Journal of Applied Health Sciences and Medicine, 5(9):9–15, 2025. DOI: 10.58614/jahsm592

Journal of Applied Health Sciences and Medicine

Journal homepage: https://www.jpub.org/journal-details.php?journal-id=39

Research Article

Lidocaine induce neurotoxicity and peripheral nerve injury in trigeminal nerve system

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Article Info

Keywords: *Lidocaine, Immunohistochemistry, Neurotoxicity, and Trigeminal nerve system.*

Received: *01.09.2025*
Accepted: *16.09.2025*
Published: *19.09.2025*

Abstract

Objectives: Local anesthetics (LAs) are widely used in dentistry for their ability to block nerve impulses, particularly lidocaine. However, LAs can commonly cause neurotoxicity in vitro and in vivo. Our study investigated the neurotoxic effects of lidocaine and the occurrence of peripheral nerve injury when lidocaine is injected for local or regional anesthesia in the oro-facial area.
Method: Sprague-Dawley rats were used in trigeminal ganglion (TG) neuron preparation and incubated with lidocaine. Cell death was assessed using a visual microscope. Nerve injury was detected by activating transcription Factor 3 (ATF3) expression in TG neurons after lidocaine injection into the trigeminal nerve endings in the infraorbital area.
Results: The rat TG neurons were killed by lidocaine after 24 hours of incubation. Cell death depends on the concentration of lidocaine, resulting in 39.83% cell death at 10 mM lidocaine, 75.20% at 20 mM lidocaine, and 89.90% at 50 mM ($p < 0.03$), compared to 1.56% in saline. The cell membrane was damaged, and the nuclei showed signs of fragmentation in DAPI staining. Nerve injury was indicated by ATF3 immunoreactivity (IR) in the nuclei of TG neurons in the maxillary area of the TG in naïve (n=10), saline (n=50), 1% lidocaine (n=95), 2% lidocaine (n=319) ($p<0.02$), and 5% lidocaine (n=433) groups ($p<0.01$).
Conclusion: Lidocaine may induce neurotoxicity and nerve injury in vitro and in vivo at clinical concentrations or in cases of overdose.

1. Introduction

The local anesthetics have been commonly used in dentistry for several years for the purpose of local or regional anesthesia in the clinical field. The local anesthetic, especially lidocaine, is activated directly on the neuronal membrane voltage-gated Na^{+} channels, blocking the propagation of action potentials. Lidocaine also excites sensory neurons and induces neuronal toxicity, leading to cell death, including direct membrane disruption, and activation of p38 mitogen-activated protein kinase involved in apoptosis [1, 2]. In human chondrocytes, the lidocaine cause delayed mitochondrial dysfunction and apoptosis [3, 4]. Lidocaine-induced increase in intracellular Ca^{+2} is a mechanism of neuronal toxicity [5]. Local anesthetics especially at high concentration of lidocaine can activate caspase3/-7 triggering apoptosis [6]. Lidocaine may cause changes in cytoplasmic calcium homeostasis and mitochondrial membrane potential [7]. The effect of lidocaine is sufficient to release Calcitonin gene-related peptide (CGRP), a key component of neurogenic inflammation, and warrants investigation into the role of TRPV1 and TRPA1 in lidocaine-induced neurotoxicity [8]. In local or regional anesthethia the Local anesthetics (LAs)

were showing peripheral nerve injury as loss or damaged of large-diameter fibers [8]. Sciatic nerve intraneural lidocaine injection induced neuropathic pain and expression of ATF-3 in DRG neurons [9, 10].

In the present study, we conducted research on lidocaine-induced neurotoxicity activated in trigeminal ganglion (TG) neurons leading to cell death and peripheral trigeminal nerve injury indicated by ATF3 immunoreactivity in TG neurons.

2. Methods

2.1. Animal

All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Seoul National University. Animal treatments were performed according to the Guidelines of the International Association for the Study of Pain. Male Sprague-Dawley rats (approximately weighing 180-200 g at the time of surgery) were used. Rats were housed at a temperature of $23 \pm 2°C$ with a 12-hour light-dark cycle and fed food and water ad libitum. The animals were allowed to habituate to the housing facilities for 1 week before the experiments, and eff orts were made to limit distress to the animals.

2.2. Lidocaine injection

After 1 week of housing, rats were given general anesthesia with sodium pentobarbital (50 mg/kg, IP). The rats were then separated into 5 groups, each consisting of 5 rats. The infraorbital area was injected with saline (2Ca/Na) and various concentrations of lidocaine (1%, 2%, and 5%) in a 2 mL solution. After the injections, the rats were housed for 3 days for the next procedures at a temperature of $23 \pm 2°C$ with a 12-hour light-dark cycle and fed with food and water ad libitum.

2.3. Lidocaine treating

The trigeminal ganglia from 5 rats were harvested and incubated in HBSS containing 0.25% trypsin (Sigma-Aldrich, St. Louis, MO, USA). The cells were washed and triturated to separate them. Cells were placed on coverslips coated with poly-L-ornithine (0.5 mg/mL; Sigma-Aldrich), and then maintained at $37°C$ under 5% CO2 [9]. After 12 hours of trigeminal neuron preparation, the cells were treated with 1%, 2%, and 5% lidocaine, and a control group was treated with saline (2Ca/Na) and $10\mu M$ capsaicin for 24 hours. Osmolarity was adjusted to 400 mOsm, and pH to 7.4. The dead cells were evaluated using a light microscope.

2.4. Immunohistochemistry

Rats were perfused with physiological saline and sequentially with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at 3 days after lidocaine injection. The trigeminal ganglion was removed and immersed in the postfixative at $4°C$ overnight and then transferred to 30% sucrose in PBS for 48 hours. Serial frozen transverse sections (14 μm thickness) were mounted on gelatin-coated slides. All immunohistochemical procedures were performed as previous at room temperature unless otherwise stated. Slides were washed in PBS and then incubated in the blocking solution containing 5% normal goat serum, 2% BSA, 2% FBS, for 1 hour at room temperature. The sections were incubated overnight at $4°C$ with rabbit anti-ATF3 antibody (1:500; Santa Cruz Biotechnology, Inc.). Sections were then washed and incubated for 1 hour at room temperature with a Cy3 conjugated goat anti-rabit IgG (H+L) antibody (1:200; Jackson ImmunoResearch, West Grove, PA, USA), for 1 hour. The sections were mounted with Vectashield. (Vector Laboratories, Inc., Burlingame, CA, USA) and visualized using a confocal microscope using the appropriate filter sets (FV-300; Olympus, Tokyo, Japan) [11].

2.5. Data analysis and statistics

The descriptive analysis and one-sample t-test were used (SPSS version 27) to compare the mean and differences with the naïve group. Differences were considered significant when the p-value was less than 0.05.

3. Results

3.1. Lidocaine induced neurotoxicity

The results of the drug test on rat TG neurons (SD rat) with total cell count of 12,920 cells in each group, after experimenting with Saline (2Ca/Na), Lidocaine at various concentration levels, and Capsaicin $10\mu M$ for 24 hours, showed that the death of neurons increased with the concentration of the drugs. The percentages of cell death were as follows: Saline 1.56%, Lidocaine 10mM 39.83%, Lidocaine 20mM 75.20%, and Lidocaine 50mM 89.90%. In comparison, Capsaicin 10μM resulted in 78.27% cell death as a positive control. These findings indicate that the death of neurons due to Lidocaine occurs at a high rate starting from a concentration of 20 mM and above $(P < 0.03)$ Figure 1 and Figure 2.

The lidocaine acts on neurons by being absorbed through the cell wall (lipid bilayer) into the cytoplasm to block sodium channels $(Na^{+}channels)$ on the cell surface. The study found that the cell membrane started to be destroyed, leading to the breakdown of neurons. Subsequently, the nucleus of the neurons were observed disintegrating into small lumps and eventually disintegrating completely. This was demonstrated in the results of the experiment using a fluorescence microscope (confocal microscope) showing the TG neuron nucleus capturing the blue fluorescence (DAPI) as depicted in the picture below Figure 3.

Comparing the mean values of the cells in each experimental group, it was found that the increase in dead cells in the group without lidocaine was not statistically significant. The other group of cells that received lidocaine were found to be statistically related $(P < 0.03)$ Table 1.

Table 1: Comparison of mean values in a one-sample t-test

Variables	Mean	SD	Sig	95% CI	
				Lower	Upper
Saline	40.40	37.43	0.73	-6.08	86.88
10 mM Li	1029.40	712.66	0.03	144.52	1914.28
20 mM Li	1943.40	1423.81	0.03	175.49	3711.31
50 mM Li	2271.40	1625.01	0.03	253.69	4289.11
10 μM cap	2026.60	1501.02	0.03	162.83	3890.37

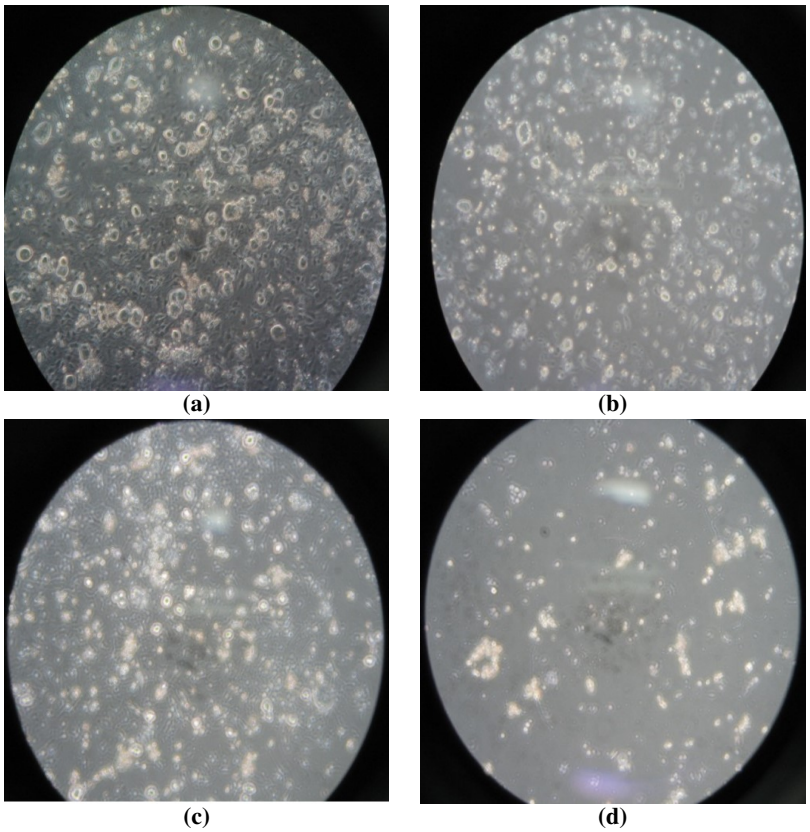

Figure 1: The expression of cell death after 24 hours of treated with lidocaine. (a) The control group treated with saline shows surviving cells. (b) The group treated with 10 mM lidocaine shows many dead cells. (c) The group treated with 20 mM lidocaine shows even more dead cells. (d) The group treated with 50 mM lidocaine shows almost all cells dead.

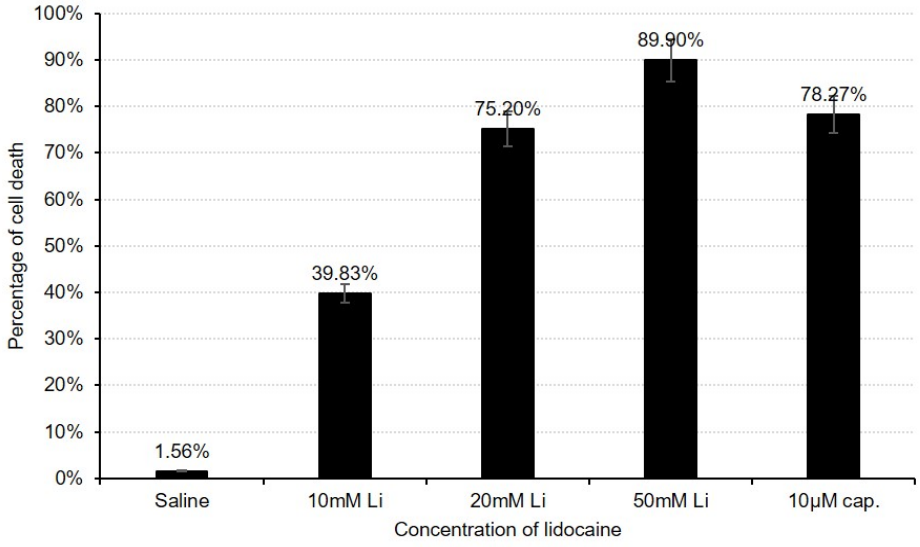

Figure 2: The percentage of cell death in various concentration of lidocaine shown in the bar chart, Capsaicin is used as the positive control.

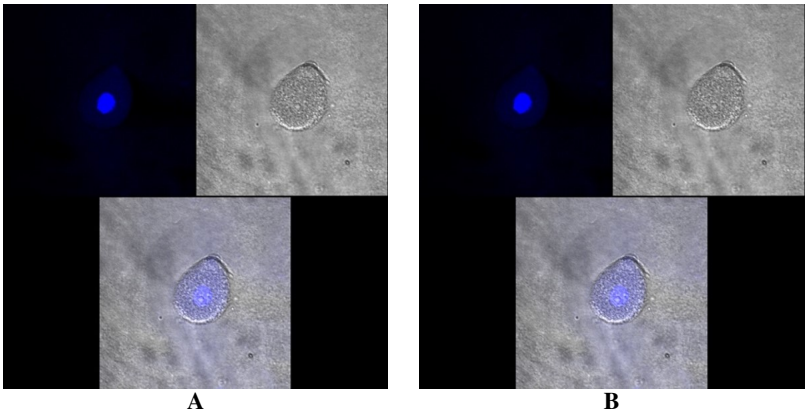

Figure 3: DAPI staining of nuclei (blue fluorescent dye). (A) The normal nucleus of the TG neuron (blue), DIC and Merged, (B) Photograph of TG neuron shows nuclear fragmentation (blue) after treated with lidocaine, DIC and merged.

3.2. Nerve injury by lidocaine

After injecting lidocaine into the trigeminal nerve in the infra-orbital area of the rats, immunohistochemistry (IHC) was performed to study Activating Factor 3 (ATF3) expression in neurons of the trigeminal ganglion after 3 days. The results showed that in the naïve group, there was minimal neuron injury compared to the injection groups. However, 2% lidocaine, 5% lidocaine, and capsaicin $10\mu M$ caused nerve injury by inducing ATF3 expression within the nuclei of TG neurons in the maxillary area of the trigeminal ganglion, as depicted in Figures 4 and 5.

When comparing the mean values of the experimental rats in each sample group, it was found that the increase of neurons with ATF3 (+) in the experimental rats without any stimulation was not related to consciousness. The rats that received lidocaine were found to be statistically related $(P < 0.01)$ Table 2.

Table 2: Comparison of mean values in a one-sample t-test

Variables	Mean	SD	Sig	95% CI	
				Lower	Upper
Naivie	2	1.581	0.23	-96	2.96
Saline	10	1.581	0.00	7.04	10.96
1% lidocaine	19	8.860	0.01	7.00	29.00
2% lidocaine	63.80	39.695	0.02	13.51	112.09
5% lidocaine	86.60	47.574	0.01	26.53	145.67

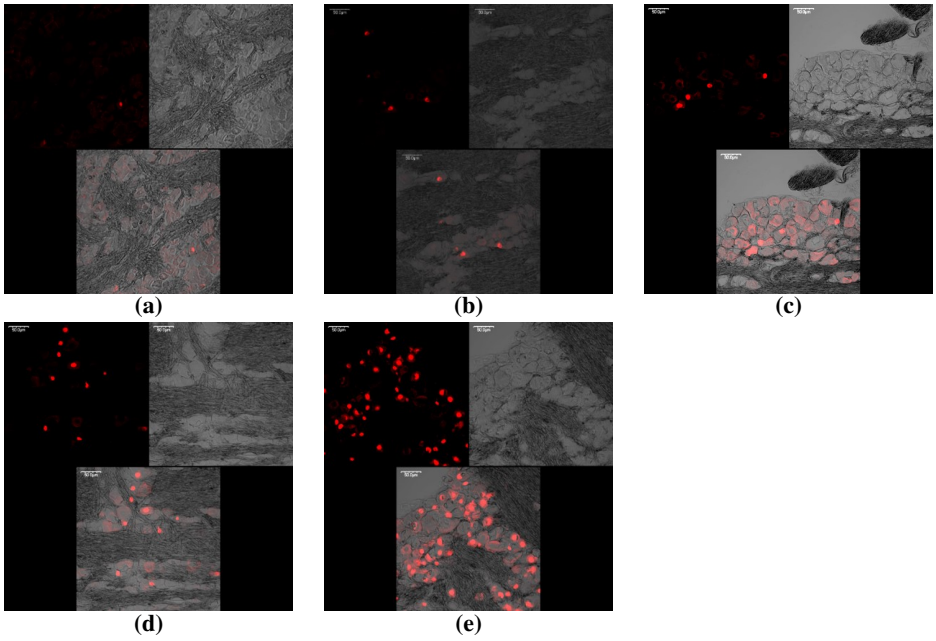

Figure 4: Expression of ATF3 immunoreactivity (ATF3 IR) in the nuclei of primary TG neurons in the maxillary nerve (V2) area after 3 days of lidocaine injection in the infraorbital region. (a) Photograph of neuron in the trigeminal ganglion showing ATF3 IR (red, Cy3 filter) treated with saline (2Ca/Na), DIC, and merged. (b) Photograph of neuron in the trigeminal ganglion showing ATF3 IR (red, Cy3 filter) treated with 1% lidocaine, DIC, and merged. (c) ATF3 IR (red, Cy3 filter) treated with 2% lidocaine, DIC, and merged. (d) ATF3 IR (red, Cy3 filter) treated with 5% lidocaine, DIC, and merged. (e) Photograph of neuron in the trigeminal ganglion showing ATF3 immunoreactivity (red, Cy3 filter) treated with capsaicin, DIC, and merged (the injured positive control).

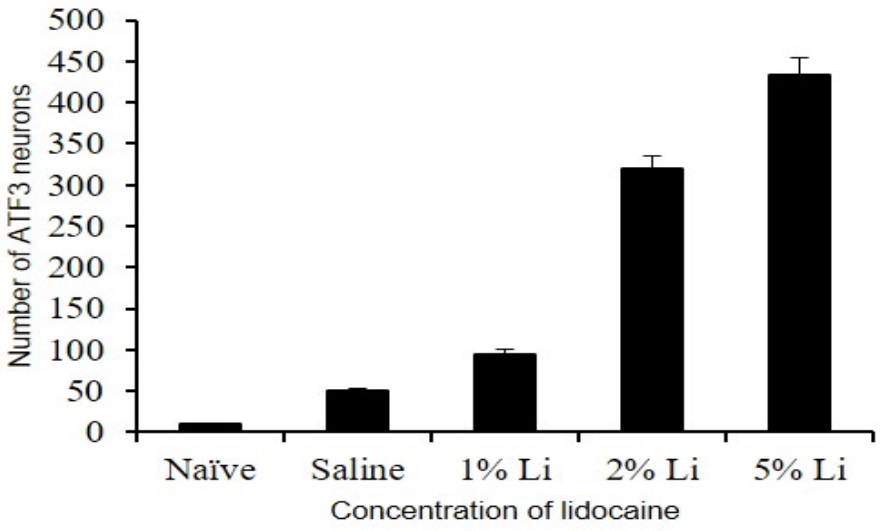

Figure 5: The bar chart of ATF3 IR expression in TG neurons change in each concentration of lidocaine analysed. ATF3-positive neurons were significantly increased in ipsilateral $(P < 0.01)$; compared with control. Scale bar = 50 μm.

4. Discussion

Lidocaine is a specific drug widely used in the field of dentistry because it blocks the nerve action potential of nerves or neurons, including sensory and motor nerves. Lidocaine has unique properties, as it dissolves in fat, allowing it to be absorbed through the cell wall or lipid bilayer into the cytoplasmic membrane. Once inside, it can block the sodium channel, preventing sodium ions from passing through. This blockade inhibits the action potential from being carried out for a period of time until the drug is metabolized, typically within 2 hours [12, 13]. Lidocaine, which is widely used today, may be toxic to the nervous system in experiments at a concentration of 10 mM or higher. After directly incubating lidocaine with the TG neurons of the experimental SD rat at various concentrations $(P < 0.03)$ and Capsaicin $10\mu M$, a large amount of cell death occurred depending on the concentration similar to previous reports in another cell line in vitro [14, 15]. In the intracellular pathway, lidocaine acts on neurons by being absorbed through the cell wall (lipid bilayer) into the cytoplasm to block sodium channels (Na+ channels) on the cell surface. The study found that the cell membrane started to be destroyed, leading to the breakdown of neurons. Subsequently, the nucleus of the neurons were observed disintegrating into small lumps and eventually disintegrating completely and even nuclei fragmentation. This action may include direct membrane disruption, activation of p38 mitogen-activated protein kinase involved in apoptosis [1, 2], delayed mitochondrial dysfunction and apoptosis [3, 4, 14], an increase in intracellular Ca^{+2} as a mechanism of neuronal toxicity [5, 15], and activation of caspase3/-7 triggering apoptosis [6]. Lidocaine may cause changes in cytoplasmic calcium homeostasis and mitochondrial membrane potential [7]. In addition to releasing intracellular Ca^{+2} ions, the effect of lidocaine is sufficient to release Calcitonin gene-related peptide (CGRP), a key component of neurogenic inflammation, warranting investigation into the role of TRPV1 and TRPA1 in lidocaine-induced neurotoxicity [8, 16]. In vitro, cell apoptosis occurred via the intrinsic pathway, but the mechanism of nucleus fragmentation in the TG neuron is still unknown. It may be caused by apoptosis factors released from mitochondria binding to the nucleus and DNA, leading to fragmentation [17, 18]. This apoptosis pathway still needs further validation.

The peripheral nerve damage induces change in gene expression in neurons, including ATF3 [19]. ATF3 is a neuronal marker of nerve injury or damage. The rat model of inferior alveolar nerve and mental nerve transection (IAMNT) showed the expression of ATF3 immunoreactivity (IR) in the TG neurons [20]. In primary neurons of dorsal root ganglion (DRG), ATF3 expression was also found in cases of peripheral axotomy, cell stress, or inflammation [21–23]. The key finding of our study indicates that peripheral nerve injury results in ATF3 immunoreactivity expression in the nucleus of TG neurons. The results indicate that even a clinical concentration of lidocaine (2%) can cause peripheral nerve injury $(P < 0.02)$. Researchers believe that the injection mat induce tissue damage, inflammation, and peripheral nerve. The data suggest that injecting lidocaine into the peripheral nerve endings may lead to nerve injury and neuropathic pain [9, 24–28]. In normal conditions, our study shows that a few cells with ATF3 immunoreactivity have been found in naïve rats. Consistent with previous reports, ATF3 has been detected in the nuclei of a very small percentage of primary sensory neurons in the dorsal root ganglion of uninjured rats [29, 30].

5. Conclusion

Lidocaine may induce neurotoxicity and peripheral nerve injury either in vitro or In vivo at clinical concentrations or in cases of overdose.

Article Information

Consent and Ethical approval: It is not applicable.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

Acknowledgement: The authors acknowledge the support of the Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea, for providing laboratory facilities and permitting the use of the

confocal laser scanning microscope. Many thanks to the Faculty of Dentistry, University of Health Sciences for supporting the grant for the trip and living costs during the experiments. Thanks to all lab members for suggesting the experimental protocol, materials, and equipment used.

Author's Contributions: Hue Vang contributed to the conception, design, data acquisition, and data interpretation, and drafted and critically revised the manuscript. Viengsavanh Inthakoun, Phimfalee Sayaxang, Phetmany Sihavong, Anhtana Photsavang, Vimonlak Bouphavanh, Khanphet Luangamat, and Maiboun Simalavong contributed to data analysis, data interpretation, and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Competing interests: Authors have declared that no competing interests exist.

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