusion of the drug, in the absence of hypotonic stimulation (600 μM 1-EBIO; current density \(= 149.1 \pm 19.6 \text{ pA/pF at } +100 \text{ mV and reversal potential } = -85.2 \pm 0.7 \text{ mV, after subtraction of basal currents; } n = 5\)). Application of 1-EBIO + cisplatin significantly decreased cell viability: viability at 24 h was 45.0 ± 2.1% of viability of cells treated with cisplatin alone (\(n = 12\); 600 μM 1-EBIO was applied for only the first 18 h and, by itself, had just a slight toxicity, which resulted in a 6.5 ± 1.0% decrease in viability compared with DMSO-treated control cells). Cisplatin + 1-EBIO increased caspase-3/7 activity compared with cisplatin alone (Fig. 8C), and the proportion of cells binding annexin V (but not staining with PI, indicating apoptosis, rather than necrosis) was significantly increased by cisplatin + 1-EBIO (Fig. 8, A and B). These results indicate that IK1 channel activity contributes to cisplatin-induced apoptotic cell death. To address whether cisplatin increases IK1 gene transcription, semi-quantitative RT-PCR was performed using RNA from untreated KB cells and KB cells treated for 24 h with cisplatin. However, the level of IK1 transcript in the cells did not appear to be altered by cisplatin (Fig. 9).

**DISCUSSION**

In the present study, we show that KB human epidermoid cancer cells express a hypotonicity-activated cation channel and identify it as the IK1 channel (Figs. 1–3). It was found that this channel functions to regulate cell volume during hypotonic stress in KB cells (Fig. 6). The IK1 channel also plays a role in mediating the cellular response to cisplatin, as seen by the decrease in cisplatin sensitivity of the cells on treatment with IK1 inhibitors (Fig. 7) and the increase in sensitivity on treatment with a channel activator (Fig. 8). Further evidence for a role of the IK1 channel in the response to cisplatin is the finding that the related cisplatin-resistant KCP-4 cells express the IK1 channel at a very low level, if at all (Figs. 4 and 5).

There is accumulating evidence for a role of changes in K\(^+\) flux in a cell’s response to cisplatin (24, 29, 30, 35). Among K\(^+\) channels, BK channels have been shown to be critical in
mediating cisplatin-induced cytotoxicity (24). Although one report has suggested that increased expression of human ether-a-go-go-related gene (HERG)-encoded K\(^+\) and two-pore domain inwardly rectifying K\(^+\) (TWIK) channels does not have a role in cisplatin resistance (25), it does not exclude a possible role of other K\(^+\) channels. It is clear that cisplatin resistance is a complicated phenomenon involving several mechanisms that may occur in different combinations and depend on the cell type (37); it is reasonable that the response to cisplatin could depend on the assortment of channels expressed in a particular cell type. To our knowledge, ours is the first report indicating a role for the IK1 channel in cisplatin sensitivity and resistance.

How does activity of the IK1 channel contribute to increased cisplatin sensitivity? Since there were no readily apparent changes in the rate of proliferation of KB cells treated with clotrimazole or TRAM-34 compared with control cells over a period of 48 h (unpublished observations), alteration of the proliferation rate is unlikely to be a major mechanism by which a change in IK1 channel activity affects the susceptibility of the cells to cisplatin. On the other hand, we found that IK1 is important for cell volume regulation, specifically, for RVD, in KB cells (Fig. 6). Activity of the VSOR Cl\(^-\) channel, also known to have a crucial role in RVD (31), also modulates the cellular response to cisplatin in KB and KCP-4 cells (15, 23). Thus the IK1 and VSOR Cl\(^-\) channels, two major components of the volume-regulatory machinery, are also involved in cisplatin sensitivity; this suggests a link between cell volume regulation and cisplatin sensitivity.

A link between cell volume regulation and apoptosis is well established. Normotonic cell shrinkage at an early stage of apoptosis, known as apoptotic volume decrease (AVD), is a prerequisite for execution of the apoptotic program (28, 32). The mechanisms of AVD and RVD are thought to share the same components, in that the same pathways for cation and anion efflux, K\(^+\) and Cl\(^-\) channels, are used in both processes (13, 32). Inhibition of these channels has been shown to prevent AVD and apoptosis (28, 33). The IK1 channel specifically has been found to be essential for Ca\(^{2+}\)-induced AVD (apoptosis induced by calcimycin) in lymphocytes (9). It seems likely that the IK1 channel could have an important role in cisplatin-induced AVD as well.

IK1 channel activity may also be necessary for the activation of downstream apoptotic signaling pathways. In microglia, IK1 channel activity was linked to activation of inducible nitric oxide synthase and the p38 MAPK pathway, which is important for microglial activation (19). The p38 MAPK pathway is one of the major pathways known to be activated by cisplatin and to mediate the effects of cisplatin (27, 40). A relationship between this pathway and IK1 channel activity in cisplatin-induced apoptosis should be a focus of future investigations.

Does cisplatin activate the IK1 channel, and, if so, by what mechanism? Cisplatin does not appear to upregulate IK1 mRNA levels (Fig. 9), so it is unlikely that changes in IK1 gene expression due to altered activity of transcription factors, such as repressor element 1-silencing transcription factor (5), activator protein-1 (12), or Ikaros-2 (12), would be involved in enhancement of IK1 channel activity. However, cisplatin treatment can cause an increase in intracellular Ca\(^{2+}\) levels (20, 38), and extracellular Ca\(^{2+}\), presumably entering the cell through plasma membrane Ca\(^{2+}\) channels, has been observed to be necessary for cisplatin-induced activation, in human carcinoma HeLa-S3 cells, of a Ca\(^{2+}\)-dependent K\(^+\) conductance inhibitable by charybdoxin (38). It is not clear, however, whether the currents were mediated by IK1 or BK channels. Additionally, reactive oxygen species (ROS), which are produced upon cisplatin treatment (4, 20, 26), may activate IK1 channels. One report provides evidence that ROS stimulate the IK1 conductance in the Calu-3 airway epithelial cell line (6). Previously, we found that ROS mediate activation of VSOR Cl\(^-\) channels in apoptosis of HeLa epithelial cancer cells (36); thus it is possible that ROS could serve as a common mediator of channel activation during apoptosis.

In summary, we have shown that the IK1 channel, which functions to regulate cell volume in KB human epidermoid cancer cells, also functions to promote apoptotic cell death in KB cells treated with the anticancer drug cisplatin. Further investigations into the signaling pathways downstream of IK1 channel activation may shed light on the mechanisms of cisplatin-induced cell death and cisplatin resistance and lead to more effective cancer therapies.

ACKNOWLEDGMENTS

We thank T. Numata, N. Takahashi, and Y. Ando-Akatsuka for valuable advice; M. Ohara, K. Shigemoto, K. Tsuchiya, and M. Yamasawa for technical assistance; and T. Okayasu for editorial help.

GRANTS

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Japan Society for the Promotion of Science.

REFERENCES


