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Research Paper

Analgesic Effect of Lidocaine in Orofacial Pain Of Rats

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ABSTRACT: *In dental treatment, lidocaine is currently used as local anesthetic, but studies on the control of orofacial pain are limited. The aim of this study was to investigate whether pre-treatment with lidocaine would involved in pain modulation in inflammatory orofacial pain.Male Sprague-Dawley white rats (240-280g) were used. The experimental group were divided into 3 groups(n=5); formalin (5 %, 50 μL), Administer 0.2%, 2% lidocaine, after administration, formalin (s.c). To induced orofacial pain, 5% formalin (50 μL) was injected under the skin on the right region of the whiskers of the experimental animals (n=5), and the act of rubbing or scratching the facial area in which the drug was injected was considered pain index. The administration of lidocaine at 2% concentration was found that the formalin-induced pain behavioral reaction was effectively reduced. The level of Nrf2 protein expression increased by formalin noticeably decreased in the medulla oblongata after lidocaine administration. Nuclear factor erythroid 2–related factor 2 (Nrf2) is an oxidative stress-mediated transcription factor. Both ginsengs significantly down-regulated the increased Nrf2 level in formalin group. These results indicate that lidocaine could be a promising regulated in the treatment of inflammatory orofacial pain.*

Keywords: *Lidocaine, Orofacial pain, Formalin, Nrf2, medulla oblongata,*

I. INTRODUCTION

Orofacial pain is involved in discomfort and pain in soft tissues including teeth and periodontal tissue in the oral cavity and the temporomandibular joint is expressed in various aspects in complex modern society and the frequency of the occurrence is increasing. Dental care institutions apply drugs in several categories for this pain control, and lidocaine is a typical amid local anesthetic for the treatment of the dental area, a derivative of acetanilid, which has a fast onset of anesthesia, no local irritation sign and long duration and is generally used for the action as a surface anesthetic[1)]. In addition, it is used in infiltration anesthesia, peripheral nerve block, and spinal and epidural anesthesia, as well.[2)] Local anesthetic is a drug that cuts off stimulus conduction of the peripheral nerve fiber and causes local sensory anesthesia, which is a drug widely used in surgical operation, dental treatment and arrhythmia treatment[2)]. Pain is generally one of the typical complications occurring during surgical treatment (Including most dental treatment) and must be controlled since it affects the patient's cooperation with treatment or prognosis after the treatment. As compared to general anesthetic, local anesthetic has less systemic absorption, so adverse reactions to various drugs are reported after relatively safer medication or administration.[3,4)] According to the results of some studies, it was reported that, when lidocaine concentration is 5 μg/ml, symptoms such as paresthesia, dizziness, excitability, abnormal behavior, fasciculation and tremor may occur and that as the concentration increases, clonic muscular contraction, consciousness degradation or convulsion may occur, so it should be administered with the exact concentration[5)].

There are a wide variety of pain control agents, and nuclear factor erythroid-2 related factor 2 (Nrf2), one of the proteins in DNA is a key systemic agent that maintains the oxdoredox reaction of a cell and induces antioxidation when exposed to oxidant stress, which has been reported that the Nrf2 pathway is activated when an inflammatory pain occurs[7,8)]. However, since there are insufficient reports according to the change of Nrf2 with the generation and control of a pain, it is necessary to check the correlation between orofacial pain and Nrf2 pathway.

Currently, lidocaine is often used as a local anesthetic in dental treatment, but there are few reports on

its application to facial area pain control. Therefore, this study induced an inflammatory pain by injecting formalin into the facial skin of experimental animals, assessed whether pre-treatment with lidocaine would affect orofacial pain relief and would observe changes in the Nrf2 pathway in the brain, the central organ of pain control and medulla oblongata, the entrance of orofacial pain signal.

II. MATERIALS AND METHODS

2.1 Experimental animal

As experimental animals, male Sprague-Dawley white mice (240-280g) were used. Maintaining a day/night circulation cycle of 12 hours and a regular environment of 23-24℃, water and feed were supplied unrestrictedly. Experimental stress by behavioral suppression was minimized as far as possible. This study was conducted according to the ethical rules of the Korean Pain Research Society concerning the experiment of the conscious animals.

2.2 Preparation of reagent

Lidocaine (Sigma, St. Louis, MO, USA) reagent was used after diluting in distilled water at 0.2% and 2% concentrations, which were concentrations immediately before the experiment. To assess the peripheral effect of lidocaine, it was administered in the same region, 10 minutes before the formalin injection. Formalin was used after diluting in physiological saline at 5% concentration.

2.3 Induction of orofacial pain

To minimize stress on the experimental animals, they were adapted in a plastic jar for more than 30 minutes, and during the test, water and food intakes were prohibited. Using an insulin syringe (0.25×8 mm), 5% formalin (50 μL) was injected under the skin on the right region of the whiskers of the experimental animals (n=5), and the act of rubbing or scratching the facial area in which the drug was injected was considered pain index.

2.4 Assessment of the pain behavioral reaction after lidocaine administration

This study would assess the impact of lidocaine on the formalin-induced pain behavior alreaction of the experimental animals. With a control group (n=5) and an experimental group (n=5), after the subcutaneous injection (S.C.) of lidocaine 50 μL diluted at 0.2 % and 2 %, respectively under the skin on the right region of the whiskers of the experimental animals, 5% formalin 50μL was injected in the same right facial area at the elapsed time of 10 minutes. Immediately from the injection, the pain behavioral reaction was observed for 45 minutes at an interval of five minutes.

2.5 Quantitative analysis of protein

Tissue extraction was performed, 30 minutes after the induction of formalin pain. The experimental animals were anesthetized via the abdominal cavity with 20% urethane (0.5 ml/kg), the blood flow to the brain was cut off by closing off the carotid artery and medulla oblongata was extracted by incising the skull. They were used after rapid freezing preservation at –70℃ till homogenization. An RIPA lysis buffer (Biosesang, Gyeonggi-do, Korea) was added to the extracted the medulla oblongata, and the tissues were homogenized, using a homogenizer (T10 Basic ULTRA-TURRAX®,IKA, Staufen, Germany) at 4℃. After the homogenization, they were centrifuged at 4℃, 13,000 rpm for 10 minutes, using a Centrifuge (1730MR, Gyrozen, Seoul, Korea). The protein absorbance of the supernatant liquid from which cell debris was removed was measured with a spectrophotometer (Human Cor., Seoul, Korea) at 595 nm. The protein concentration was quantitated, using a Bio-Rad Protein assay kit (Bio-Rad,Hercules, CA, USA) by standardization of bovine serum albumin (BSA). The quantitated protein, distilled water (D.W), LDS sample buffer (4X, Bolt™, Thermofisher Scientific, Seoul, Korea) and reducing agent (10X, Thermofisher Scientific, Seoul, Korea) were mixed and heated at 70 ℃ for 10 minutes, and after keeping and centrifugation at –20 ℃for two minutes, it was mixed again in a vortex. The prepared protein samples were loaded on polyacrylamide gel (Bolt®4-12 % Bis-Tris Plus Gels, 15 well. Thermofisher Scientific, Seoul, Korea) at 18.5$\mu\ell$ and run at 200 V for 22 minutes. To move the proteins from the gel to PVDF membrane (0.45 μm, 26.5 cm x 3.75 m), they were transferred at 20 V for one hour, and to check the overall protein patterns, they were checked with Ponceau solution, and then, cleansed in D.W for 10 minutes. They were blocked for two hours on a kneading machine (CR100, FINEPCR, Gyeonggi-do, Korea) with a blocking buffer (1X PBST, 5 % skim milk, sodium azide), and after washing with 1X PBST (10X PBST, D.W, 0.1 % Tween 20), a GAPDH mouse monoclonal Ab (1:2.000, Santa Cruz Biotechnology, INC., Texas, USA) and an Nrf2 rabbit polyclonal Ab (1:500, Santa Cruz Biotechnology, INC., Texas, USA) were kept overnight at 4 ℃ over five times for seven minutes with the primary antibody (Ab). After washing with 1X PBST five times for seven minutes, the secondary Ab was attached on the kneading machine at room temperature for two hours, and the expression was analyzed, using ECL prime (Amersham

Pharmacia Biotech, Buckinghamshire, UK) diluted at 1:1. The primary Ab was used after dilution in a blocking buffer, and the secondary Ab of GAPDH and Nrf2 were used after diluting a rabbit anti-mouse IgG (Abcam, MA, USA) and a goat anti-rabbit IgG (Abcam, MA, USA), respectively in 5% skim milk, excluding sodium azide at a ratio of 1:10,000. The degree of expression was presented, using Image J (1.50i, USA).

2.6 Statistical analysis

For a statistical analysis of the results of the experiment, an analysis of variance of the data measured repeatedly in multiple groups and an LSD post-hoc test were used. For a statistical comparison, the standard value of statistical significance was set as $p<0.05$. All results were indicated as the average ± standard deviation (SEM).

III. RESULTS OF EXPERIMENT

3.1 Facial area pain control effect according to lidocaine administration

In the orofacial pain model, induced with formalin injected under the skin of the facial area of the experimental animals, changes in the pain behavioral reaction according to lidocaine administration was shown in Figures 1 and 2. In the administration of lidocaine at 0.2% concentration, there was no significant difference in the primary pain behavioral reaction between the formalin injection group and the drug infusion group; however, in the secondary pain behavioral reaction, the pain behavioral reaction of the drug infusion group was noticeably reduced from that of the formalin injection group, and in the administration of lidocaine at 2% concentration, the secondary pain behavioral reaction was significantly relieved in the drug infusion group as compared to that of the formalin injection group (Fig. 1). As these pain behavioral reactions were checked through changes by lapse of time, in the administration of lidocaine at 0.2% concentration, at the elapsed time of 30 minutes after drug injection, the pain behavioral reaction was reduced most effectively, and similarly, in the administration of lidocaine at 2% concentration, at the elapsed time of 20-40 minutes that came under the secondary pain behavioral reaction, it was effective for pain control (Fig. 2). It was noted through these results that pain was controlled when lidocaine was administered for facial area pain control and that administration at 2% concentration was more effective for pain control than that at 0.2% concentration.

3.2 Impact of lidocaine administration on the Nrf2 pathway activation

Concerning whether the Nrf2 pathway activation would be involved in the generation and control of orofacial pain, after lidocaine administration, whether the aspect of Nrf2 protein expression changes in inflammatory pain reaction relief was observed (Fig. 3 a,b). Nrf2 protein expression was checked in the medulla oblongata of the brain. In the preliminary experiment, it was found that after formalin injection, Nrf2 expression significantly increased, and through this, it was noted that the Nrf2 pathway was involved in the generation of orofacial pain. It was observed that in the lidocaine administration group, the level of Nrf2 protein expression was significantly reduced as compared to the formalin group, and the correlation of the Nrf2 pathway with facial area pain control was checked.

IV. DISCUSSION

As the time for social activities increased from past years, orofacial pain occurs for various causes including stress, and the facial area is the most sensitive area in the human body. If an inflammatory or neuroapthic pain is induced by several external stimuli, the discomfort affects even the emotional or mental part[9]. Since the effect of drugs to treat this may be highly dependent on the concentration of the administration of the drugs, it is necessary to adjust the concentration of the administration, considering whether the drug can reach the morbid region fast, at the amount that can show a good effect at the lowest concentration. In general, it is known that the toxic action of lidocaine is dose-dependent, and the threshold of the toxic action on the central nervous system differs depending on the patient's condition, but the average blood concentration is approximately 5μg/ml[10]. Lidocaine at a blood concentration 1-5μg/ml has an excellent effect on the suppression of excitability induced by the cerebral cortex to suppress the expression of convulsion while at 5-12μg/ml, it selectively shuts off the inhibitory mechanism which may cause nystagmus, hallucination, tremor or convulsion, and at concentrations beyond this, a powerful inhibitory action occurs in the central nervous system, which may cause a coma or respiratory paralysis[11]. On the other hand, Song et al. reported that the pain rating scale significantly decreased with lidocaine at 2μg/ml and there was a little side effect[12]. Like these results, this study proved that lidocaine could effectively be applied to the generation and control of orofacial pain by verifying that the formalin-induced orofacial pain behavioral reaction would significantly be relieved by the administration of lidocaine at 0.2% or 2% concentration.

Nrf2 is an inflammatory pain control agent, and the fact that it is a decisive factor for antioxidation protein expression has been proven through a series of studies. It has been reported that Nrf2 is in charge of the detoxification of environmental hazardous substance in normal cells by leading the maintenance of the

antioxidation system and its increase in a stress situation and of the main function in cell protection in an oxidant stress situation[13,14]. In addition, it is known that, as Nrf2 expression increases, an antioxidation effect through the increase of the expression of antioxidation factors prevents the activation of inflammatory factors and shows an anti-inflammatory effect[15]. Likewise, in the results of this study, it was found that if orofacial pain occurred by formalin injection, the level of Nrf2 expression increased, and the level of Nrf2 expression decreased with the pain relief by lidocaine treatment, and based on these results, it is suggested that the Nrf2 pathway is involved in the generation and control of orofacial pain.

V. CONCLUSION

The present study would assess the impacts of lidocaine administration on the generation and control of pain in the inflammatory pain model induced in the facial skin of the experimental animals with formalin. After the administration of lidocaine, 10 minutes before the injection of formalin in the facial area, changes in the pain behavioral reaction in the same region were observed. Then, the brain and medulla oblongata of the experimental animals were extracted, and changes in the Nrf2 pathway activation were analyzed, utilizing a quantitative analysis of protein. To summarize the results of the experiment, first, as a result of formalin injection in the facial skin, orofacial pain behavioral reaction significantly increased. As a result of the administration of lidocaine,10 minutes before formalin injection, it was found that the formalin-induced pain behavioral reaction was effectively reduced. In addition, it turned out that the administration of lidocaine at 2% concentration was more effective for the secondary pain behavioral reaction relief than that of lidocaine at 0.2% concentration. Lastly, the level of Nrf2 protein expression increased by formalin noticeably decreased in the medulla oblongata after lidocaine administration.

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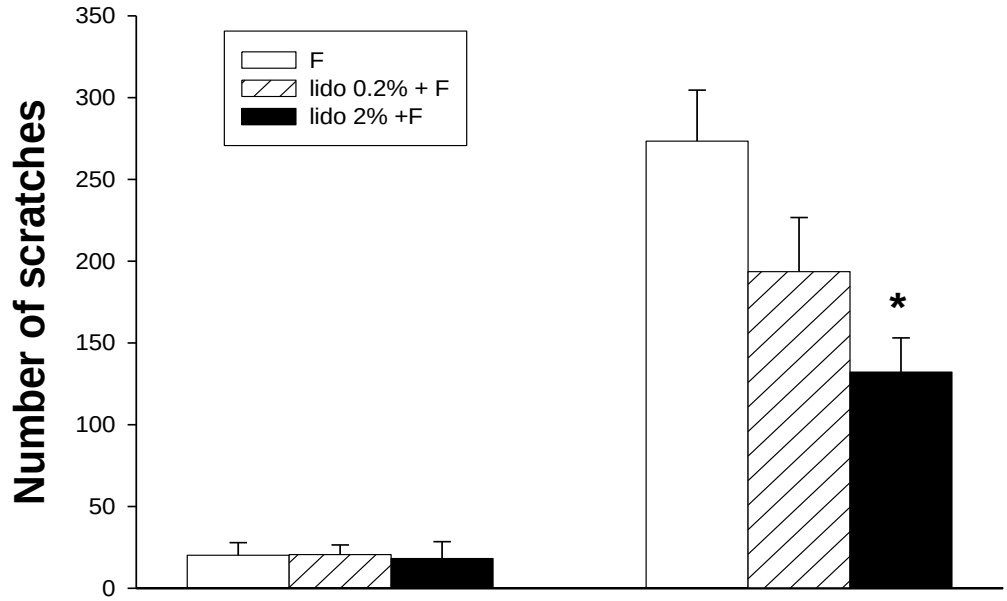


Fig 1. Effect of lidocaine (0.2% and 2%) Intraperitoneal, on pain behavioral response formalin-induced. lidocaine-adiministrated group was significantly reduced behavioral responses as compared with formalin-treated group. *P<0.05, 5% formalin VS. lidocaine.

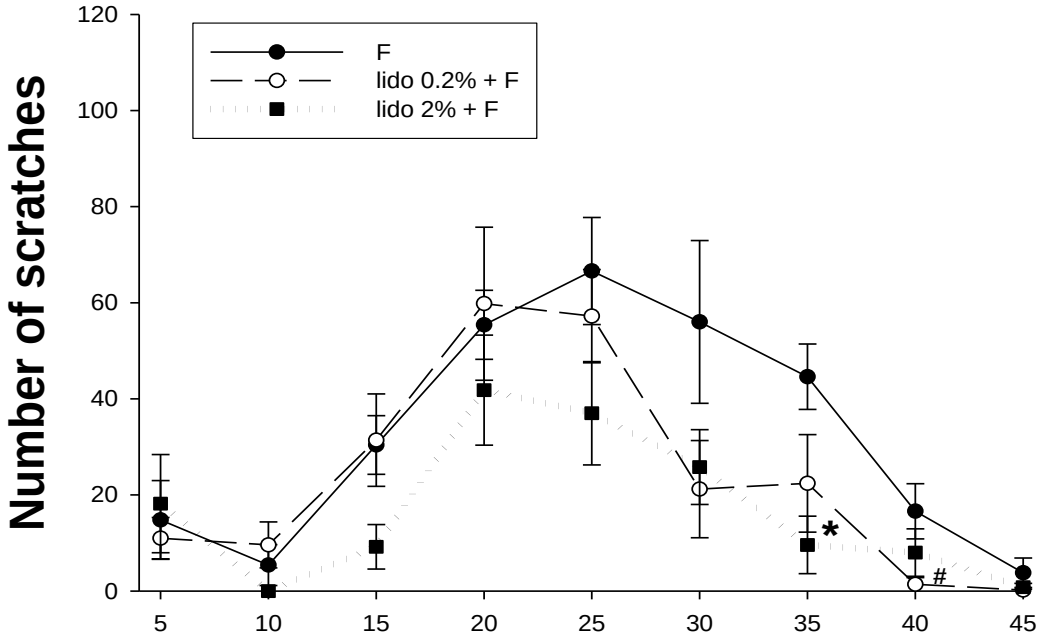
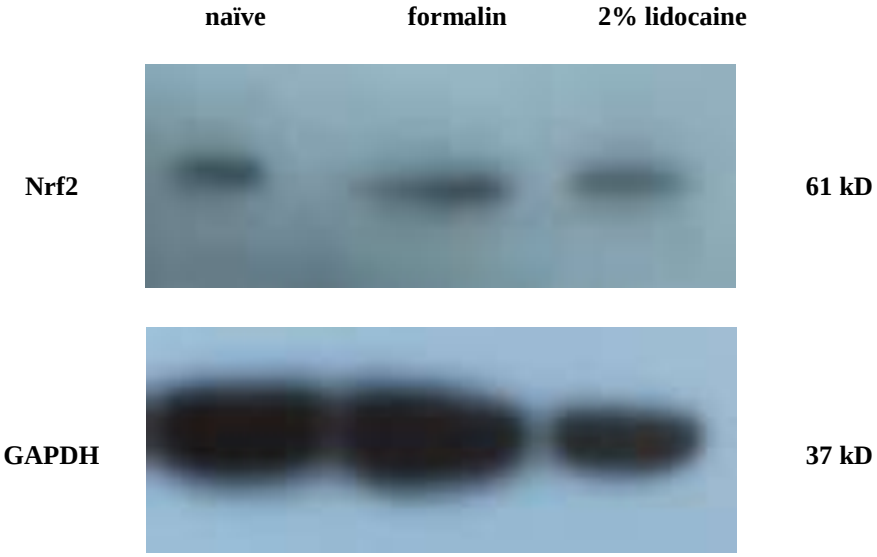


Fig 2. lidocaine effect of group was significantly reduced behavioral responses on second phase(11-45min), but not on first phase(0-10min) as compared with 5% formalin-treated group. *p<0.05, 5% formalin VS. 0.2% lidocaine. #p<0.05, 5% formalin VS. 2% lidocaine.

A



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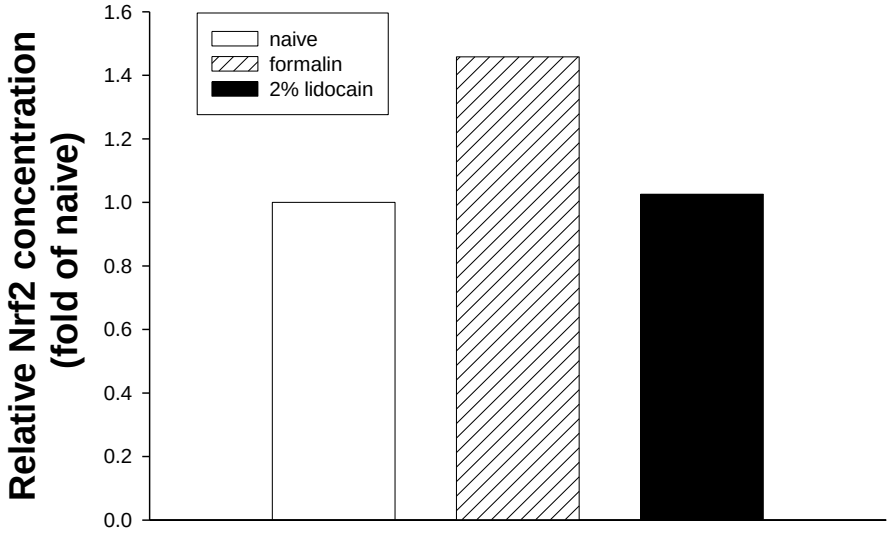

Fig 3. Nrf2 protein expression in the medulla oblongata. a. **naïve** , b. formalin, c. 2% lidocaine + formalin,