



Short Communication

Astemizole inhibits cell proliferation in human prostate tumorigenic cells expressing *ether à-go-go-1* potassium channels

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Received April 30, 2017; Accepted December 14, 2017; Published December 17, 2017

Doi: <http://dx.doi.org/10.14715/cmb/2017.63.12.4>

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Abstract: Prostate cancer (PC) is the main cause of cancer mortality in men worldwide. Therefore, novel treatments for PC are needed. *Ether à-go-go-1* (Eag1) potassium channels display oncogenic properties, and have been suggested as early tumor markers and therapeutic targets for different cancers. These channels are overexpressed in many human tumors including PC. Astemizole targets several molecules involved in cancer including Eag1 channels, histamine receptors and ABC transporters. Here we studied Eag1 mRNA expression and protein levels in the non-tumorigenic and non-invasive human prostate RWPE-1 cell line, and in the tumorigenic and highly invasive human prostate WPE1-NB26 cell lines. The effect of astemizole on cell proliferation and apoptosis was also studied. The human prostate cell lines RWPE-1 and WPE1-NB26 were cultured following the provider's instructions. Eag1 mRNA expression and protein levels were studied by real time RT-PCR and immunocytochemistry, respectively. Cell proliferation and apoptosis were studied by a fluorescence AlamarBlue® assay and flow cytometry, respectively. No difference in Eag1 mRNA expression was observed between the cell lines. However, high Eag1 protein levels were observed in the invasive WPE1-NB26 cells, in contrast to the weak protein expression in RWPE-1 cells. Accordingly, astemizole decreased cell proliferation at nanomolar concentrations only in the invasive WPE1-NB26 cells. Our results suggest that astemizole may have clinical relevance for prostate cancer treatment in patients with high Eag1 protein levels.

Key words: Astemizole; Prostate cancer; Ion channels; Eag1; Anti-histamine.

Introduction

Prostate cancer (PC) remains a major cause of cancer mortality worldwide (1). Thus, novel therapeutic approaches are urgently needed. Potassium channels have generated great interest as promising tools in oncology (2). One of the most studied ion channels in cancer is the voltage-gated potassium channel *ether à-go-go-1* (Eag1, KCNH1, Kv10.1) (3). This channel shows restricted distribution in normal tissues but is detected in over 70% of human tumors including PC, as well as in an immortalized cell line from a normal prostate and in some PC cell lines (4-7). Astemizole is an anti-histamine with anti-cancer properties that targets several molecules involved in cancer including Eag1 and Herg potassium channels, histamine receptors, and ABC transporters (8). We wondered if there is differential Eag1 expression in a model of PC-progression cell lines, namely, the human prostate epithelial cell lines RWPE-1 (normal, immortalized, non-tumorigenic, and non-invasive) and WPE1-NB26 (tumorigenic and highly invasive, derived from RWPE-1 cells) (9). We also investigated if astemizole may have anti-proliferative and pro-apoptotic effects on these cells.

Materials and Methods

Cell lines and reagents

The human prostate cell lines RWPE-1 and WPE1-NB26 were obtained from the American Type Culture Collection (Manassas, VA) and cultured following provider's instructions. Astemizole and DMSO were purchased from Sigma Chemical Co (St. Louis, MO). The anti-Eag1 antibody was purchased from Novus International (Littleton, CO).

Real-time RT-PCR

Total RNA was extracted with TRIzol. Real-time PCR was performed using the TaqMan™ system (Applied Biosystem, Foster City, CA, USA) and reagents kit (Life Technologies). The TaqMan probes used were: Eag1 (part number Hs00924320_m1) and h-cyclophilin-A (part number Hs04194521_s1, constitutive gene). Data were analysed with the $2^{-\Delta\Delta C_t}$ method.

Immunocytochemistry

Cells were seeded on charged glass slides, boiled for antigen retrieval and processed as described (10). The anti-Eag1 antibody was used at a 1:500 dilution (Novus International Inc. USA) overnight at 4°C. Sections were

counterstained with hematoxylin (Dako, Denmark). The slides were observed using an Olympus IX51 microscope, and photos were taken with an Olympus-DP70 camera.

Cell Proliferation assays

Cells (5×10^3) were cultured for 3 days and then incubated for 96 h in culture medium alone or with astemizole or DMSO. Then, the cells were rinsed and incubated for 5 hours in medium with 10-vol% Alamar-Blue® (AbD Serotec Ltd.). Following incubation, the medium was transferred to 96-well black-polystyrene microplates. Fluorescence (excitation 530 nm, emission 590 nm) was measured on a FLUOROSKAN ASCENT microplate reader (Labsystems).

Apoptosis experiments

Apoptosis was studied as described (11). Briefly, 8×10^4 cells were grown and treated with astemizole as described above for the proliferation experiments. Camptothecin (apoptosis inducer) and methanol (necrosis inducer) were used as positive controls, and were added 24 hours before the end of the treatment with astemizole. Apoptosis was determined with the Annexin V-FITC kit (Invitrogen Co.) binding to phosphatidylserine and DNA staining by propidium iodide (PI). Experiments were carried out with the flow cytometer CYAN ADP (Dako, Glostrup, Denmark). Percentages of viable (FITC and PI negative), apoptotic (FITC positive and PI negative), and late apoptotic (FITC and PI positive) cells were obtained by quadrant analysis using Summit 4.3 software.

Statistical analysis

Analysis of variance followed by the Tukey-Kramer test was used to compare data between groups. *P* values < 0.05 were considered statistically significant. The analysis was conducted using the GraphPad Prism software version 5.0.

Results

Differential Eag1 expression and effect of astemizole on prostate cell lines

RWPE-1 (normal, immortalized, non-tumorigenic, and non-invasive) and WPE1-NB26 (tumorigenic and highly invasive) cells displayed similar Eag1 mRNA expression as assessed by real-time PCR (Fig. 1A). However, while RWPE-1 cells showed very low Eag1 protein levels (Fig. 1B), the invasive WPE1-NB26 cells displayed high Eag1 protein expression in the cytoplasm and nucleus (brown immunostaining, Fig. 1C). Accordingly, astemizole decreased RWPE-1 cell proliferation only at the highest concentration tested (2 μ M, Fig. 1D), but decreased WPE1-NB26 cell proliferation in a concentration-dependent manner, starting from nanomolar concentrations (Fig. 1E). Then, we wondered if astemizole may also induce apoptosis. We did not observe pro-apoptotic effects of astemizole in any cell line in the nanomolar range. Then, we selected the most astemizole-sensitive cell line and studied the effect of a higher concentration (2 μ M). Figure 1F shows that this concentration of astemizole induced apoptosis in the WPE1-NB26 cells.

Discussion

Previous studies demonstrated Eag1 expression in PC human samples (6), as well as in an immortalized cell line from normal prostate cells and in some PC cell lines (4, 5). Using the PC-progression model of RWPE-1 cells (9) we observed that both the normal non-tumorigenic and non-invasive RWPE-1 cells and the tumorigenic and invasive WPE1-NB26 cells displayed Eag1 mRNA expression. Although we expected higher Eag1 mRNA expression in the tumorigenic cells, the Eag1 mRNA expression in the non-tumorigenic cells may be due to the presence of the HPV-18 genome in this cell line (9) because HPV oncogenes induce Eag1 expression (10).

Interestingly, while RWPE-1 cells displayed very low Eag1 protein levels, the tumorigenic and invasive WPE1-NB26 cells showed high Eag1 protein levels. Although it is common to find limited correlations between mRNA and protein levels in different systems (12), more studies are needed to determine this seeming lack of correlation. For instance, Eag1 expression and channel activity are regulated through the cell cycle and by steroid hormones (10, 13-15). Consequently, it will be interesting to know how Eag1 is regulated by these different factors and conditions in prostate cells. In addition, the half-life of Eag1 mRNA and protein in this

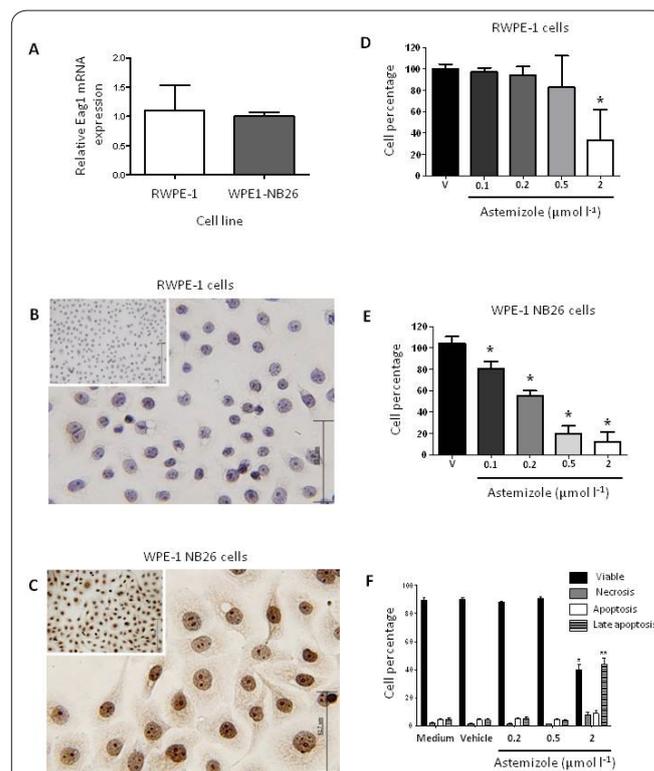


Figure 1. Eag1 expression and effects of astemizole on the proliferation and apoptosis of prostate cells. Relative Eag1 mRNA expression did not show differences between cell lines (A). Eag1 protein level was weakly detected in RWPE-1 cells (B), while WPE1-NB26 cells showed strong Eag1 protein expression (brown immunostaining, C). Magnification = 400x, insert = 200x. Astemizole inhibited cell proliferation of RWPE-1 cells only at the highest concentration tested (D), but clearly had higher potency in the invasive WPE1-NB26 cells (E), * *p* < 0.05 vs vehicle. Apoptosis was increased by astemizole in the WPE1-NB26 cells at the highest concentration tested (F), * *p* < 0.05 vs viable or ** late apoptosis in all the other groups.

type of tumor should be also studied.

It was noteworthy that we observed that the WPE1-NB26 cells were more sensitive to astemizole in the cell proliferation studies. This differential effect may be associated to the high Eag1 protein expression observed in the invasive cells. However, because astemizole targets several proteins involved in cancer, the precise molecular mechanism of the anti-proliferative effect of astemizole on prostate cancer cells should be elucidated. For instance, Eag1-silencing experiments and co-incubation of astemizole with specific antagonists of histamine receptors or ABC transporter inhibitors should be tested. Nevertheless, the stronger effect of astemizole on the proliferation of cells displaying high Eag1 protein expression suggests that this compound may have clinical relevance in tumors with high Eag1 protein levels. This is especially important because Eag1 has been associated with cancer progression from early stages. For instance, Eag1 channel expression is regulated by some cancer etiological factors like human papillomavirus oncogenes and estrogens, and it is expressed in low- and high- grade intraepithelial cervical lesions and non-cancerous breast tissue adjacent to breast tumor tissue (2, 10).

We did not observe pro-apoptotic effects of astemizole in any cell line in the nanomolar range. Additional studies including longer incubation times with astemizole, cell cycle arrest, earlier stages of apoptosis, and investigation of other mechanisms of death are needed.

Although *in vivo* studies and clinical trials are needed to reposition astemizole for PC therapy, our results suggest that this anti-histamine may be a new treatment for PC patients having tumors with high Eag1 levels.

Interest conflict

The authors declare that they have no conflict of interest.

Author's contribution

GB-R, EH-G, EV, MdGC-L, VZ-G, and EG performed experiments. All authors contributed to experiment design and analysis of data, as well as in the manuscript preparation and revision.

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