Original Article
Lidocaine suppresses glioma cell proliferation by inhibiting TRPM7 channels

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Abstract: Background: Malignant glioma is the most common brain cancer with devastating prognosis. Recurrence of malignant glioma following surgery is very common with few preventive and therapeutic options. Novel targets and therapeutic agents are constantly sought for better outcome. Our previous study established that inhibition of transient receptor potential melastatin 7 (TRPM7) channels resulted in significant decrease of human glioma cell growth and proliferation. As local anesthetic lidocaine has been shown to inhibit TRPM7 currents, we hypothesize that lidocaine may suppress glioma cell proliferation through TRPM7 channel inhibition. Methods: TRPM7 currents were recorded in rat C6 glioma cells using the whole cell patch clamp technique. Cell growth and proliferation were assessed under microscopic examination and biochemical assays. Results: Lidocaine inhibits TRPM7-like currents in a dose-dependent and reversible manner. At 1 and 3 mM, it inhibits ~30% and ~50% of TRPM7 currents. At these concentrations, it is effective in inhibiting the proliferation of C6 cells. As expected, the TRPM7 inhibitors gadolinium and 2-Aminoethoxydiphenyl borate have similar effects on TRPM7 currents and proliferation of C6 cells. Similar to its effect on C6 cells, lidocaine inhibits the proliferation of A172 cells, a human glioblastoma cell line. Conclusions: Lidocaine significantly inhibits the proliferation of glioma cells. The effect of lidocaine is mediated, at least in part, by inhibiting TRPM7 channels.

**Keywords:** Lidocaine, local anesthetic, TRPM7, glioma, proliferation

Introduction

Malignant glioma is the most common primary brain tumor with devastating prognosis [1]. Maximal resection is essential for the treatment but surgery itself is a risk factor for recurrence [2]. Recurrence of malignant glioma following surgery is very high with few preventive and therapeutic options. One major advance over last two decades is the development of temozolomide that increased the survival time only by about 2.6 months after surgery when adding to radiotherapy [3, 4]. Even so, around 70% malignant glioma is not responsive to temozolomide [5]. Therefore, it is hoped that potential new targets and drugs are able to reduce the growth and proliferation of glioma cells and increase the survival rate of cancer patients.

The melastatin-like transient receptor potential 7 (TRPM7) is a member of TRPM family mediat-ing the entry of Ca^{2+} and Mg^{2+} [6]. High expression of TRPM7 has been found in a number of human cancer tissue and cell lines, including head and neck cancer, breast cancer, ovarian cancer, prostate cancer and pancreatic cancer [7-11]. TRPM7 plays an important role in maintaining the homeostasis of Ca^{2+} and Mg^{2+} in cancer cells regulating the cell function and cycle [12]. Thus it appears to be an attractive target to suppress the cancer cell proliferation and potentially reduce the recurrence of cancer. Our previous studies have demonstrated that TRPM7 play an important role in the proliferation, migration and invasion of human malignant glioma cells [13], suggesting that TRPM7 might serve as a promising target for malignant glioma treatment.

Lidocaine is a widely used local anesthetic that can be administered to local or regional tissue, epidural or intrathecal space to induce nerve conduction block. It has been shown that lido-
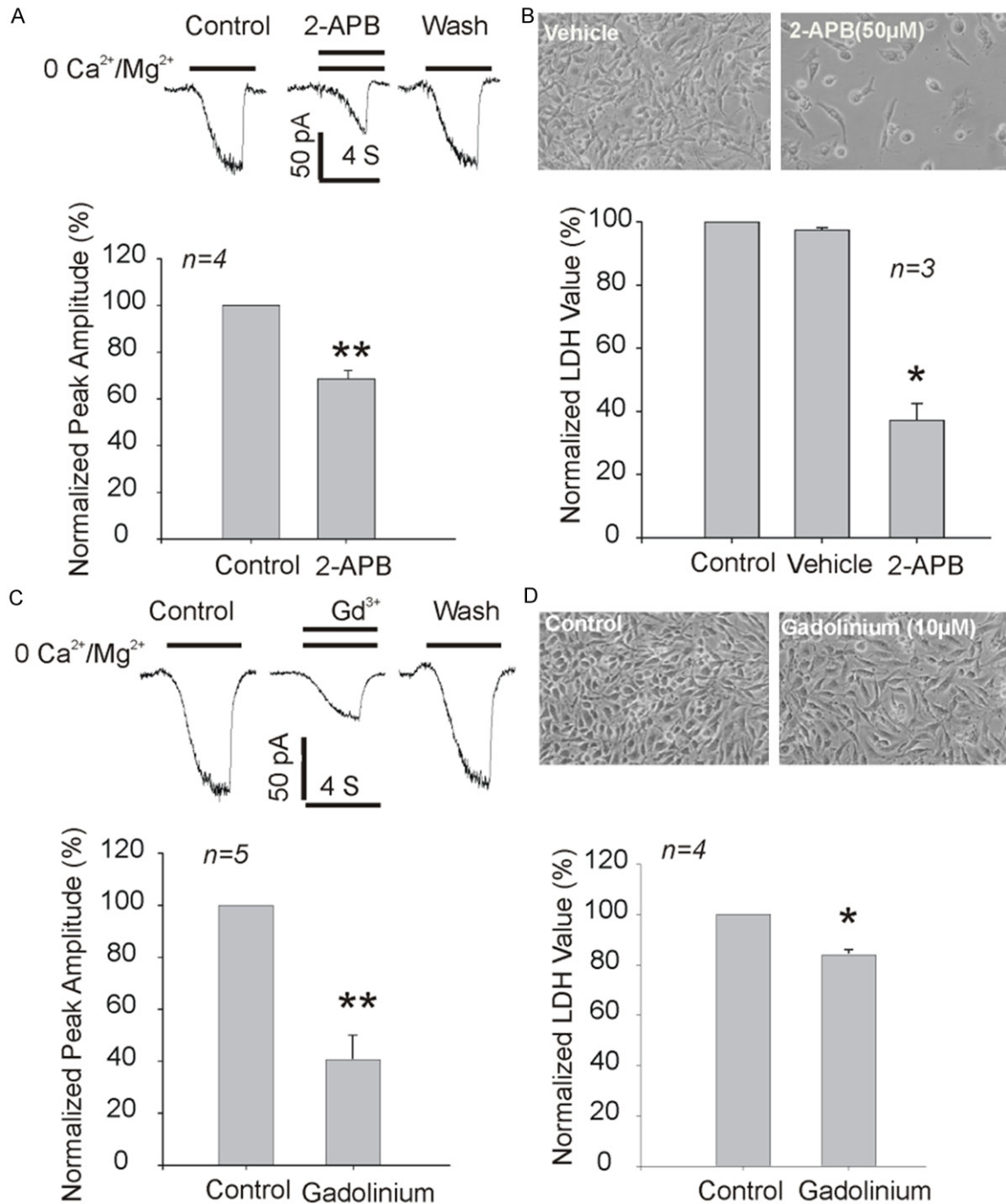
Figure 1. 2-APB and Gadolinium inhibit TRPM7 currents and proliferation of C6 glioma cells. A: Representative traces and summary data showing the inhibition of TRPM7 currents by 50 μM 2-APB. B: Effects of 2-APB on the growth and proliferation of C6 glioma cells as measured by total LDH release. Data were from three independent experiments. C: Representative traces and summary data showing the inhibition of TRPM7 currents by 10 μM Gadolinium. D: Effects of Gadolinium on the growth and proliferation of C6 glioma cells as measured by total LDH release at 48 h. Data were from four independent experiments. *P<0.05 and **P<0.01, unpaired t-tests.

caine is neuroprotective against ischemia [14] and Zn^{2+} induced neurotoxicity [15]. Lidocaine is the only local anesthetic that can be adminis-

tered intravenously. Intravenous infusion of lidocaine has been found safe and effective in reducing postoperative ileus and pain following

colon resection [16]. We have found that lidocaine inhibits TRPM7 currents in HEK293 cells and cultured neurons [15]. A safe and beneficial profile of lidocaine makes it attractive to test its potential effect on cancer cell biology. In the current study, we demonstrated that lidocaine inhibits the proliferation of malignant glioma cells at least in part, by inhibiting TRPM7 channel currents.

Materials and methods

Cell culture

C6 rat glioma and A172 Human glioblastoma cell lines were cultured in Dulbecco's modified Eagle's medium (MEM, Sigma, St. Louis, MO) plus 10% fetal bovine serum, 50 units/ml penicillin, and 50 µg/ml streptomycin at 37°C. Cells were plated in 35 mm poly-L-ornithine coated dishes for electrophysiological recording and proliferation assay 2 days after plating.

Electrophysiology

Whole-cell patch clamp recordings were performed as described previously [15]. Data were acquired using an AXOPATCH 200B amplifier with pCLAMP 8.1 software and filtered at 2 kHz and digitized at 5 kHz using Digidata 1322A (Axon Instruments, Foster City, CA). Unless otherwise specified, cells were voltage-clamped at -60 mV. Patch electrodes were constructed from thin-walled borosilicate glass (1.5 mm diameter; WPI, Sarasota, FL) on a two-stage puller (PP83, Narishige, Tokyo, Japan). Pipettes had a resistance of 2-4 MW when filled with the intracellular solution (see below). For rapid changes of extracellular solutions, a multibarrel perfusion system (SF-77, Warner Instruments, Hamden, CT) was used. (WPI, Sarasota, FL). All experiments were performed at room temperature.

LDH assay

Lactate dehydrogenase (LDH) assay was performed as described [15]. At the end of the experiments, 0.5% Triton X-100 was used to permeabilize the cells for obtaining the maximal releasable LDH. After 30 min, 50 µl medium was collected from each well. 50 µl of assay mixture from Cytotoxicity Detection Kit (Roche Applied Science) was then added to each sample. Following 30 min incubation at room temperature in dark, the absorbance at 492 nm was read on a multi-well plate reader, subtracting background absorbance measured at 620 nm.

Solutions and chemicals

Standard extracellular solution contained (in mM): 140 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 20 HEPES, 10 glucose (pH 7.4 adjusted with NaOH; 320-335 mOsm). For divalent-free external solutions, $CaCl_2$ and $MgCl_2$ were removed with osmolarity adjusted with sucrose. Patch electrodes contained (in mM): 140 CsF, 10 HEPES, 1 $CaCl_2$, 11 EGTA, 2 TEA (pH 7.3 adjusted with CsOH, 290-300 mOsm). 2-Aminoethoxydiphenyl borate (2-APB) was purchased from Calbiochem (San Diego, CA); gadolinium chloride was from Sigma.

Statistics

Data are expressed as mean ± S.E. Student's t-test was employed as appropriate for the analysis of statistical significance.

Results

2-APB and Gd^{3+} inhibit TRPM7 currents and the proliferation of rat C6 glioma cells

The presence of functional TRPM7 channel in rat C6 glioma cells was confirmed by whole-cell patch clamp. Reducing the extracellular Ca^{2+} concentration from 2 to 0 mM evoked a non-desensitizing inward current (**Figure 1A** and **1C**), which characteristic is consistent with our previous report of TRPM7 currents expressed in HEK-293 cells [15]. This current was inhibited by *2-APB and* Gd^{3+} (**Figure 1A** and **1C**), the nonspecific TRPM7 inhibitors, further confirming the presence of *TRPM7 currents in rat C6 glioma cells.* We then examined whether *2-APB and* Gd^{3+} have inhibitory effect on the proliferation of C6 glioma cells. We found that the proliferation of C6 glioma cells were significantly suppressed by *2-APB and* Gd^{3+} (**Figure 1B** and **1D**), which findings are agreeable with our previous results in human glioma cells.

Lidocaine inhibited TRPM7 currents in C6 glioma cells

We further examined the effect of lidocaine on TRPM7 currents in C6 glioma cells. As expected, the evoked non-desensitizing inward current was blocked by lidocaine in a dose-depen-
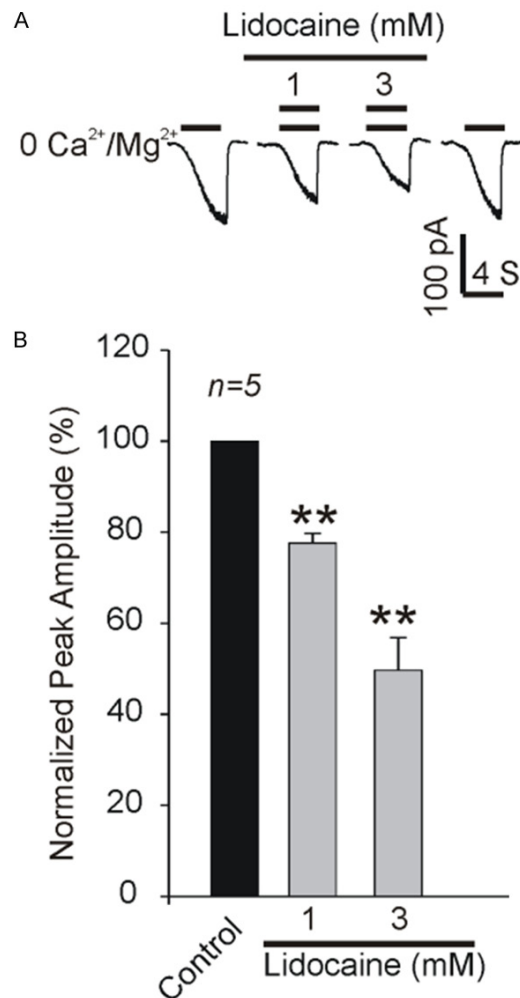
Figure 2. Inhibition of TRPM7 currents by lidocaine in C6 glioma cells. Representative traces (A) and summarized data (B) showing the inhibition of TRPM7 currents by 1 and 3 mM lidociane in C6 glioma cells. **P<0.01, n=5 cells.

dent and reversible manner (**Figure 2A**). Around 20% and 50% of TRPM7 currents were inhibited by 1 and 3 mM lidocaine, respectively (**Figure 2B**).

Lidocaine inhibited proliferation of C6 glioma cells

As TRPM7 plays an important role in glioma cell proliferation, we speculated that lidocaine might have an inhibitory effect on the proliferation of glioma cells by negative modulation of TRPM7 channel function. As expected, microscopic examination showed that lidocaine significantly suppressed the growth of C6 cells in a dose dependent fashion (**Figure 3A**). The inhi-
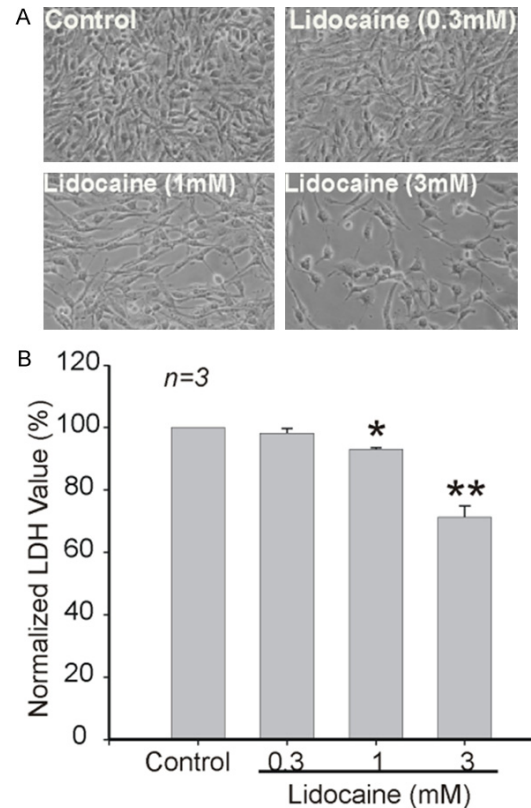

Figure 3. Inhibition of growth and proliferation of C6 glioma cells by lidocaine. A: Representative images showing the growth and proliferation of C6 glioma cells in the absence or presence of variable indicated concentrations of lidocaine for 48 h. B: Effects of lidocaine on the growth and proliferation of C6 glioma cells as measured by total LDH release. *P<0.05, **P<0.01. Data were from three independent experiments.

bition of proliferation was supported by LDH assays, which showed 30% reduction of total LDH at 3 mM (**Figure 3B**).

No voltage-gated Na+ and ASIC currents were recorded in C6 glioma cells

Lidocaine is a potent voltage-gated Na+ channel inhibitor and has recently been identified as an ASIC inhibitor [17]. ASIC was reported to play a role in some glioma cells [18]. The inhibition of lidocaine on those channels might contribute to its inhibition on the proliferation of C6 rat glioma cells. However, our findings show that there are no voltage-gated Na+ and ASIC currents in this cell line (**Figure 4A** and **4B**). A small and non-desensitized current was induced by lower pH of 4.5, however, it was not inhibited by high concentration of amiloride, excluding the
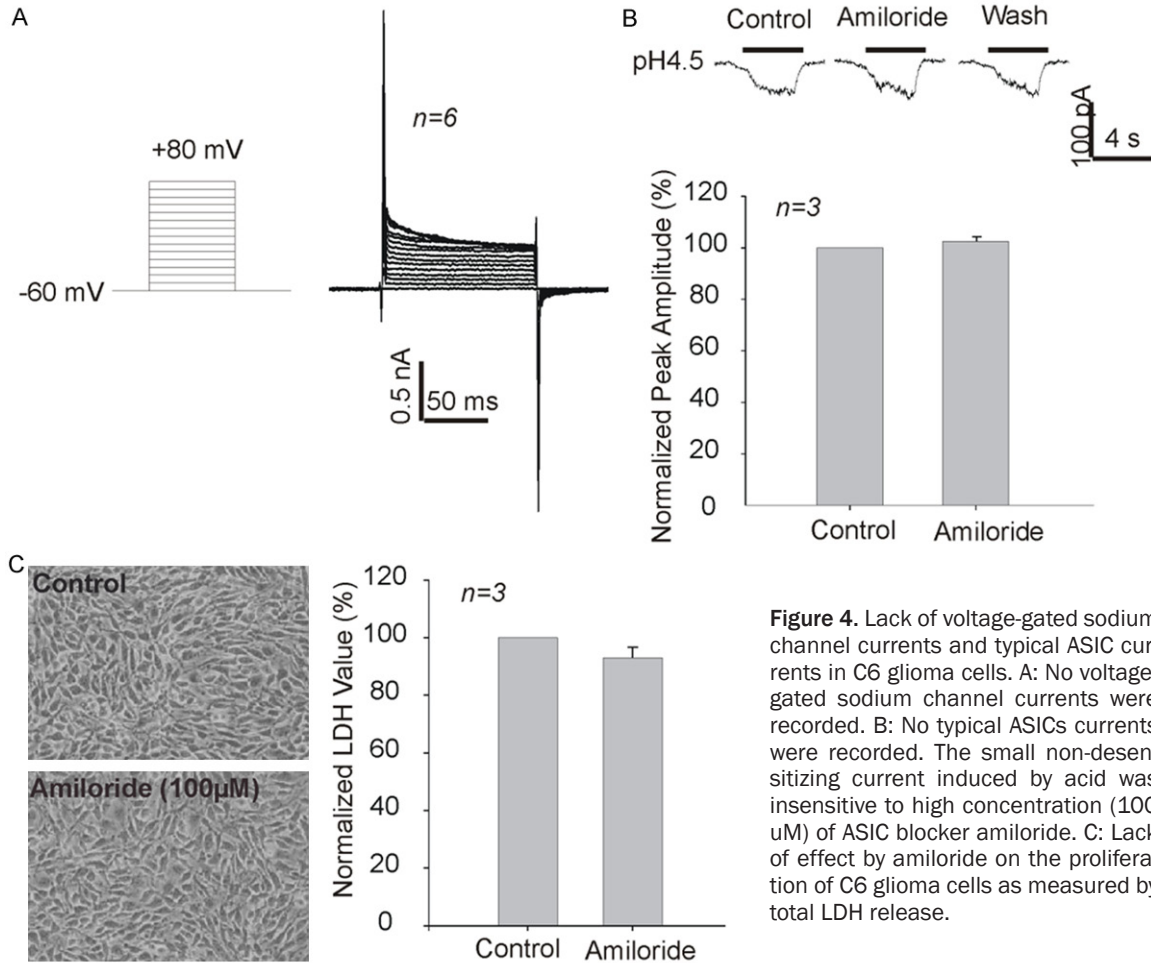
Figure 4. Lack of voltage-gated sodium channel currents and typical ASIC currents in C6 glioma cells. A: No voltage-gated sodium channel currents were recorded. B: No typical ASICs currents were recorded. The small non-desensitizing current induced by acid was insensitive to high concentration (100 uM) of ASIC blocker amiloride. C: Lack of effect by amiloride on the proliferation of C6 glioma cells as measured by total LDH release.

possibility of ASIC current (**Figure 4B**). In addition, amiloride has no significant inhibitory effect on the proliferation of C6 glioma cells (**Figure 4C**).

The inhibitory effect on glioma cell proliferation by lidocaine was further extended to human A 172 glioma cells. Visual examination under microscope indicated a significant inhibition occurred at 0.3 mM and the inhibition increased at higher concentration of 1 and 3 mM (**Figure 5**). Interestingly, A172 cells seem to be more sensitive to lidocaine treatment, suggesting that other potential mechanisms exist in this cell line.

Discussion

Most of glioma are malignant and account for 81% of malignant tumors in CNS and overall 29% of all primary brain and CNS tumors [1]. Glioblastoma (also known as glioblastoma mul-tiforme, grade IV astrocytoma) is the most common form of glioma with dismal prognosis. Current standard of care for primary glioblastoma is surgical resection, combined with chemotherapy and radiation. In addition to chemotherapy with temozolomide, a number of target therapies on various growth hormones and intracellular pathways have been attempted with limited success [19]. Recurrence after surgical resection is inevitable, and estimated survival is only 15-18 months upon diagnosis and 5 year survival is only 5%.

TRPM7 has been shown to be overexpressed in a number of human cancer cells and mediate cell proliferation, migration, invasion and metastasis, including head and neck cancer cells [7], breast cancer cells [7, 8], ovarian cancer [7, 9], prostate cancer [10], and lung cancer [20]. Inhibition of TRPM7 currents or knockdown of TRPM7 expression by small interference RNA was able to suppress their malignant
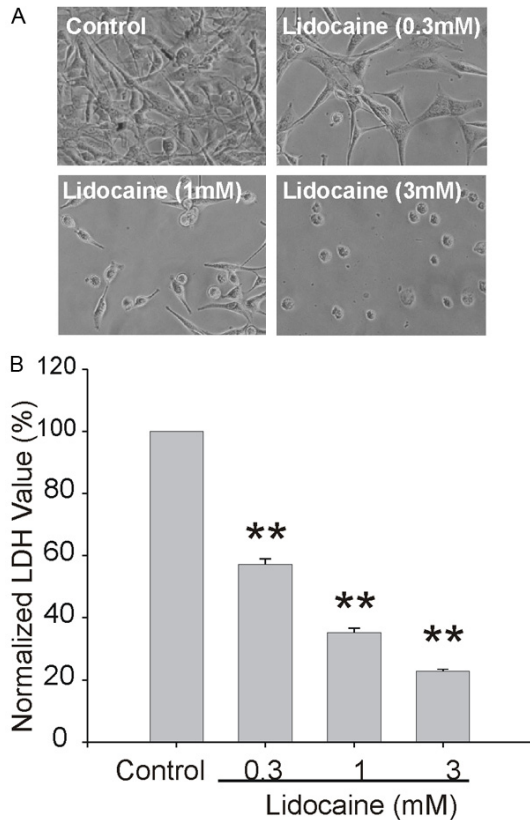
Figure 5. Effects of lidocaine on the growth and proliferation of A172 human glioma cells. A: Representative images showing the proliferation of A172 human glioma cells in the absence or presence of variable indicated concentrations of lidocaine for 48 h. B: Effects of lidocaine on the growth and proliferation of A172 human glioma cells as measured by total LDH release. **$P<0.01$.

biological behaviors. Therefore, TRPM7 may serve as a prognostic marker and a potential therapeutic target [21].

In this study, we first confirmed the existence of TRPM7 channels in rat C6 cells and then tested the effects of lidocaine on TRPM7 currents and cell proliferation. Lidocaine inhibited the TRPM7 currents in a dose dependent manner. About 20% inhibition occurred at 1 mM and 50% inhibition at 3 mM, which was quite significant as two known TRPM7 inhibitors 2-APB (50 μM) and Gadolinium (10 μM) had about 30% inhibition and 60% inhibition respectively.

We further showed that lidocaine caused significant reduction in the proliferation of C6 cells. The role of TRPM7 in mediating the effect of lidocaine is supported by our results showing that both TRPM7 inhibitors exhibited a similar

inhibitory effect on C6 cell proliferation. The suppressing effect of lidocaine on glioma cell proliferation was also demonstrated in A172 human glioma cells.

We have shown previously that lidocaine inhibits ASICs, another Na^+ influx channels [17]. However, there was no typical ASICs current was recorded in C6 cells. In addition, ASIC inhibitor amiloride had no effects on the proliferation of C6 glioma cells. Thus, we can exclude the possibility that lidocaine suppresses the proliferation of glioma through its effect on ASICs.

Further study is needed to disclose the mechanism of lidocaine on TRPM7 channels. The action site appears to be extracellular, as our previous study showed that lidocaine analog QX-314 which does not cross cell membrane, was able to inhibit TRPM7 currents in cultured cortical neurons [15]. It may be possible to develop new local anesthetics with higher potency, efficacy, and specificity targeting TRPM7, which may have novel therapeutic applications.

Lidocaine is one of the most commonly used local anesthetics for topical, local and neuraxial anesthesia. It is routinely administered intravenously for attenuating sympathetic response during tracheal intubation. It has been used intravenously as anti-arrhythmia agent. At anti-arrhythmia dose, lidocaine was found to be neuroprotective against ischemia in rats [14, 22]. Intravenous administration of lidocaine was found to reduce postoperative cognitive dysfunction in patients underwent coronary bypass surgery [23, 24]. Systemic administration of lidocaine also reduced the perioperative opioid requirement [25], and effective in reducing postoperative ileus and pain following colon resection [16]. Clinical preparation of lidocaine usually ranges from 0.5% to 5%, e.g., 18.5 to 185 mM. The observed effective concentrations (0.3 mM-3 mM) might be reachable by direct application, however, we need to be cautious about interpreting our result in clinical application since it is more than ten times of the serum concentration 6-12 μM for 48 hours of lidocaine infusion[24] and the toxic effects of lidocaine need to be considered. Structure modification of lidocaine to enhance the glioma suppressing effects might be a solution. Surgery is associated with related stress

induced hormone and immune function change that renders patient vulnerable for recurrence [2]. Considering its devastating prognosis, even a small decrease of the recurrence of glioma would be significant. Application of a well-known local anesthetic with safe and beneficial profile during surgical resection of glioma might be beneficial with little risk.

In summary, we demonstrated that local anesthetic lidocaine significantly inhibited the proliferation of glioma cells, and indicated that the effect of lidocaine was mediated by inhibiting TRPM7 currents.

Acknowledgements

Disclosure of conflict of interest

None.

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