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PERIPHERAL AND CENTRAL EFFECTS OF DAPT IN OROFACIAL INFLAMMATORY PAIN

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ABSTRACT

The aim of this study was to investigate whether peripheral and central administration of DAPT, Notch singnaling inhibitor, is involved in pain modulation in inflammatory orofacial pain. The inflammatory orofacial pain was induced by the injection of 5% formalin into right vibrissa pad of rats. The pain behavioral response was measured the number of grooming or scratching on the orofacial area for 9 successive 5 minutes intervals. DAPT was administrated into the identified vibrissa pad(500, 1250, 2500 μM/ 50㎕) or intracisternal space(5, 50, 500 μM/ l0㎕) 10 min before formalin injection. The nociceptive responses were reduced in the 2nd phase (11~45 minutes), particularly 20, 30 minutes after formalin injection following administration of DAPT into vibrissa pad (1250, 2500 μM/ 50㎕). Intracisternal (5, 50μM/ l0㎕) administration of DAPT alleviated the formalin-induced pain behaviors in the 2nd phase, especially 25~40 minutes after formalin injection. Therefore, DAPT could be a promising analgesic agent in the treatment of inflammatory orofacial pain.

KEYWORDS: DAPT, Notch signaling, Orofacial pain.

INTRODUCTION

Orofacial pain is the main reason of patient's visit to the dentistry. People often look for the cause of the pain mostly in the teeth and surrounding tissues. However, diverse and complex degrees, pattern, and range of pains have been reported recently and, consequently, many studies have evaluated the causes and epileptogenesis of them in various aspects.[1,2] Inflammatory orofacial pain occurs because the chemical mediators are activated at the inside and outside of the tissue cells and surrounding inflammatory cells, which are damaged by the noxious stimuli applied to the peripheral tissues (e.g., teeth, surrounding tissues and temporomandibular joints), stimulate nerve endings, and excite the sense center through the interneuronal signaling pathway of the pain signal.[3]

Various medications are applied to control orofacial pain depending on the degree and pattern of the pain. Generally, non-steroid anti-inflammatory medications are used because they can inhibit Cyclooxygenase-1, which is a common major factor of inflammatory response. However, the amount of the usual dose is limited due to side effects.[4] For this reason, it is important to study new therapeutic targets for pain and molecular mechanisms that can minimize the side effects, yet still showing the efficacy of medications.

The signaling system of Notch protein is known to play an important role in the physiological and pathological processes of the human body. It is expressed by combining one of the Notches (Notch-1, -2, -3, -4) with Jagged (Jagged-1, -2) or Delta (Delta-1, -3, -4), which a ligand.[5] The abscission of the extracellular domain and the notch intracellular domain (NICD) occurs consecutively because of the reaction between ADAM-family metalloprotease and γ-secretase, which are generated by the contact between Notch and ligand. The separated NICD is displaced into the nucleus and combined with recombination signal binding protein Jκ (RBP-Jκ). As a consequence, RBP-Jκ, which is separated from a supplementary inhibitory factor, aggregates with a supplementary activation factor to induce the expression of target genes such as hairy and enhancer of split (HESs family) and HEYs family.[6,7]

It is known that the expression of Jagged-1, a Notch ligand, is upregulated by NF-κB.[8,9] Previous studies have reported that mitogen-activated protein kinase(MAPK) increases Notch activation and it provides that Notch signal interacts with the delivery system of inflammation and pain signals. However, the role of the Notch signal related to the control of orofacial pain has been poorly studied.

Therefore, this study aimed to analyze the role of Notch signal in the formalin-induced inflammatory pain model of the orofacial region using experimental animals. First, this study confirmed the pain behavior responses induced by formalin and tried to evaluate the effects of the Notch

signal control on the pain behavior responses by administering a Notch signal inhibitor at the central and peripheral nerves.

MATERIALS AND METHODS

1. Experimental Animals

This study was carried out with satisfying the principle of animal experiment and after being approved by the animal experiment ethics committee of 00 University. Sprague-Dawley male rats (240-280g) supplied from Hyochang Science (Daegu, Korea) were used for the experiment. The day/night cycle (12hrs each) and temperature (23-25℃) were maintained constant throughout the experimental period. The water and feed supplies were not restricted.

2. Formalin-induced orofacial pain model

The orofacial pain model of the experimental animals was performed following the conventional experimental procedure.[2] Experiment animals were adapted to a plastic container for more than 10 minutes and water and food intake were prohibited during the experiment. Using 30 gauge needle insulin syringes, 30μl of 5% formalin was injected subcutaneously at the whisker area and rubbing or scraping the treated area after the injection was considered as a pain index. The accumulated responses were recorded for 45minutes at 5minutes interval. The pain response behavior shows a biphasic form, indicating that it increases immediately after the formalin injection, gradually decreased afterward, and then it increases again approximately after 10 minutes. Therefore, it was evaluated for the first phase response (0-10minutes) and the second phase response (11-45minutes) separately. Formalin was diluted in physiological saline to 5%.

3. Administration of DAPT to central and peripheral nerves

DAPT (500, 1250, and 2500μM/50㎕) was injected subcutaneously at the same site 10minutes before administering formalin for the peripheral treatment. DAPT (5, 50, and 500 μM/ l0㎕) was administered to the cisterna cerebellomedullaris using Hamilton syringe (Hamilton Co., Reno, NV, USA) 10minutes before administering formalin for the central nerve treatment. Fiver percentage DMSO was used as a vehicle and the same dose was used for the peripheral and central treatments. The concentration of DAPT was determined by Xie et al.[10]

4. Catheterization for administering DAPT to the central nerve

The surgery to apply DAPT to the cisterna cerebellomedullaris was conducted as follows.[11] The rats were put under general anesthesia by injecting Zoletil(1ml/kg) and xylazine(0.25ml/kg) mixture solution intramuscularly into the hind leg. The hair on the back of the head was shaved and fixed to a stereotaxic frame (Model 1404; David Kopf Instruments, Tujunga, CA, USA). Afterward, the skin was cut from the crown of the head to the bottom of the occipital bone. After opening the surrounding muscles including the posterior head muscle and confirming the micro-leakage of the cerebrospinal fluid in the intracisternal space by making a small hole using a 26 gauge syringe needle, a polyethylene catheter (8 cm, PE10; CalyAdams, Parsippany, NJ, USA) was inserted into approximately 2mm. The catheter was rotated on a mini-implant screw planted in the parietal bone and fixed with a dental self-cure composite resin (Dentsply, York, PA, USA) to prevent the catheter from falling out during the experiment. The tip of the catheter was sealed with a stainless steel wire (0.32 mm) to prevent the cerebrospinal fluid from leaking. The incision was sutured and gentamycin (0.05 ml/kg) was injected intramuscularly to prevent infection. The subjects were confirmed to be awakened from the anesthesia within 2 hours after operation and they were used for the experiment after recovering from the operation at least for three days.

5. Statistical Analysis

The experimental results were analyzed using SPSS Statistics ver. 19.0 (IBM Co., Armonk, NY, USA) and Sigmaplot 2001. The one-way ANOVA followed by LSD post-hoc test was conducted. The measurement was expressed as mean ± standard error and the significance was determined at $p<0.05$.

RESULTS

1. Regulating the Response Behaviors against Orofacial Pain through Peripheral Administration of DAPT

In the orofacial pain model induced by formalin administered subcutaneously to the face, the effects of DAPT, a Notch inhibitor, on the pain behavior response are presented in Fig. 1 and 2. The pain behavior responses were not different between the formalin group and the formalin treatment after vehicle peripheral treatment in the first and second phases. After administering DAPT to the peripheral nerve, the number of pain behavior response was 33.33±3.53 and 26.330±13.33 times for 1250 and 2500μM/50㎕, respectively. Compared to the vehicle administration prior to formalin group (92.83±25.84 times), the pain behavior response decreased significantly in the first phase. In the second phase, the number of pain behavior responses was 176.33±35.54 and 164.83±34.14times in the formalin group after administering DAPT at the concentrations of 1250 and 2500μM/50㎕, respectively. It was significantly smaller than that of the vehicle administration prior to formalin group (416.54±54.35 times) (Fig. 1). When the changes in the pain behavior responses by time were evaluated, the pain behavior responses of formalin group after DAPT peripheral administration (1250 and 2500μM/50㎕) decreased significantly at 5, 20, and 25minutes compared to the vehicle administration prior to formalin group (Fig. 2).

2. Regulating the Response Behaviors against Orofacial Pain through Central Nerve Administration of DAPT

In the orofacial pain model, the effects of DAPT administration to the central nerve on the pain behavior response are presented in Fig. 3 and 4. After administering DAPT to the central nerve, the first phase pain behavior response was 12.663±4.65 and 14.00±7.05times at 5 and 50μM/l0$\mu\ell$ in the formalin group, which was significantly smaller than the vehicle administration prior to formalin group (57.57±9.47 times). However, pain regulation effects were not found at the high concentration condition (500μM/l0$\mu\ell$). The results of the second phase pain behavior response showed that the number of the pain behavior responses was 212.83±43.11, 163.00±34.01, and 197.83±16.57times after DAPT administration at 5, 50, and 500μM/l0$\mu\ell$, which was significantly less than the vehicle administration prior to formalin group (336.71±30.59times). However, the pain regulation effect at a high concentration (500μM/l0$\mu\ell$) was not significantly different compared to 50μM/l0$\mu\ell$ (Fig. 3).

In terms of changes by time, the pain behavior responses of the formalin group after DAPT administration to the central nerve (50μM/l0$\mu\ell$) was significantly lower than the formalin group after vehicle administration at 5 and 20 minutes (Fig. 4).

SUMMARY AND DISCUSSION

This study aimed to evaluate the change in the pain behavior response according to the DAPT, an inhibitor of a Notch signal application in the orofacial pain model of experimental animals. It has been reported that the Notch, one of the human membrane proteins, induces various diseases such as cancer[12] and osteoporosis[13] depending on the intensity of the signal by interacting with diverse signal delivery pathways. It is also known that it interacts with the expression of mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), TLR, transforming growth factor (TGFβ), and NO, which are major molecules of inflammation and pain signals.[14]

In the process of delivering inflammation and pain signals, the regulation of Notch signal has been confirmed not only at the molecular level but also as a result of behavioral responses through animal experiments. This study validated the changes in the pain behavior response by regulating the Notch signal by applying DAPT, which inhibit γ-secretase in the Notch signaling system. The results of this study agree with previous studies on the role of the Notch signaling. Previous studies have reported that, in the neuropathic model, experimental animals were induced to express notch intracellular domain (NICD) along with the increased mechanical allodynia[15] and the application of N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester (DAPT), a Notch signal inhibitor, alleviated mechanical allodynia and reduced the expression of NICD.[16]

The Notch protein is involved in cell proliferation, differentiation, and apoptosis, and it is activated in association with many diseases. In the central nervous system, the Notch signaling pathway regulates the activity of microglia,[17] and affects the expression of mitogen-activated protein kinase (MAPK) and NO.[18] In the neuropathic pain model induced by the ligation of the cervical vertebrae and the nerve peroneus communis, the administration of DAPT into the intraspinal gap alleviated mechanical allodynia and reduced the expression of NICD.[19] The results support the role of the Notch signal in the central nerve. Moreover, the application of DAPT, a γ-secretase inhibitor, prevents the loss of cell viability induced by lysophosphatidylcholine (LPC) to the human umbilical vein endothelial cell (HUVE) and reduces the expression of Notch1, HES-1, and Monocyte Chemoattractant Protein-1 (MCP-1).[20] Moreover, Chen[21] reported that, intheliver fibrosis model, the administration of DAPT to the abdominal cavity protected hepatocyte and improved liver fibrosis, implying the peripheral effects of DAPT. The results of this study also showed that the subcutaneous administration of DAPT to the cisterna cerebellomedullaris and face had the orofacial pain regulation effects, proving the effects of DAPT on the central and peripheral nerves, which agreed with the results of previous studies.

In summary, the administration of γ-secretase, an inhibitor of the Notch signal, to the central and peripheral nerve had significant effects on the orofacial pain control. Although molecular biologic studies are required in relation to the sub-target factors interacting with the Notch signal, the results of this study proved that the activation of the Notch signal plays an important role in mediating the orofacial inflammation and pain signaling process. Moreover, these results implied the possibility of applying DAPT as a pharmacological agent in the control of pain signals.

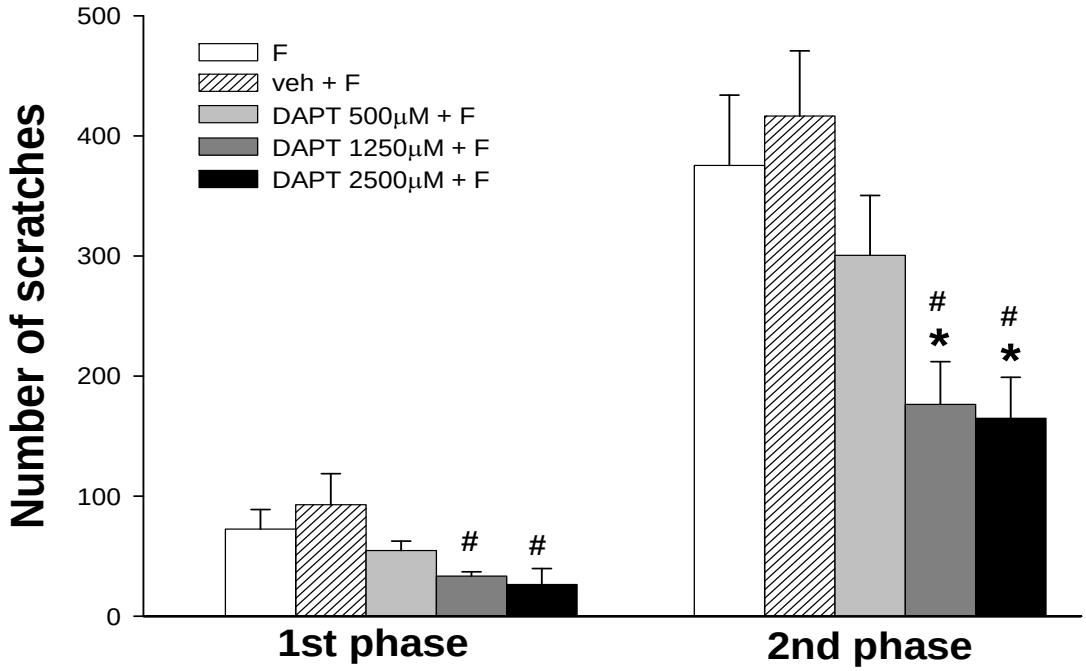

Fig. 1: Peripheral effects of DAPT, Notch inhibitor, on nociceptive behavior.

The nociceptive responses were reduced following peripheral administration of DAPT (1250, 2500 μM/ 50㎕) into rat's vibrissa pad 10 min before formalin injection (n=6). *$p < 0.05$, vs. F, #$p < 0.05$, vs. veh + F, veh : vehicle, F: formalin.

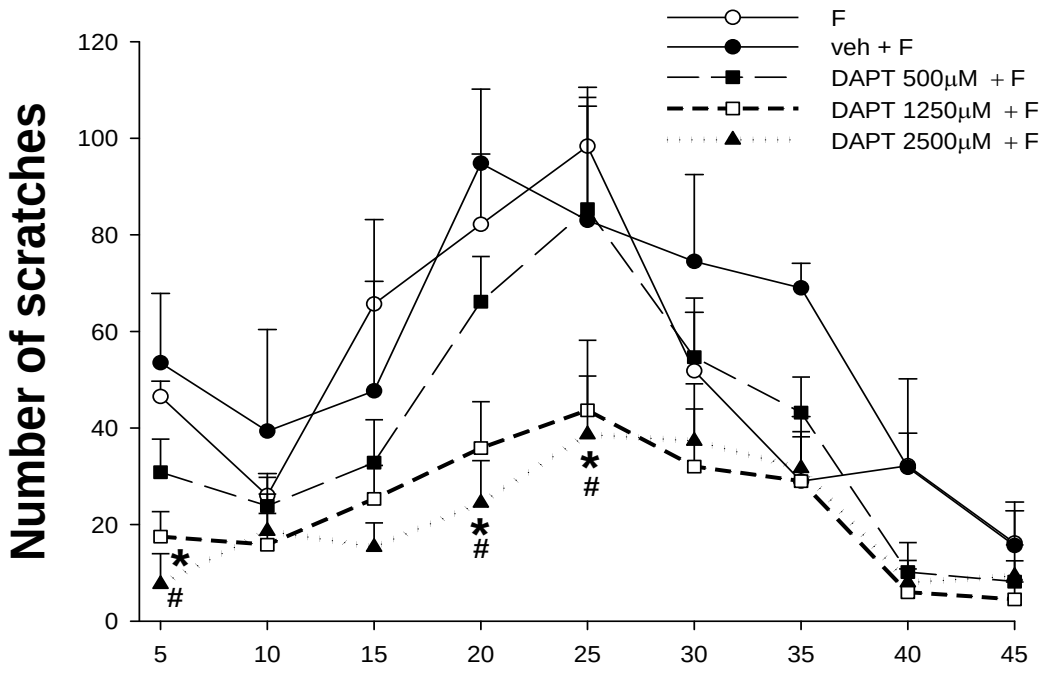

Fig. 2: Changes in nociceptive responses following peripheral administration of DAPT.

Peripheral administration of DAPT significantly reduced the nociceptive responses after induction of orofacial pain(n=6). *$p < 0.05$, veh + F vs. DAPT 1250 μM + F, #$p < 0.05$, veh + F vs. DAPT 2500 μM + F, veh : vehicle, F: formalin

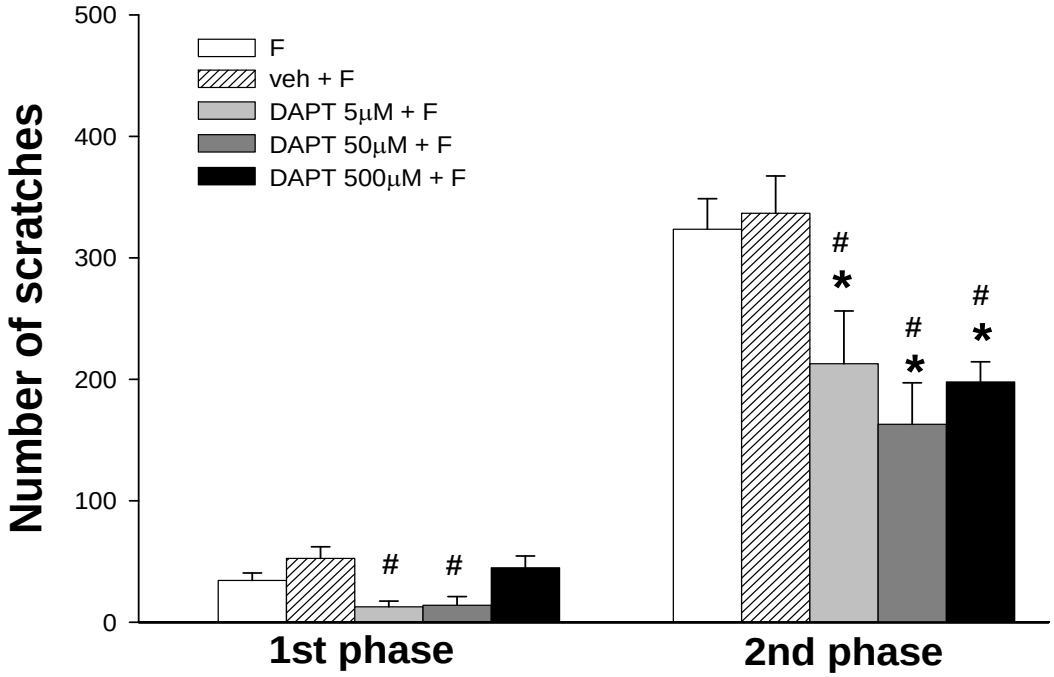

Fig. 3: Central effects of DAPT, Notch inhibitor, on nociceptive behavior.

The nociceptive responses were reduced following central administration of DAPT (1250, 2500 μM/ 50㎕) into rat's vibrissa pad 10 min before formalin injection (n=6). *p < 0.05, vs. F, #p < 0.05, vs. veh + F, veh : vehicle, F: formalin.

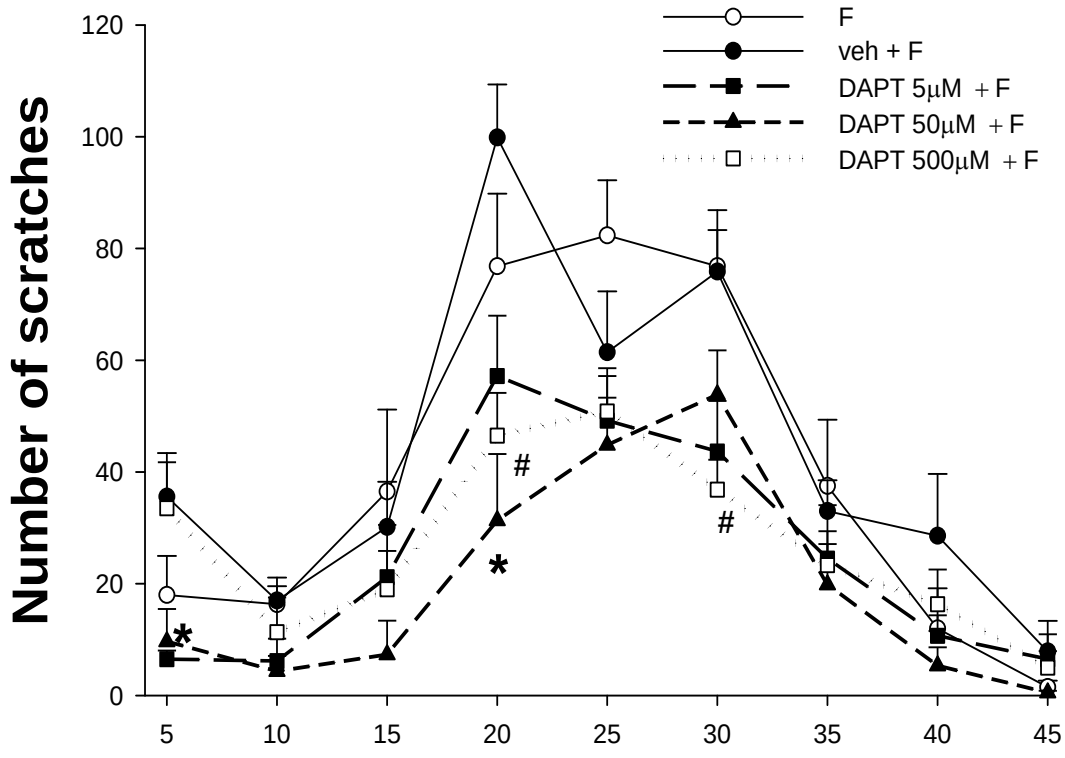

Fig. 4: Changes in nociceptive responses following central administration of DAPT.

Central administration of DAPT significantly reduced the nociceptive responses after induction of orofacial pain(n=6). *p < 0.05, veh + F vs. DAPT 1250 μM + F, #p < 0.05, veh + F vs. DAPT 2500 μM + F, veh : vehicle, F: formalin.

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