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ORIGINAL ARTICLE

Rapid Relief Mechanism of Allergic Rhinosinositis after "Assisted Drainage" Therapy

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ABSTRACT

Rhinosinusitis is mostly affected by viral infections and allergy and resolves without antibiotics usage. However, antibiotics are still frequently used as drug of choice for rhinosinusitis. If conservative treatments failed, surgical procedures had to be done. Neurogenic inflammation is involved in rhinosinusitis. Possibly because rhinosinusitis-induced chronic gingivitis successfully relieved by the "assisted drainage therapy" (ADT). This new periodontal therapy consists of scaling and root planing followed by subgingival massage. However, the mechanism had not been clearly verified. **Objective:** To verify the mechanism of allergic rhinosinusitis symptoms relief by ADT. **Methods:** Randomized control time series design experimental study was conducted in two groups of Wistar rats. Allergic sensitization was performed by injections and inhalation of ovalbumin (OVA). Gingivitis was induced by synthetic *Porphyromonas gingivalis* lipopolysaccharide (*Pg*$LPS_{1435/1450}$). One group was subjected to ADT before inhalation and another groups without ADT. Immunohistochemistry for biomarkers of allergic reaction (LTC4 and ECP) and neurogenic inflammation (CGRP and VIP) performed. **Results:** After OVA inhalation, allergic reaction and neurogenic inflammation biomarkers had significantly lower in rats subjected to ADT before inhalation than without ADT (p=0.001). **Conclusion:** ADT significantly decreased nasal allergic reaction and neurogenic inflammation biomarkers, therefore could be proposed as an adjunct therapy of allergic rhinosinusitis.

ABSTRAK

Mekanisme berkurangnya gejala rinosinusitis alergi setelah terapi *assisted drainage.* Rinosinusitis terutama disebabkan oleh virus dan alergi, serta akan sembuh dengan sendirinya tanpa antibiotik. Akan tetapi, antibiotik masih sering dipakai sebagai obat pilihan untuk rinosinusitis. Bila terapi konservatif gagal maka perlu dilakukan tindakan bedah. Selain alergi, keradangan neurogenik juga terlibat dalam rinosinusitis. Beberapa laporan kasus menduga bahwa gingivitis kronis memicu rinosinusitis dan suatu terapi yang disebut sebagai *assisted drainage* (ADT) dapat mengurangi gejala dalam hitungan menit. Terapi periodontal temuan baru tersebut merupakan pembersihan karang gigi yang disertai dengan pemijatan subgingiva, namun demikian mekanismenya masih perlu diverifikasi. **Tujuan:** Untuk melakukan verifikasi mekanisme kerja ADT dalam mengurangi gejala rinosinusitis dengan cepat. **Metode:** Studi eksperimental *randomized control time series design* dilakukan pada dua kelompok tikus Wistar. Sensitisasi alergi dilakukan dengan injeksi dan inhalasi ovalbumin (OVA). Gingvitis diinduksi dengan lipopolisakarida *Porphyromonas gingivalis* (*Pg*$LPS_{1435/1450}$) sintetis. Pada satu kelompok dilakukan ADT sebelum inhalasi dan kelompok lain tanpa ADT. Pemeriksaan jaringan hidung dilakukan dengan pemeriksaan imunohistokimia pada biomarker reaksi alergi (LTC4 dan ECP) serta keradangan neurogenik (CGRP dan VIP). Analisa data menggunakan ANOVA. **Hasil:** Beberapa menit setelah inhalasi OVA, ekspresi biomarker reaksi alergi dan keradangan neurogenik lebih rendah secara signifikan (p=0,001) pada tikus yang mendapat perlakuan ADT sebelum inhalasi dibandingkan tanpa inhalasi. **Simpulan:** ADT mampu secara signifikan mengurangi biomarker reaksi alergi dan keradangan neurogenik dalam hitungan menit, sehingga dapat diusulkan sebagai terapi ajuvan rinosinusitis alergi.

Key words: allergic rhinosinusitis, assisted drainage therapy, gingivitis

INTRODUCTION

Rhinosinusitis could be acute or chronic. Symptom that persisted for more than 12 weeks is categorized as chronic rhinosinusitis (CRS).[1] It is a disease presented by chronic symptoms such as nasal obstruction, rhinorrhea, hyposmia and facial pain, highly prevalent and has a considerable impact on quality of life.[2] Chronic rhinosinusitis affects approximately 30 million Americans and results in an estimated 11.6 million physician visits and $4.3 billion in health care costs annually. CRS mostly related to viral infections and allergy, and self limited without the use of antibiotics. However, in the USA there were 13 million antibiotics prescription for rhinosinusitis.[3]

Pathophysiology of CRS has not been established, allergic reaction as well as neurogenic involvement have been hypothesized. Many factors may involved in its allergic involvement, such as IgE, leukotriene and eusinophils. The IgE activates mastcells or basophils via cross-linking of the IgE receptors.[9] Leukotriene, an important mediator involved in allergy, is a product released by mast cells and basophils degranulation after stimulation by lippopolysaccharide produced by bacteria.[9] It increases airway hyperrensponsiveness as well as mucous secretion, especially leukotriene C4 (LTC4). The presence of eosinophils in rhinosinusitis is owing to its ability to infiltrate the sinus mucosa. Its activation can be detected with eosinophilic cationic protein (ECP) release.[10]

Based on the neurogenic switching hypothesis, sensory and parasympathetic nerves activation play a role in the interaction of immunogenic and neurogenic inflammation. This might be involved in the pathophysiology of CRS and accounted for nasal symptoms.[4,11] The maxillary sensory nerve involves in CRS by secretion of neuropeptide calcitonin gene-related peptide (CGRP) that causes sneezing as well as increases secretion. The vasoactive intestinal peptide (VIP) from parasympathetic nerve increases secretion as well as vasodilatation.[12,13]

Conservative treatments of CRS are decongestants, corticosteroids, antibiotics and diathermy. Un-fortunately, if failed, surgical procedures such as adenoidectomy had to be done.[2] Interestingly, in accordance with the traditional theory of CRS etiology, recent studies suggest a positive bacterial culture is found in only about 0.5% to 2% of viral RS cases, thus the paradigm is changing; causes other than viral infection i.e. allergy, or other causes must be found.[3] Therefore, it is not surprising that despite its widespread prevalence, the pathophysiology has not fully understood until recently, and no optimal treatment has been identified.[1]

Whilst until now medical community is still uncertain in CRS pathophysiology, "good news" emerged from dental literatures which suggested that CRS may also connected to dental infection. It was reported that CRS in children could be caused by chronic gingivitis. In previous case reports, the assisted drainage therapy (ADT) was able to relief clinical symptoms of severe CRS i.e. nasal congestion and headache in minutes.[4,5] The assisted drainage therapy (ADT) was named after a periodontal treatment, which developed by Utomo and proposed as a new invention in periodontology and medicine, especially in asthma management.[4-6] It consists of scaling and root planning (SRP) accompanied by gingival sulcus massage. Human study confirming the rapid effect of ADT in relieving of CRS symptoms had not been done; nevertheless, a study of rapid relief of ashmatic symptoms, a comorbidity of rhinosinusitis, in allergic children had been conducted in 2007.[6] The concept of one airway one disease and axon reflex in asthma and rhinosinusitis pathophysiology may elucidate its similar results.[7,8]

Therefore, the objective of this study was to verify the mechanism how ADT rapidly relief allergic CRS symptoms by measuring the allergic reaction (LTC4 and ECP) and neurogenic inflammation (VIP and CGRP) biomarkers in allergic Wistar rats. Finding of these markers might increase our knowledge on the pathophysiology of CRS.

METHODS

In this study, Wistar rats were initially allergic-induced with ovalbumin (OVA), then to create chronic gingivitis model, upper gingiva was injected with synthetic *Porphyromonas gingivalis* lipo-polysaccharide with mass ion 1435 and 1450 (*Pg*LPS$_{1435/1450}$).[14] The assisted drainage therapy was done to certain groups to verify its effect towards the rapid relief of CRS symptoms, based on the modulation of allergic reaction (LTC4 and ECP), as well as neurogenic inflammation (VIP and CGRP) biomarkers. Laboratory examination for gingiva and nasal tissues was done with peroxidase im-munohistochemistry. Statistical analysis was done with analysis of variance (ANOVA) using SPSS 15.

Male Wistar rats (120-150gr) were randomly selected into two experimental groups: (A)without the assisted drainage therapy, (B)with the assisted drainage therapy and one control group. Each group was divided into 3 subgroups (A1-3 and B1-3) which each consisted of 6 rats. Subgroups 1, 2 and 3 were allergic rats with intra sulcus (i.s.) injection of *Pg*LPS$_{1435/1450}$ received one of three doses 0.3, 1.0 and 3.0µg/mL respectively. These 3 doses represented low (0.3µg/mL), cut off point (1.0µg/mL) and high (3.0µg/mL).[15] Control group was injected with phosphate buffered saline (PBS) intraperitoneally (i.p.). The chronologic of randomized control series design research was shown in Figure 1.

Allergic sensitization and inhalation in rats was done according to Toward and Broadley's method.[16] In day 1 and 14 Wistar rats were injected i.p. with OVA (Sigma Aldrich, Germany), 10µg in 10mg $AIOH_3$ diluted until

1mL volume, each dose was 200µL. In day 21, they were subjected to OVA inhalation, 1mg/mL in sterile saline 1mL dose using nebulizer (OMRON™, USA) for 30 minutes. Intra sulcus (i.s) injections to induce gingivitis as well as to modulate allergic reaction with 0.3, 1.0 and 3.0µg/mL *Pg*LPS$_{1435/1450}$ (Astarte Biol, USA) were done in day 11 and 12 according to Dumitrescu's method in upper right molar (Figure 2), 4 days after stimulation resulted in chronic gingivitis (verified based on the change of gingival histopathological sample).[17] Control group were injected with PBS i.p.

The assisted drainage therapy, using sickle shaped scaler (Figure 3 and 4) was done for 3 minutes between the upper first and second molars (Figure 2) 20 minutes before OVA inhalation. After OVA inhalation, rats were sacrificed 30-40 minutes later. Each step (control, day 14, day 21 etc.), tissue samples were taken by dissecting the nasomaxillary tissue above the injected area (Figure 2), thus every subgroup must consist 18 rats. Sacrifice was done according to the euthanasia protocol. The local and systemic effect of ADT towards gingiva and nasal tissue were examined with peroxidase immunohistochemistry using diamino-benzidine (DAB).

This research and its laboratory examinations were conducted in the Biology Department Brawijaya University Malang, October 2008 until February 2009. The research protocol had been approved by the Animal Care and Use Ethical Committee, Faculty of Veterinary Medicine Airlangga University, Surabaya. Statistical analysis was done with Analysis of Variance (ANOVA) to reveal the interaction between variable doses of OVA, *Pg*LPS$_{1435/1450}$ and the assisted drainage therapy.

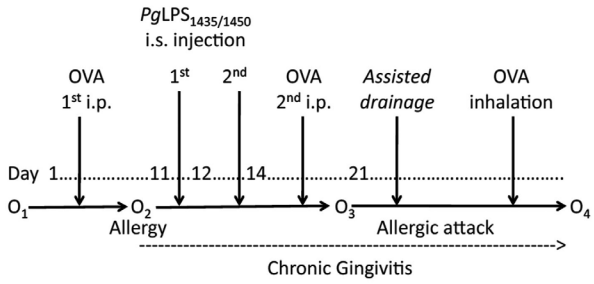

Figure 1. Randomized control series study design of OVA-induced allergic rats and allergic rats induced with *Pg*LPS$_{1435/1450}$
Notes:
O_1 rats which will be allergic induced with ovalbumin 1st i.p. injection in day 1
O_2 allergic rats will be induced with *Pg*LPS$_{1435/1450}$ i.s. injection in day 11 and 12 to create chronic gingivitis as well as ovalbumin 2nd i.p. injection in day 14
O_3 allergic rats with chronic gingivitis which will be treated with the assisted drainage therapy before olbumin inhalation in day 21
O_4 rats with allergy and chronic gingivitis that had been induced with OVA inhalation, without or with the assisted drainage therapy

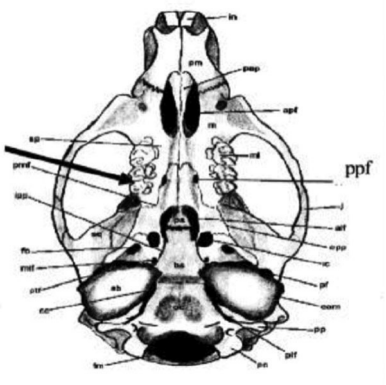

Figure 2. Location of injection and the Assisted Drainage Therapy (ADT) in Wistar rats

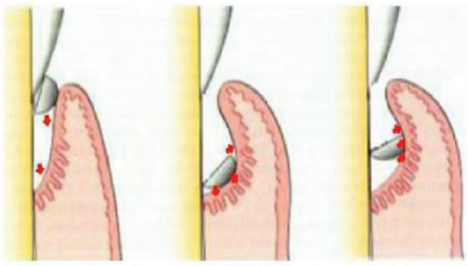

Figure 3. Assisted drainage therapy, scaling root planing with gingival sulcus massage (arrow) (adapted from Newman et al, 2006)[19]

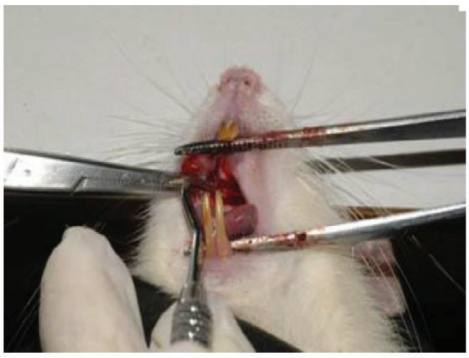

Figure 4. The assisted drainage therapy in rat

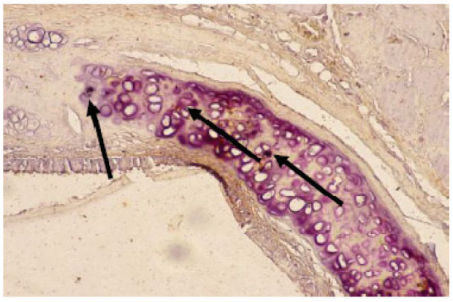

Figure 5. Nasal CGRP expression. After *Pg*LPS$_{1435/1450}$ i.s 2nd injection before OVA i.p. injection (day 14). 400x

RESULTS

Immunohistochemical examination
Allergic reaction and neurogenic inflammation biomarkers expressions of gingiva and nasal immunohistochemistry samples were counted per view with light microscope (Olympus™ CX-31). The image of CGRP expressions in nasal tissue was shown in Figure 5.

Effect of PgLPS $_{1435/1450}$ injection towards allergic rats
After intraperitoneal ovalbumin allergic sensitization and intrasulcular injection of *Pg*LPS$_{1435/1450}$ 0.3, 1.0 and 3.0 µg/mL, in day 11 and 12, there were several modulation of allergic and neurogenic inflammation biomarkers of nasal tissue in day 14 and 21. Nasal tissue examination results and statistical significance were shown in Table 1 and Table 2.

Local and systemic effects of ADT on day 21 using OVA inhalation
In order to verify the ability of ADT to create systemic effect; hence, from gingival stimulation which resulted in decrease of nasal symptoms, gingival and nasal tissue examinations should be evaluated. The local effect of ADT towards gingival tissue in allergic rats, which were subjected to OVA inhalation without ADT, revealed that they had significant higher expressions (p=0.001) of all allergic reaction (LTC4 and ECP) and neurogenic inflammation (VIP, CGRP) biomarkers if compared with group received ADT. Nasal tissue examination results and statistical significance were shown in Table 3. In this study, "rapid effect" would be verified, therefore euthanasia should be done as soon as possible, in this study it had been done approximately 60 minutes after ADT.

Effects of OVA, LPS and ADT in nasal tissue
Viewing the modulation of biomarkers compared to control which caused by treatments of OVA, *Pg*LPS $_{1435/1450}$ and ADT in nasal tissue was easily evaluated. Control and the results in rats' nasal tissue examinations with 3.0µg/mL *Pg*LPS$_{1435/1450}$ intra-sulcular injection could be seen in Figure 6a-d.

DISCUSSION

Several mechanisms have been proposed related to the inter relationship between CRS, migraine headache and allergy that are: (1) autonomic symptoms caused by parasympathetic cranial activation; (2) the neurogenic switching mechanism which were confirmed by other study.[11,13] The auto-nomic nervous system role is important in CRS since it provides the general innervation to the nose, with the parasympathetic nerves supplying the resting tone and controlling secretion; the sphenopalatine ganglion was account for this action.[12,13]

It was interesting that even in difficult allergic CRS, a child patient who was actually scheduled for nasal surgery, ADT rapidly relief her symptoms in minutes. Successful treatment result was confirmed by an ENT specialist one week later. It was true that according to the neurogenic switching mechanism, the trigger of sensory and parasympathetic stimulation could be initiated from chronic gingivitis.[4,19]

This study confirmed that the induction of chronic gingivitis by synthetic *Pg*LPS$_{1435/1450}$ is via toll-like receptor-2 (TLR2) and TLR4.[14] *Pg*LPS only stimu-lates TLR4, not TLR2, which is dependable for the induction of the allergy related T-helper2 (Th2) cytokines.[20] This study confirmed that *Pg*LPS$_{1435/1450}$ increased allergic reaction and neurogenic inflammation in nasal tissue, although the difference was not significant (Table 1).

In day 14, significant increase of inflammation biomarkers were observed in all groups (Table 1). It was logical since higher LPS dose induced greater inflammatory response. This result was in line with other study which reported the increase of leukotriens from mast cells after *Pg*LPS injection.[9]

Table 1. Allergic reaction and neurogenic inflammation in allergic rats (nasal tissue, day 14)

Dependent variable	Allergic Only Mean ± SD	Allergic Rats with *Pg*LPS$_{1435/1450}$ injection Mean±SD					
Allergic reaction		**0.3µg/mL n = 6**	***p***	**1.0µg/mL n = 6**	***p***	**3.0µg/mL n = 6**	***p***
LTC4	2.167±1.722	2.833±0.983	0.951	5.000±1.265	*0.140	7.833±3.189	*0.001
ECP	3.000±0.894	7.167±0.983	0.089	5.167±3.061	0.577	7.667±4.082	0.048
Neurogenic inflammation							
VIP	2.833±1.169	5.833±2.787	*0.142	7.50±0.548	0.010	9.50±2.881	*0.001
CGRP	2.167±0.753	9.50±4.722	0.002	6.833±2.137	0.070	7.50±2.074	*0.032

*significant (p<0.05)

Table 2. Allergic reaction and neurogenic inflammation in allergic rats (nasal tissue, day 21)

Dependent variable	Allergic Only Mean ± SD	Allergic Rats with *Pg*LPS $_{1435/1450}$ injection Mean±SD					
Allergic reaction		**0.3µg/mL n = 6**	***p***	**1.0µg/mL n = 6**	***p***	**3.0µg/mL n = 6**	***p***
LTC4	17.50±3.507	7.167±0.753	*0.001	9.833±5.879	*0.014	7.167±2.137	*0.001
ECP	19.00±2.280	10.50±1.871	*0.001	10.833±3.869	*0.001	11.667±0.817	*0.001
Neurogenic inflammation							
VIP	15.00±0.894	12.167±1.472	0.218	10.333±1.751	*0.016	13.333±3.724	0.649
CGRP	14.667±1.633	12.167±1.472	0.193	11.667±1.033	0.090	15.167±2.927	0.976

*significant (p<0.05)

Table 3. Allergic rats OVA inhalation with ADT vs without ADT (nasal tissue, *Pg*LPS$_{1435/1450}$ 3.0µg/mL)

Dependent variable	Allergic Pre-inhalation	Allergic OVA Inhalation without ADT		Allergic OVA Inhalation with ADT		without ADT vs. with ADT
Allergic reaction	**Mean ± SD**	**Mean ± SD**	***p***	**Mean±SD**	***p***	***p***
LTC4	7.167±2.137	22.00±1.414	*0.001	11.833±1.472	*0.001	*0.001
ECP	11.667±0.817	17.833±0.753	*0.001	17.00±3.033	*0.003	*0.001
Neurogenic inflammation						
VIP	13.333±3.724	19.333±2.160	*0.009	6.00±3.225	*0.004	*0.001
CGRP	15.167±2.927	18.167±4.119	0.177	3.167±1.941	*0.001	*0.001

*significant (p<0.05)

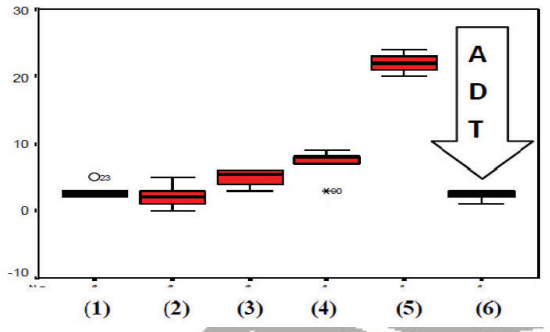

Figure 6A. Expression of LTC4 in allergic only and allergy with PgLPS1$_{1435/1450}$ 3.0µg/mL injection

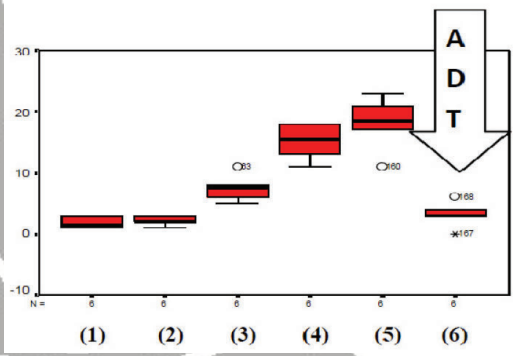

Figure 6C. Expression of CGRP in allergic only and allergy with *Pg*LPS$_{1435/1450}$ 3.0µg/mL injection

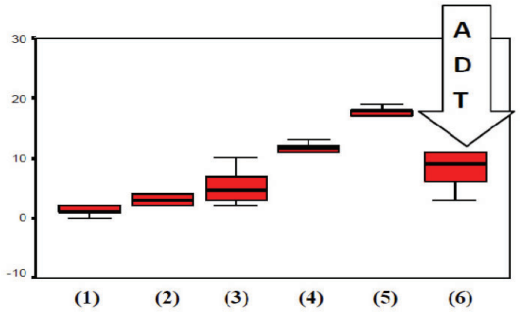

Figure 6B. Expression of ECP in allergic only and allergy with *Pg*LPS$_{1435/1450}$ 3.0µg/mL injection

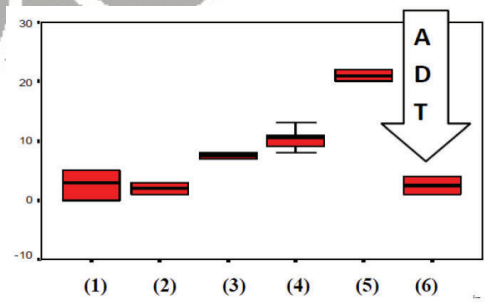

Figure 6D. Expression of VIP in allergic only and allergy with *Pg*LPS$_{1435/1450}$ 3.0µg/mL injection

The effects of OVA, LPS (3.0µg/mL) and ADT in nasal tissue in Boxplots. Notes: (1) Control (pre-treatment); (2) before OVA 2nd i.p. injection (day 14): (3) after *Pg*LPS$_{1435/1450}$ 2nd i.s. injection, before 2nd OVA i.p. injection (day 14); (4) OVA pre-inhalation (day 21); (5) OVA inhalation without ADT; (6) OVA inhalation with ADT (arrow). The four boxplots showed that OVA i.p. injection, OVA inhalation and *Pg*LPS$_{1435/1450}$ i.s. injection were able to increase allergic reaction and the neurogenic inflammation biomarkers in the nasal tissue. On the opposite, ADT decreased allergic reaction and the neurogenic switching mechanism (Figure 6).

Interestingly, in day 21, opposite results were shown. The *Pg*LPS injection have decreased all biomarkers that caused by OVA sensitization (Table 2). This result might mimics the endotoxin tolerance phenomenon, that resulted from the resistance of host immune response towards prolonged LPS stimulation.[21] It was hypothesized that endotoxin tolerance resulted in the diminished interleukin (IL)-12p40 production and increased of IL-10 has led to the shifting to the allergy-related immune response marked by expression of Th2.[21] The result of our study supported the endotoxin tolerance theory and prolonged *Pg*LPS in term of oral infection, increased allergic reaction making higher role of the Th2 immune response.

Table 1 and 2 showed the results of allergic sensitization with OVA i.p. injection in Wistar rats with and without *Pg*LPS$_{1435/1450}$ i.s. injection in nasal tissue. At day 14, all rats received *Pg*LPS$_{1435/1450}$ injections had higher expressions of all biomarkers compared with allergic group only. On the contrary, in day 21 all biomarkers had lower expressions in all doses compared with allergic group only, although it was not always significant.

Immunogenic and neurogenic inflammations in oral tissue could be triggered by LPS via TLRs, it was confirmed in this study that after *Pg*LPS$_{1435/1450}$ i.s. injection, gingival biomarkers expressions for allergic reaction and neurogenic inflammation increases significantly (p=0.001). In neurogenic inflammation, continued release of VIP and CGRP is able to activate mast cells and basophils to release histamine and tryptase, that in turn stimulatesnerve endings, so called the neurogenic switching mechanism.[11,19] In this study, it was also verified that *Pg*LPS$_{1435/1450}$ gingival i.s. injection was able to stimulate distant nerve tissue based on the increase of VIP and CGRP expressions in the nasal tissue. In this study, the verification of mast cells and basophils activations in this study was indicated by the increase of LTC4 expressionand eosinophils activation by ECP expression (Table 1).

The result of this study was inconsistent with previous study in neonate Balb/c mice, which reported that only low dose (<1.0µg/mL) of *Pg*LPS increased allergy via serum IgE examination after 2 months.[15] Since this study was conducted only 21 days and in older rats, the result could be different. Nevertheless, it supported our hypothesis that all oral infections should be eliminated to reduce allergy.

Instant resolution of rhinosinusitis symptoms after the ADT was suspected due to rapid decrease of the allergic and neurogenic inflammation in allergic Wistar rats in this study. The most important systemic effect of ADT in allergic rhinosinusitis was its ability to improve sinusitis symptoms verified by significant decrease (p=0.001) of LTC4, ECP, VIP and CGRP expressions in nasal tissue within minutes after OVA inhalation (Table 3 and Figure 6a-d). This phenomenon could be explained by the occurrence of "synaptic plasticity" (change in response strength of transmission over synaptic pathways) during ADT which able to slow down the nerve transmission.[22]

Table 3 showed that in nasal tissue of allergic rats that were subjected to OVA inhalation without ADT, had significant higher expression of all allergic reaction and neurogenic inflammation biomarkers compared with OVA inhalation with ADT. The local effect of ADT towards gingival tissue in allergic rats which were subjected to OVA inhalation without ADT revealed that they had significant higher expressions (p=0.001) of all allergic reaction (LTC4 and ECP) and neurogenic inflammation (VIP and CGRP) biomarkers compared with ADT. The systemic effect of ADT towards nasal tissue was shown in Table 3. The assisted drainage therapy by massaging the gingival sulcus for about 3 minutes is suggested to increase local tissue temperature.

One study revealed that increase of tissue temperature will decrease tissue pH and increase of extracellular Ca^{2+} level.[22] Disturbance of Ca^{2+} regulation which is important for pre-synapse stimulation resulted in the occurrence of "synaptic plasticity" for several minutes which then lowering the synapse impulse flow.[23] This condition resulted in an immediate "cut off" of the neurogenic switching mechanism after ADT verified by the rapid significant decrease of VIP (p=0.001), and CGRP (p=0.001) expressions in the gingival tissue (data not shown) and nasal tissue (Table 3). It also answered the main question: "Why the assisted drainage therapy was able to improve nasal congestion within minutes?"

Other solution for answering the question was considering the half-life of a substance. The half -life of allergic reaction biomarkers LTC4 was 7 minutes and ECP was 65 minutes.[9,24] Since the time lag between ADT and rat euthanasia was approximately 60 minutes, it was logical that significant difference had already occurred.

The half-life for neurogenic switching mediators were very fast (VIP = 2-5mins and CGRP = 6-10mins).[19] Therefore, it was logical that ADT was able to cut off the neurogenic switching mechanism, either by

increasing the local tissue temperature or drainage of inflammatory mediators that came out concomitantly within the oozed blood after ADT had been done.

Since nasal cavity also innervated by the maxillary nerve (CNV2); stimulation of this nerve can reflexively influence nasal engorgement, respiration rate, nasal secretion, and sneezing. Since most trigeminal stimulants were lipid soluble, such as volatile chemicals; the stimulations were possible.[12] It was suggested that stimulated CNV2 and the SPG may caused multiple chemical sensitivity syndrome (MCS) that was sensitive to humidity, cold weather and volatile which were not related to allergy.[12] It is in line with a study that showed "allergic" rhinosinusitis symptoms are not related to the increase of IgE, since IgE-mediated allergy is present only in a proportion of rhinosinusitis patients.[25] Therefore, despite the presence of classical IgE-mediated allergy, based on elevated allergen-specific serum IgE levels and positive skin prick tests, currently there is no direct evidence for allergy as a major cause of sinonasal inflammation in CRS.

Consequently, other than allergy only, the role of oral infection was important in rhinosinusitis. Sensitization of the CNV2 in the oral cavity which also innervated nasal tissue was confirmed by the increase of CGRP expressions in gingival and nasal tissue after *Pg*$LPS_{1435/1450}$ injection. Stimulation of CNV2 in the gingiva may propagate antidromically (opposite to the direction of regular impulse) to the nasal cavity and able to stimulate parasympathetic nerve directly via the SPG.[12,26] This action was verified by the increase of VIP expression in nasal tissue which was the main secretion of parasympathetic nerve, and also mimicking the "axon reflex" mechanism in CRS and asthma connection.[8,13] The connectivity of stimulation between nasal sensory and parasympathetic nerves also confirmed by other study.[27]

CONCLUSION

Based on the significant decrease of allergic and neurogenic biomarkers of CRS within minutes after the "assisted drainage" therapy, it was concluded that this therapy was an effective procedure for rapid relief of CRS which were suspected to be induced by periodontal infection. Additionally, this study also explained: (1) the unknown pathophysiology of CRS which may cause by the sensitization of SPG which initiated from chronic gingivitis of maxillary teeth and (2) that oral periodontopathic bacteria product, the *P. gingivalis* LPS, was able to increase allergic reaction, therefore elimination of oral infection was important to reduce allergy. Consequently, in order to minimize unnecessary as well as prolonged treatments and medications for the CRS, the collaboration of medical practitioners, especially otolaryngologist and dental practitioners is important.

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