NeuroToxicology 54 (2016) 53–64

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

GNB2 is a mediator of lidocaine-induced apoptosis in rat pheochromocytoma PC12 cells

Yonghong Tan[1,*], Qiong Wang[1], Baisong Zhao, Yingjun She, Xiaobao Bi

Department of Anesthesiology, Guangzhou Women and Children's Medical Center, Guangzhou 510623, Guangdong, China

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 23 March 2016
Accepted 23 March 2016
Available online 24 March 2016

Keywords:
Lidocaine
GNB
Cell cycle
Apoptosis
2-DE

ABSTRACT

Lidocaine has been recognized to induce neurotoxicity. However, the molecular mechanism underlying this effect, especially the critical molecules in cells that mediated the lidocaine-induced apoptosis were unclear. In the present study, PC12 cells were administrated with lidocaine for 48 h. Using MTT assay and flow cytometry, we found lidocaine significantly decreased the cell proliferation and S phases in PC12 cells with treatment concentrations, and significantly enhanced cell apoptosis with treatment concentrations. Two-dimensional gel electrophoresis (2-DE) analysis and LC–MS/MS were used to identification of protein biomarkers. Six proteins were identified. Among them, three were up-expressed including ANXA6, GNB2 and STMN1, other three were down-expressed including ubiquitin-linke protein 7 (UBL7), DDAH2 and BLVRB. Using qRT-PCR, we confirmed that lidocaine up-regulated the mRNA expression of STMN1, GNB2, ANXA6 and DDAH2, and found that the GNB2 had the largest change (about increased by 6.4 folds). The up-regulation of GNB2 by lidocaine was also validated by western blot. After transfected with 100 μM GNB2-Rat-453 siRNA, the expression of GNB2 in PC12 cells was almost completely inhibited; and the cell proliferation and cells in S phases were significantly enhanced, cell apoptosis including both early apoptosis and later apoptosis were significantly reduced in the presence of 0.5 mM lidocaine for 48 h. Therefore, neuronal apoptosis was induced by lidocaine and this effect was mediated by GNB2. Further research is needed to assess the clinical relevance and exact mechanism of neuronal apoptosis caused by lidocaine.

1. Introduction

Lidocaine is an amino amide-type of local anesthetic, which was first synthesized in 1943 (Hong et al., 2011). Lidocaine can be applied to the skin or to mucous membranes to reduce the immediate feeling of pain and produce numbness, with a rapid onset of action and intermediate efficacy (Li and Han, 2015). Thus, lidocaine was widely used as a local or topical anesthetic in both emergency departments and dental offices. However, lidocaine has shown to be neurotoxic in patients who are administration of an excessive dose of this agent (Lambert et al., 1994; Rigler et al., 1991) or subject to an unintended intravascular administration (Kawasaki et al., 2010). The major side effects of lidocaine had been shown in the central nervous system (such as drowsiness, dizziness, apprehension, euphoria, and tinnitus), gastrointestinal tract, and cardiovascular system (such as hypotension, bradycardia, and cardiovascular collapse) (Onizuka et al., 2012). In addition, lidocaine is hepatically metabolized and then causes damage to the liver (Werdehausen et al., 2009). In patients recovering from spinal anesthesia with lidocaine, transient neurological symptoms was described (Talakoub et al., 2015). Some studies reported that lidocaine is also toxic to articular chondrocytes (Chopra et al., 1971; Gianelly et al., 1967; Karpie and Chu, 2007). However, effect of lidocaine on neuro-cell growth and apoptosis, and the molecular mechanisms underlying this effect, especially the critical molecules in cells that mediated the lidocaine-induced apoptosis were unclear.

It was implicated that lidocaine induced morphological changes such as cell axon collapse and cell swelling (Kasaba et al., 2003; Onizuka et al., 2011). Lidocaine has been indicated to promote cell

* Corresponding author at: Department of Anesthesiology, Guangzhou Women and Children's Medical Center, No. 9, Jinsui Road, Guangzhou 510623, Guangdong, China.
E-mail addresses: yonghtan@hotmail.com, yonghongtandoctor@yeah.net (Y. Tan), wangtans@hotmail.com (Q. Wang), zhaobaisong819@163.com (B. Zhao), yjsheh@yeah.net (Y. She), bobjuan@sina.cn (X. Bi).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuro.2016.03.015
0161-813X/

apoptosis through the mitochondrial pathway and even necrosis (Mete et al., 2015). By using mouse neuroblastoma cell as a model, it was found that there was an inverse relation between local anesthetic concentrations and cell viability. Local anesthetics produced toxic effects by neurite inhibition at low concentrations and by apoptosis at high concentrations (Mete et al., 2015).

The purpose of this study was to evaluate the effect of lidocaine on neuronal apoptosis and to investigate the target for lidocaine in Pheochromocytoma-12 (PC12) cells using proteome analysis. PC12 cells have been widely used in both neurobiological and neurotoxicological studies (Das et al., 2004; Gozal et al., 2005). In addition, role of the identified target in lidocaine-regulated neuronal apoptosis in PC12 cells was further evaluated by transfecting its siRNA.

2. Materials and methods

2.1. Cell culture

PC12 cells were cultured in high-glucose DMEM (Gibco, USA) containing 5% FBS and 10% horse serum; 100 μg/ml of streptomycin and 100 U/ml of penicillin were added. The cells were grown at 37 °C with 5% CO_2 humidified atmosphere. Various concentration of lidocaine was prepared using 1% alcohol (vehicle) and used to

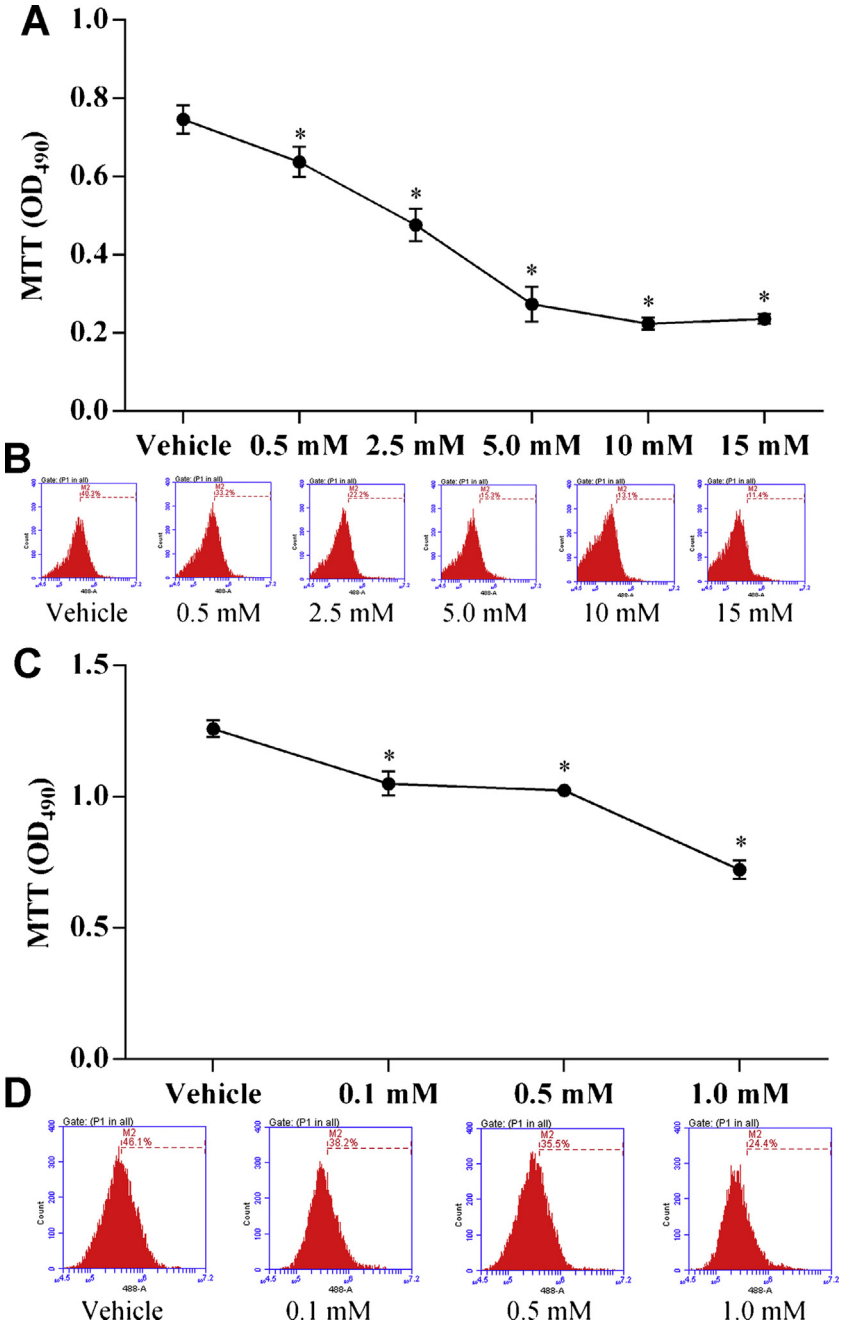

Fig. 1. Lidocaine suppressed cell proliferation of PC12 cells. After treated with various concentration of lidocaine for 48 h, the cell proliferation of PC12 cells were determined. (A and B) The cell proliferation of PC12 cells in the presence of vehicle (0.1% alcohol), 0 mM, 0.5 mM, 2.5 mM, 5 mM, 10 mM and 15 mM lidocaine. The 50% inhibitory concentrations of lidocaine was calculated, and the cell proliferation of PC12 cells in the presence of vehicle (0.1% alcohol), 0.1 mM (lower than IC_{50}), 0.5 mM (=IC_{50}), and 1 mM lidocanine (higher than IC_{50}) (C and D). Cell proliferation was performed using both MTT assay (A and C) and Click-iT EdU flow cytometry assay (B and D). *$P < 0.05$ vs. vehicle.