REVIEWS

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Andrzej Teisseyre[A–D], Justyna Gąsiorowska[B, E], Krystyna Michalak[A, C, E–F]

Voltage-Gated Potassium Channels Kv1.3 –Potentially New Molecular Target in Cancer Diagnostics and Therapy*

Department of Biophysics, Wroclaw Medical University, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Voltage-gated potassium channels, Kv1.3, which were discovered in 1984, are integral membrane proteins which are activated ("open") upon change of the cell membrane potential, enabling a passive flux of potassium ions across the cell membrane. The channels are expressed in many different tissues, both normal and cancer. Since 2005 it has been known that the channels are expressed not only in the plasma membrane, but also in the inner mitochondrial membrane. The activity of Kv1.3 channels plays an important role, among others, in setting the cell resting membrane potential, cell proliferation, apoptosis and volume regulation. For some years, these channels have been considered a potentially new molecular target in both the diagnostics and therapy of some cancer diseases. This review article focuses on: 1) changes of expression of the channels in cancer disorders with special regard to correlations between the channels' expression and stage of the disease, 2) influence of inhibitors of Kv1.3 channels on proliferation and apoptosis of cancer cells, 3) possible future applications of Kv1.3 channels' inhibitors in therapy of some cancer diseases. In the last section, the results of studies performed in our Laboratory of Bioelectricity on the influence of selected biologically active plant-derived compounds from the groups of flavonoids and stilbenes and their natural and synthetic derivatives on the activity of Kv1.3 channels in normal and cancer cells are reviewed. A possible application of some compounds from these groups to support therapy of cancer diseases, such as breast, colon and lymph node cancer, and melanoma or chronic lymphocytic leukemia (B-CLL), is announced (**Adv Clin Exp Med 2015, 24, 3, 517–524**).

Key words: cancer, apoptosis, Kv1.3 channel, proliferation, polycyclic compounds.

It is known that voltage-gated potassium channels of the Kv1.3 type are integral membrane proteins, which are activated ("open") upon change of the cell membrane potential, enabling a passive flux of potassium ions across the cell membrane. Kv1.3 channels, originally named as "n" ("normal") voltage-gated potassium channels, were discovered in 1984 in human T lymphocytes [1]. The biophysical properties of these channels were studied in more detail by Cahalan and colleagues in 1985 [2]. The channels appeared to be a "delayed rectifier" like voltage-gated potassium channels with a slow and complex inactivation [2]. The channel encoding gene was isolated from mouse and rat genomic DNA in 1990 [3, 4]. Because of a significant (60–70%) structural homology with voltage-gated potassium channels encoded by the *Shaker* gene in *Drosophila Melanogaster*, mammalian "n" type channels were classified as related to the *Shaker* channel family. The name "Kv1.3" was first introduced in 1991. It describes a mammalian *Shaker*-related voltage-gated potassium channel encoded by the KCNA3 gene. This gene was found in humans, rats and mice [5]. At first, the Kv1.3 channels were supposed to be expressed in T lymphocytes only. Currently it is known that

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Kv1.3 channels are expressed abundantly in many tissues, both normal and cancer [6–8]. Studies performed in the last 10 years provide evidence that Kv1.3 channels are expressed not only in the plasma membrane, but also in the inner mitochondrial membrane [9, 10]. The activity of Kv1.3 channels plays an important role in cell functioning, particularly in setting the resting membrane potential, cell proliferation, apoptosis and volume regulation [10, 11]. It has been shown that the blocking of Kv1.3 channels inhibited cell proliferation by arresting the cell cycle in the G_1 phase [11]. Kv1.3 channels are inhibited by many chemically unrelated compounds. Among the inhibitors are some heavy-metal cations, small-molecule organic compounds and venom-isolated oligopeptides [5, 11–13]. The most potent specific inhibitors inhibit the channels at subnanomolar concentrations [5, 11–13]. Specific blockers of Kv1.3 channels, such as 5-(4-phenoxybutoxy)psoralen (PAP-1), are currently being tested in pre-clinical trials as potential selective immunosuppressants of autoantigen-specific effector memory T lymphocytes (T_{EM}) in T-lymphocyte-mediated autoimmune diseases, such as sclerosis multiplex, type I diabetes, rheumatoid arthritis and psoriasis [11–13].

Another question that is important for possible clinical applications of Kv1.3 channel inhibitors is related to the role of inhibition of these channels in the induction of the apoptosis of Kv1.3-expressing cancer cells. Studies performed in recent years have demonstrated that expression of Kv1.3 channels is required for the induction of apoptosis, of both normal human T lymphocytes and leukemic T cell line Jurkat, by ceramide, tumor necrosis factor (TNF), sphingomielinase, staurosporine [10, 14] and actinomycin-D [15]. Lack of expression of Kv1.3 channels in the murine cytotoxic T lymphocyte cell line CTLL-2 was related to a significantly reduced ability of these cells to undergo apoptosis induced by all the factors mentioned above [10, 14, 15]. It was shown that induction of the expression of Kv1.3 channels in CTLL-2 cells by Kv1.3 vector transfection restored the apoptotic sensitivity of these cells [14, 15]. Moreover, suppression of Kv1.3 channel expression by application of Kv1.3-targeting siRNA significantly reduces the ability of both normal and leukemic T lymphocytes to undergo apoptosis induced by staurosporine [14].

It has been shown that the activity of Kv1.3 channels in the cell membrane was up-regulated upon activation of the death receptor pathway of apoptosis by Fas ligands in the human leukemic T cell line Jurkat [16]. The up-regulation of the channel activity, which occurred *via* caspase 8-dependent pathway, generated a sustained outward current that was required to promote the efflux of potassium ions and cell shrinkage – hallmarks of cell apoptosis [16]. On the other hand, it was shown that inhibition of mitochondrial Kv1.3 channels by a pro-apoptotic protein Bax cumulated in the mitochondrial space upon apoptotic stimulation was required to activate the intracellular (mitochondrial) pathway of apoptosis by means of cytochrome c release, reactive oxygen species production and depolarization of the inner mitochondrial membrane in the Jurkat T cell line and in Kv1.3-expressing CTLL-2 cells, but not in Kv1.3-deficient CTLL-2 cells [14]. It was shown that Bax inhibited Kv1.3 channels at nanomolar concentrations by binding to the extracellular mouth of the channel pore, such as occurs in the case of specific peptide inhibitors of these channels, for example, margatoxin (MgTX) [14]. Experiments performed on isolated mitochondria showed that inhibition of mitochondrial Kv1.3 channels by Bax caused a transient hyperpolarization of the inner mitochondrial membrane, followed by a pronounced depolarization, cytochrome c release and production of reactive oxygen species (ROS) [14]. All these processes are hallmarks of activation of the mitochondrial pathway of apoptosis. The effect of Bax on isolated mitochondria was mimicked by MgTX. This confirms the notion that inhibition of mitochondrial Kv1.3 channels is the first key event in the induction of mitochondrial apoptotic pathway in the case of Kv1.3 expressing cells [14].

Kv1.3 Channels in Cancer Diagnostics and Therapy

Recently, Kv1.3 channels are considered a new, potentially attractive molecular target in both cancer diagnostics and therapy [6–8, 17–20]. First of all, several studies have demonstrated altered expression of Kv1.3 in some cancer specimens in comparison with normal tissue [6–8, 17, 18]. An increased expression of Kv1.3 channels was observed in the case of breast, colon, smooth muscle (leiomyosarcoma), skeletal muscle (alveolar rhabdomyosarcoma) and lymph node cancer [8, 17] and in mature neoplastic B cells in chronic lymphocytic leukemia (B-CLL) [20]. A significantly increased expression of Kv1.3 channels that was positively correlated with tumor aggressiveness was recently observed in the case of leiomyosarcoma and alveolar rhabdomyosarcoma [8, 18]. In the case of breast cancer, contradictory data has been obtained [17, 21, and 22]. A significantly increased expression of the channels' protein was shown in cancer cells when compared to normal tissue [17]. Tumorigenic mammary epithelial M13SV1R2 and M13SV1R2-N1 cells expressed

significantly more of the channels' proteins than normal human mammary epithelial cells M13SV1 [21]. Moreover, the channel expression was significantly higher in highly tumorigenic M13SV1R2-N1 cells than in weakly tumorigenic M13SV1R2 cells [21]. On the other hand, a markedly reduced expression of Kv1.3 channels at both mRNA and protein levels was detected in a case of breast adenocarcinoma and there was an inverse correlation between the channels' expression and tumor grade [22]. It was shown that the Kv1.3 gene promoter was methylated in 42.3% of the samples (22/52) and the methylation was associated with poorly differentiated tumors and younger patients [22]. Moreover, the Kv1.3 gene promoter was methylated in the MCF-7 breast carcinoma cell line whereas the methylation was absent in a primary culture of normal breast cells (HMEpC) [22]. The data suggests that DNA methylation is responsible for the observed decrease of Kv1.3 gene expression [22]. A significantly reduced expression of the channels was also observed in kidney, bladder, pancreas, lung, brain (astrocytoma, oligodendroglioma, and glioblastoma), stomach and prostate cancer [8, 18, 22–24]. In the case of prostate cancer, there is also an inverse correlation between the expression of the channels and the stage of the disease [23]. Moreover, in prostate cancer, the channels' expression is significantly higher in the weakly metastatic LNCaP and AT-2 cell lines than in the strongly metastatic PC3 and Mat-Ly-Lu cell lines [8, 25, 26]. A significant reduction of the expression of Kv1.3 channels was also observed in the case of pancreas adenocarcinoma, especially in patients with metastasis, where the channels were almost absent [7, 8, 24]. It has been shown that the reduction of the channels' expression was also probably a consequence of the methylation of the promoter region of the Kv1.3-encoding gene in pancreatic cells. This process was enhanced in patients with metastasis. The survival distribution function showed that patients with a methylated promoter region of the Kv1.3 gene in pancreatic cells have significantly less chance to survive than patients with an unmethylated promoter region of this gene [7, 24]. Thus, expression of the Kv1.3 channels can be considered a potential diagnostic marker in some cancer diseases. However, in some cases, such as in brain cancer (astrocytoma, oligodendroglioma, glioblastoma), no clear correlation between the channels' expression and stage of the disease has been obtained yet [8].

It is well-established that cancer cells are characterized by uncontrolled proliferation combined with resistance to apoptosis induced by endogenous apoptotic stimuli. On the other hand, it has been shown that the activity of Kv1.3 channels is involved both in cell proliferation and in apoptosis. It is also known that the activity of the channels is inhibited by many specific and non-specific inhibitors [11–13]. Therefore, it is of importance to prove whether inhibition of Kv1.3 channels in cancer cells could inhibit proliferation and induce apoptosis of cancer cells.

Available data provides evidence that an application of a specific inhibitor of Kv1.3 channels, margatoxin (MgTX), significantly inhibited proliferation of the weakly metastatic prostate cancer cell line AT-2, but was ineffective in the case of the strongly metastatic prostate cancer cell line Mat-LyLu [27]. On the other hand, a non-specific inhibitor of the channels, 4-amino-piridine (4-AP), significantly inhibited the proliferation of both cell lines [27]. It was also shown that inhibition of Kv1.3 channels by a non-specific inhibitor, tetraethylamine (TEA), inhibited the proliferation of mammary epithelial M13SV1R2 and M13SV1R2-N1 cells. The inhibition was significantly higher in the highly tumorigenic M13SV1R2-N1 cells than in the weakly tumorigenic M13SV1R2 cells and in normal cells [21]. Moreover, MgTX inhibited the proliferation of lung cancer cells *in vitro* and *in vivo* [28]. It was shown that MgTX inhibited cancer cell proliferation by arresting the cell cycle in the G_1 phase [23]. The cell viability upon an application of MgTX was significantly lower in cancer cells than in normal cells [28]. Therefore, both specific and non-specific inhibitors of Kv1.3 channels may potentially be used in therapy of some cancer diseases.

Moreover, inhibition of Kv1.3 channels by specific membrane-permeant inhibitors, such as 5-(4-phenylobutoxy)psoralen(Psora-4),5-(4-phenoxybutoxy)psoralen (PAP-1) and clofazimine, induced apoptosis of Kv1.3-expressing cancer cells – murine cytotoxic T lymphocyte cell line CTLL-2 transfected with Kv1.3 vector (CTLL-2--Kv1.3), human T cell line Jurkat T, human osteosarcoma SAOS-2 and mouse melanoma B16F10 cell line, but not of Kv1.3-lacking CTLL-2 cells (CTLL-2-pJK) and human chronic myelogenous leukemia cell line K562 [19]. The knock-down of Kv1.3 channels by applying the siRNA technique eliminated the sensitivity of Jurkat T cells to apoptosis induced by the inhibitors of Kv1.3 channels [19]. The induction of death of Kv1.3-expressing cancer cells by the inhibitors of Kv1.3 channels occurred by activation of the intracellular (mitochondrial) pathway of apoptosis. This process involved an activation of caspase-9 (but not caspase-8), activation of caspase-3, release of mitochondrial cytochrome c, and increase of mitochondrial reactive oxygen species (ROS) production, depolarization of the inner mitochondrial membrane and

cleavage of poly-ADP ribose-polymerase (PARP) [19]. The induction of cancer cell apoptosis by the membrane-permeant inhibitors of Kv1.3 channels occurred in a concentration-dependent manner. The process of apoptosis induction required higher concentrations of these inhibitors than was needed to completely inhibit Kv1.3 channels. The ability to induce apoptosis of intact cancer cells was limited to membrane-permeant Kv1.3 channel inhibitors, since potent but membrane-impermeant compounds, such as MgTX (EC_{50} < 1 nM), applied at a concentration 1000-times higher than needed to completely inhibit Kv1.3 channels, were ineffective. This would seem to confirm that the inhibition of mitochondrial, but not plasma membrane Kv1.3 channels were required to induce the apoptosis of Kv1.3-expressing cancer cells by activating its intracellular (mitochondrial) pathway [19]. In agreement with this evidence, the ability of membrane-permeant Kv1.3 channel inhibitors to induce apoptosis of cancer cells was significantly augmented when the inhibitors were co-applied with inhibitors of membrane multi-drug resistance transporters. This would seem to prevent the channel inhibitors' molecules from being removed from cancer cells by membrane multi-drug resistance proteins. Moreover, clofazimine, which was the least potent Kv1.3 channel inhibitor of the three compounds tested (EC_{50} = 300 nM for clofazimine, compared to 3 nM for Psora 4 and 2 nM for PAP-1), was the most effective inducer of apoptosis of Kv1.3-expressing cancer cells (EC_{50} ~ 1 μM for clofazimine in the presence of multi-drug resistance transporter inhibitors compared to > 10 μM for both Psora-4 and PAP-1) [19]. This was probably due to the ability of this compound to block both Kv1.3 channels and membrane multi-drug resistance transporters [19]. Importantly, the results obtained demonstrate that membrane-permeant Kv1.3 channel inhibitors could induce apoptosis of Kv1.3-expressing cancer cell lines even in the absence of Bax or Bak pro-apoptotic proteins, such as in the case of Bax/Bak-deficient human Jurkat leukemic T cells and Bax/Bak-double knock-out murine embryonic fibroblasts (MEF DKO cells) [19]. A mutational inactivation-mediated deficiency of the pro-apoptotic proteins Bax and Bak often observed in tumor cells can protect them from mitochondria-mediated apoptosis induced by anticancer drugs, such as etoposide, bleomycin or cisplatin [29]. It was shown that the survival of patients suffering from both colon and gastric cancers after surgery was significantly diminished in the Bax-deficient group compared to the group of patients with a normal function of Bax [29]. Therefore, membrane-permeant Kv1.3 channel inhibitors that could induce apoptosis of Bax/Bak deficient cancer cells might offer a novel option in the treatment of chemotherapeutic-resistant malignancies. Finally, experiments performed on syngenic C57BL/6 mice showed that the inhibition of Kv1.3 channels by clofazimine applied *in vivo* not only prevented growth of an induced melanoma, but also reduced the tumor size by 90% after 6 days of treatment [19]. Histological examination and studies on cell apoptosis of the brain, heart, lungs, small intestine, kidney, liver and spleen tissues showed no significant abnormalities in these organs in clofazimine-treated mice in relation to untreated animals. The effect of clofazimine was much more efficient than in the case of a much more potent (EC_{50} < 1 nM compared to 300 nM for clofazimine), but membrane-impermeant, Kv1.3 channel inhibitor – MgTX [19]. This was probably due to the fact that clofazimine could simultaneously inhibit cancer cell proliferation (by inhibition of plasma membrane Kv1.3 channels) and induce apoptosis of these cells (by inhibition of the channels in the mitochondria), whereas MgTX could only inhibit cell proliferation without being able to induce apoptosis [19, 28]. In fact, application of MgTX or other membrane-impermeant Kv1.3 channel inhibitors, such as *Stichodactyla helianthus* (Shk) or charybdotoxin (ChTX) could even slightly increase the number of viable cancer cells [19].

Recently published data showed that the inhibition of Kv1.3 channels over-expressed in human neoplastic B cells in chronic lymphocytic leukemia (B-CLL) by Psora-4, PAP-1 and clofazimine induced apoptosis of these cells by activation of the intracellular (mitochondrial) pathway of this process [20]. In agreement with the results of previous studies, the more potent, but membrane-impermeant Kv1.3 channel inhibitor *Stichodactyla helianthus* (Shk) was ineffective. Moreover, the ability to induce apoptosis of B-CLL cells was significantly augmented when the inhibitors were co-applied with inhibitors of membrane multi-drug resistance transporters. Finally, clofazimine was the most effective inducer of apoptosis of B-CLL cells (EC_{50} ~ 1 μM in the presence of multi-drug resistance transporter inhibitors compared to > 10 μM for both Psora-4 and PAP-1) [20]. Interestingly, an application of the Kv1.3 channel inhibitors caused a selective apoptosis of B-CLL cells without affecting T lymphocytes from patients with leukemia and B and T lymphocytes from healthy donors. The authors showed that such a selective apoptosis of cancer cells was a consequence of both the inhibition of Kv1.3 channels and a chronically increased production of reactive oxygen species (ROS) by the mitochondria of cancer cells [20]. It was shown that a pre-treatment of B-CLL cells with

membrane-permeant ROS scavengers: superoxide dismutase (PEG – SOD) and catalase (PEG – CAT) eliminated the apoptosis of cancer cells induced by the Kv1.3 channel inhibitors. On the other hand, a mild mitochondrial oxidative stress induced by a pre-treatment of normal B lymphocytes with a sub lethal dose of a membrane-permeant pro-oxidant agent, 7-O-(4-triphenylphophoniumbutyl) quercetin iodide (Q-7BTPI), sensitized normal B cells to a pro-apoptotic activity of the Kv1.3 channel inhibitors [20]. A mild mitochondrial oxidative stress in combination with an application of the Kv1.3 channel inhibitors did not induce apoptosis of the Kv1.3-deficient murine CTLL-2 pJK cancer cell line. This demonstrates that both the mild mitochondrial oxidative stress observed in cancer cells and an inhibition of Kv1.3 channels is necessary for a selective induction of cancer cell apoptosis by the Kv1.3 channel inhibitors [20]. It was also shown that an application of the Kv1.3 channel inhibitors caused a selective apoptosis of B-CLL cells in 29 patients regardless of the stage of the disease and despite an increased expression of anti-apoptotic Bcl-2 proteins in B-CLL cells in most cases [20]. Importantly, it was shown that injections of clofazimine into mice at therapeutic doses (5 μg/g mouse) did not significantly change blood cell counts in the animals treated. The results of these studies show that small-molecule membrane-permeant inhibitors of Kv1.3 channels could be considered as efficient pharmacological agents to be potentially applied in therapy of malignant tumor diseases characterized by an overexpression of Kv1.3 channels. Clofazimine, which has been used since the 1960's in medical practice as an antibiotic (in the treatment of leprosy, for example), may be considered a most promising candidate [20].

Polycyclic Compounds as Inhibitors of Kv1.3 Channels

There are many other Kv1.3 channel inhibitors that are membrane-permeant small-molecule organic compounds and could simultaneously inhibit cancer cell proliferation (by inhibition of plasma membrane Kv1.3 channels) and induce apoptosis of these cells (by inhibition of the channels in the mitochondria). To this group belong, among others, some biologically-active plant-derived polycyclic compounds from the groups of flavonoids, chalcones and substituted stilbenes and some of their natural and synthetic derivatives. These plant-derived compounds are present in everyday diet products and are characterized by a low cytotoxicity. The ability of the compounds, characterized by good bioavailability, to inhibit proliferation and induce apoptosis of cancer cells by inhibition of Kv1.3 channel activity could potentially be applied to support therapy of some tumor diseases, such as breast, colon and lymph node cancer, melanoma or chronic lymphocytic leukemia (B-CLL), in which the Kv1.3 channels are overexpressed in comparison to healthy tissues. The possibility of an effective elimination of cancer cells by low-toxic compounds present in everyday diet should be considered important to the study of both the biology of cancer and the development of new strategies for cancer treatment. Taking into account both the ever-increasing number of people suffering from malignant tumors and the limited effectiveness of the therapeutic strategies currently applied, the enhancement of knowledge concerning the cellular mechanisms of cancer cell destruction seems to be a scientific problem of social significance.

Experiments carried out during the last 10 years provide evidence that some biologically-active plant-derived polycyclic compounds from the groups of flavonoids and stilbenes and some of their natural and synthetic derivatives are inhibitors of Kv1.3 channels in both normal and cancer cells. It was shown that a compound from the group of isoflavones, genistein, and a compound from the group of substituted stilbenes, resveratrol, are both inhibitors of Kv1.3 channels in human T lymphocytes isolated from the peripheral blood of healthy donors [30, 31]. Genistein, a plant-derived isoflavone known as a potent protein tyrosine kinase (PTK) inhibitor, also inhibits cell proliferation in breast cancer cell lines MCF-7 and MDA-MB-231 [32], colon cancer cell line HT-29 [33] and induces apoptosis in colon cancer cell line HT-29 [33]. Resveratrol is a natural anti-cancer agent which is present at its highest concentrations in red grapes and wine. Resveratrol is also involved in the inhibition of lipid peroxidation, chelation of copper ions, scavenging of free radicals, alteration of eicosanoid synthesis, inhibition of platelet aggregation, anti-inflammation, vaso-relaxation, estrogenic activity and cardio-protection [34]. The inhibition of Kv1.3 channels by genistein and resveratrol occurred in a concentration-dependent manner and was not complete – the channels were inhibited to about 23% and to about 18% of their control activity at maximal concentrations of the compounds, equal to 80 μM and 200 μM in the case of genistein and resveratrol, respectively [30, 31]. The estimated values of the half-blocking concentrations (EC_{50}) were from 10 to 40 μM for genistein and about 40 μM for resveratrol [30, 31]. The

inhibition of Kv1.3 channels by both genistein and resveratrol was accompanied by a significant slowing of the Kv1.3 currents' activation rate, whereas the inactivation rate remained unchanged upon an application of the compounds [30, 31]. The inhibitory effects of genistein and resveratrol were additive [31]. This may suggest that the mechanisms of Kv1.3 channel inhibition by genistein and resveratrol were distinct [31]. It was shown that the inhibitory effect of genistein on human T lymphocyte Kv1.3 channels occurred in a PTK-independent pathway, probably as a result of direct interactions of the compound with the channels' proteins [30]. The results have also demonstrated that resveratrol, which is an activator of protein kinase C (PKC), inhibits Kv1.3 channels in a PKC-independent pathway, probably by direct interactions with the channels' protein [31]. The ability to inhibit Kv1.3 channels in normal human T lymphocytes is shared by two synthetic methoxy- derivatives of a flavonoid naringenin (4', 7-dimethylether and 7-methylether) and a synthetic tetramethoxy-derivative of a substituted stilbene-piceatannol [35]. It was shown that application of naringenin-4', 7-dimethylether, naringenin- 7-methylether and tetramethoxy- piceatannol at a 30 μM concentration inhibited Kv1.3 channels to about 4%, 29% and 31% of the control activity, respectively [35]. On the other hand, it was shown that naringenin and piceatannol did not inhibit Kv1.3 channels in normal human T lymphocytes when applied at 30 μM and 40 μM concentrations, respectively [35]. Another compound from the group of flavonoids, aromadendrin, was also not an inhibitor of Kv1.3 channels in normal human T lymphocytes when applied at 30 μM concentration [35]. Interestingly, most of the tested compounds, like genistein and synthetic methoxy-derivatives of naringenin and piceatannol, have an ability to inhibit the activity of membrane multidrug resistance proteins MRP1 [36–38]. Although the results showed no correlation between the ability of selected flavonoids to inhibit membrane multidrug resistance proteins MRP1 and Kv1.3 channels in normal human T lymphocytes, it was shown that inhibition of both types of membrane proteins could occur, for genistein and methoxy-derivatives of piceatannol and naringenin, at comparable concentrations [30, 35]. This would suggest that an inhibition of the channels could be accompanied by a simultaneous inhibition of membrane multidrug resistance proteins MRP1 [35]. It seems possible that such a simultaneous inhibition of Kv1.3 channels and the membrane multidrug resistance proteins could occur in the case of Kv1.3-expressing cancer cells.

Studies performed recently provide evidence that a prenyl-derivative of naringenin – 8-prenylnaringenin – inhibits Kv1.3 channels both in normal human T lymphocytes and in human T cell line Jurkat when applied at low micromolar concentrations [39]. This compound is a natural derivative of naringenin that is isolated from common hops (*Humulus lupulus*). This compound was identified as a potent phytoestrogen, with estrogenic activity higher than that of the known phytoestrogens like coumesterol, genistein and daidzein [40]. Other studies showed that 8-prenylnaringenin could also inhibit cell proliferation and induce apoptosis in the breast cancer cell line MCF-7 [40]. The results of electrophysiological studies indicate that 8-prenylnaringenin inhibits Kv1.3 channels in a concentration-dependent manner [39]. The estimated EC_{50} value is about 3 μM. In contrast to what was observed earlier in the case of genistein and resveratrol, the inhibitory effect of 8-prenylnaringenin was complete. A complete inhibition of Kv1.3 channels occurred at concentrations higher than 10 μM [39]. The inhibitory effect of 8-prenylnaringenin was reversible. In contrast to the inhibitory effects of genistein and resveratrol, the inhibition of Kv1.3 channels by 8-prenylnaringenin was accompanied by a significant acceleration of channel inactivation without any pronounced change in the activation rate. 8-prenylnaringenin is the most potent inhibitor of the Kv1.3 channels of the naringenin derivatives tested to date. The potency of the inhibition may be due to the presence of a prenyl group in the molecule of this flavonoid [39].

Altogether, the available results demonstrate that:

1) some of the studied flavonoids and stilbenes inhibit Kv1.3 channels,

2) the inhibitory effects are not complete at micromolar concentrations, except for 8-prenylnaringenin,

3) differences in chemical structures between the channels' inhibitors and non-inhibitors are very subtle,

4) the mechanism of the channels' inhibition is probably different in the case of each tested compound,

5) the inhibitory effects of some compounds are additive.

Nevertheless, the influence of most biologically-active plant-derived polycyclic compounds on the activity of Kv1.3 channels in cancer cells still remains unknown. Even in the case of the compounds already tested, such as genistein and resveratrol, a study on the influence of these compounds on Kv1.3 channels in cancer cells is needed. It also remains unknown whether there is a relationship between the inhibition of Kv1.3 channels in cancer cells by biologically active polycyclic compounds

and the ability of these compounds to induce apoptosis of Kv1.3 channel-expressing cancer cells. Studies on the influence of selected biologically-active plant-derived polycyclic compounds on the activity of Kv1.3 channels in cancer cells and on the ability of these compounds to induce apoptosis of Kv1.3 expressing cancer cells are ongoing.

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Address for correspondence:

Andrzej Teisseyre
Department of Biophysics
Wroclaw Medical University
Chałubińskiego 10
50-368 Wrocław
Tel.: +48 71 784 14 14
E-mail: andrzej.teisseyre@umed.wroc.pl

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