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ORIGINAL ARTICLE

Neurogenic Inflammation Involves in Systemic Spread of Oral Infection

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ABSTRACT

Focal infection theory proposed in early 1900's stated that dental infection caused systemic disorders. Nevertheless, the theory was abandoned since large number of teeth were extracted with no satisfying result. Recent reports revealed that oral infections were able to spread systemically. However, there is no rationalization available to explain how assisted drainage therapy (ADT), a periodontal therapy that could relief migraine and asthma within minutes. Oral neurogenic and immunogenic inflammation interaction involving pro-inflammatory markers such as calcitonin gene-related peptide (CGRP), TNF-α; and antiinflammatory vasoactive intestinal peptide (VIP) was still under investigation. **Objective:** To verify the spread of oral inflammation to distant organ after performing ADT by analysing CGRP, VIP and TNF-α expressions. **Methods:** Two different concentration of *Porphyromonas gingivalis* lipopolysaccharide (*Pg*$LPS_{1435/1450}$) was injected intragingivally into two groups of 12 Wistar rats. After four days, 12 rats were given ADT and all samples were subsequently sacrificed 40 mins after ADT. Immunohistochemistry analysis using CGRP, VIP and TNF-α on the nasal and bronchus tissue was performed. ANOVA was used for statistical analyisis of the difference between CGRP, VIP and TNF-α expression between experimental groups. **Results:** *Pg*LPS injections slightly increased CGRP, VIP and TNF-α expressions in the control group. Rats undergone ADT had lower CGRP and TNF-α but higher VIP expressions. **Conclusion:** Neurogenic inflammation involved in systemic spread of oral infection. ADT was able to downregulate inflammation in distant organ posibly by stimulating VIP.

ABSTRAK

Inflammasi neurogenik berperan pada penyebaran infeksi oral. Teori infeksi fokal rongga mulut telah diusulkan sejak awal 1900an, infeksi gigi menyebabkan berbagai penyakit sistemik. Namun, teori ini mulai ditinggalkan setelah banyak gigi telah dicabut tanpa memberikan hasil yang memuaskan. Penelitian terbaru membuktikan bahwa infeksi rongga mulut dapat menyebar secara sistemik. Walaupun demikian, penemuan terdahulu tidak dapat membuktikan bagaimana terapi periodontal "assisted drainage" (ADT), dapat mengurangi gejala migren dan asma dalam hitungan menit. Penelitian terkait interaksi keradangan imunogenik dan neurogenik yaitu mediator pro-inflamasi *calcitonin gene-related peptide* (CGRP), TNF-α dan *vasoactive intestinal peptide* (VIP) masih jarang dilakukan. **Tujuan:** Melakukan verifikasi penyebaran keradangan neurogenik mulut ke organ yang jauh setelah melakukan ADT melalui ekspresi CGRP, VIP dan TNF-α. **Metode**: 24 tikus wistar jantan disuntik intragingival dengan lipopolisakarida *Porphyromonas gingivalis* (*Pg*$LPS_{1435/1450}$). Setelah empat hari, 12 tikus diberikan ADT, kemudian semua sampel dikorbankan 40 menit setelah ADT. Ekpresi CGRP, VIP dan TNF-α dianalisis dengan imunohistokimia. Analisis statistik menggunakan ANOVA dilakukan untuk menganalisis perbedaan nilai ekspresi CGRP, VIP, dan TNF-α tiap kelompok uji. **Hasil:** Injeksi *Pg*LPS meningkatkan CGRP, VIP an TNF-α walau tidak selalu bermakna pada kelompok kontrol. Ekspresi CGRP dan TNF-α menurun, tetapi ekspresi VIP meningkat pada kelompok ADT. **Simpulan:** Peradangan neurogenik terlibat dalam penyebaran keradangan rongga mulut ke seluruh tubuh yang dimungkinkan karena ADT mengurangi peradangan organ lain melalui stimulasi VIP.

Key words: assisted drainage therapy, focal infection, immunogenic, neurogenic inflammation

INTRODUCTION

Focal infection theory postulates that a myriad of diseases could be caused by microorganisms that arise endogenously from a focus of infection.[1] In the 20th century, this concept was pioneered by William Hunter, in a publication and a 1910 talk at McGill University, Montreal. He said that dental restorations "built in, on, and around diseased teeth which form a veritable mausoleum of gold over a mass of sepsis to which there is no parallel in the whole realm of medicine." It emphasized the importance of cooperation between dentists and physicians, as well as the necessity of ensuring that the focus of infection is completely eliminated.[2] Since then, it became a common practice to extract all endodontically or periodontally involved teeth to eliminate focal infection. However, the concept was eventually forgotten by medical and dental society in the 30's since there were no clear result.[3]

Interestingly, this theory is currently being carefully reconsidered. At the landmark conference at the University of North Carolina in 1997, it was devoted to this theme, that periodontal disease can contribute conditions such as cardiovascular the disease and that periodontal therapy may contribute to control of diabetes.[2] Nevertheless, the concept of oro-systemic connections mainly based on immunological mechanism. The effect of neurogenic mechanism that responsible to amplify immunogenic inflammation was rarely investigated.

Moreover, immunological concept of oral focal infection could not explain the rapid improvement of asthma symptoms after periodontal treatment. The assisted drainage therapy (ADT), a new periodontal therapy, that is consisted of scaling root planing combined with subgingival massage. Previous studies have shown that ADT significantly improved forced expiratory volume in one second (FEV1) in allergic asthma children in Dr Soetomo Hospital Surabaya.[4] The asthmatic symptoms diminished within 5 minutes following ADT. The antiinflammation mechanism of ADT was still uncovered. One of the possibilities would be the involvement of neurogenic inflammation that give fast response after treatment. Oral neurogenic inflammation article was considered rare.[5] Therefore, the progress of oral neurogenic research towards systemic diseases was slow. In medicine, there was a concept called "neurogenic switching" introduced by Meggs in 1997. It revealed the interaction between mast cells (MC) and sensory nerve.[6] It proposed that distant inflammation may caused by the propagation or spread of this interaction.

Inflammation is a critical process in the oral cavity, especially in gingival inflammation and pulpitis. Nevertheless, the cellular process involved in oral inflammation is not well delineated. Recent evidence from other organs as well as the mouth suggests that neurogenic inflammation involving MCs may be a critical factor.[7] Spreading of inflammation was caused by amplification of local inflammation via mast cell-nerve interaction involving proinflammatory mediators calcitonin gene-related peptide (CGRP) which able to stimulate MCs whose product tumor necrosis factor-α (TNF-α) is able to stimulate nerve endings, thus became a "vicious circle".[6] In order to achieve homeostasis, which is the healthy stage of the body, naturally the body also releases antiinflammatory mediator such as vasoactive intestinal peptide (VIP).[8] However, the role of VIP in mast cell-nerve interaction is not clearly understood. The objective of this study was to verify the spread of neurogenic inflammation in periodontal tissue to distant organ by examining nasal and bronchus tissue as well as ADT role in the downregulation of inflammation via stimulating antiinflammatory mediator release.

METHODS

This research was conducted in the Biology Department Brawijaya University Malang. The research protocol had been approved by the Animal Care and Use Ethical Committee, Faculty of Veterinary Medicine, Airlangga University, Surabaya. Intragingival injection *Pg*LPS$_{1435/1450}$ (Astarte Biologics, WA, USA) was performed on twenty four male wistar rats with weight ranging from 120-150 grams.[9,10] One group of 12 rats subjected to 0.3 µg/mL (low dose), other group of 12 rats subjected to 3.0 µg/mL (high dose), and six rats was injected with phosphate buffered saline (PBS) which served as control group. Exact dose of *Pg*LPS$_{1435/1450}$ achieved by diluting 100 µg *Pg*LPS$_{1435/1450}$ with PBS. After four days of injection, 6 rats of each experimental group was treated with assisted drainage therapy (ADT) (Figure. 1). The assisted drainage therapy, a procedure of subgingival massage using the blunt side/back of sickle shaped scaler, was done for 3 minutes between the upper first and second molars. The rats in all groups were sacrificed 30-40 minutes after ADT. Nasal and bronchus tissue samples of were taken for analysis. Demineralization of the tissue samples was performed for 7-10 days with ethylenediaminetetraacetic acid (EDTA). The TNF-α (Santa Cruz Biotech, USA); (2) CGRP (Santa Cruz Biotech, USA); and (3) VIP (Santa Cruz Biotech, USA) monoclonal antibodies were used for peroxidase immunohistochemistry analysis according to the manufacturer instruction. Statistical analysis was done with Analysis of Variance (ANOVA) to analyse the interaction between TNF-α, CGRP and VIP as inflammation biomarkers with concentration of *Pg*LPS$_{1435/1450}$ doses and ADT. The number of cells with positive expression of neurogenic and immunogenic biomarkers on nasal and bronchus tissue samples were counted per view using light microscopy (Olympus™ CX-31).

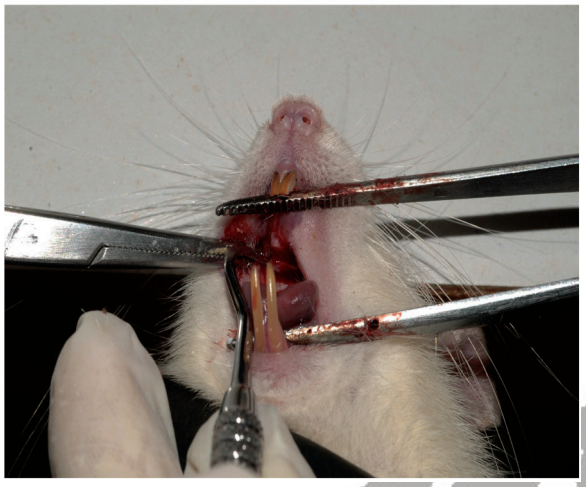

Figure 1. Assisted drainage therapy in Wistar rat

Table 1. Immunogenic and neurogenic inflammation in nose after *Pg*LPS$_{1435/1450}$ injection

Dependent variable	Control Mean ± SD	*Pg*LPS$_{1435/1450}$ 0.3 µg/mL n = 6	*p*	*Pg*LPS$_{1435/1450}$ 3.0 µg/mL n = 6	*p*
Immunogenic					
TNF-α	5.00±0.89	29.83±1.17	0.001	34.83±2.48	0.001
Neurogenic					
CGRP	1.83±0.98	6.33±2.58	0.014	2.67±1.21	0.992*
VIP	2.67± 2.34	3.67± 1.51	0.773*	3.67±1.37	0.077*

Table 2. Immunogenic and neurogenic inflammation in bronchus after PgLPS$_{1435/1450}$ injection

Dependent variable	Control Mean±SD	*Pg*LPS$_{1435/1450}$ 0.3 µg/mL n = 6	*p*	*Pg*LPS$_{1435/1450}$ 3.0 µg/mL n = 6	*p*
Immunogenic					
TNF-α	10.00±2.76	20.33±3.01	0.001	21.67±1.63	0.001
Neurogenic					
CGRP	2.33±1.86	11.50±1.38	0.001	12.33±1.37	0.001
VIP	1.33±0.82	5.00±1.26	0.002	8.00±1.41	0.001

Table 3. The effect of assisted drainage toward inflammations in nose

	Before	After ADT *Pg*LPS$_{1435/1450}$ 0.3 µg/mL n = 6	*p*	Before	After ADT *Pg*LPS$_{1435/1450}$ 3.0 µg/mL n= 6	*p*
Immunogenic						
TNF-α	20.17±0.98	15.00±2.28	0.001	25.67± 3.20	20.33 ± 3.01	0.014
Neurogenic						
CGRP	6.33±2.58	7.50±4.23	0.577*	5.50±2.88	2.33±1.37	0.035
VIP	3.67± 1.51	8.33±2.66	0.004*	6.00±1.26	8.83±3.12	0.067*

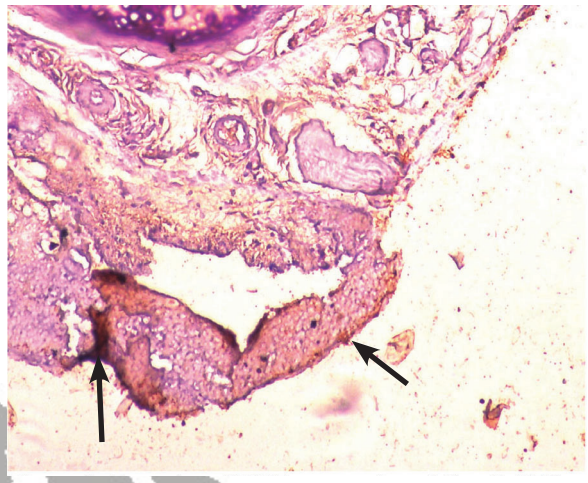

Figure 2. Bronchus CGRP expression. After *Pg*LPS$_{1435/1450}$ injection (day 4). Brown stain. Magnification: 400x

Table 4. The effect of assisted drainage towards inflammations in bronchus

Dependent variable	Before ADT *Pg*LPS$_{1435/1450}$ 0.3 µg/mL n = 6		After AD	Before ADT *Pg*LPS$_{1435/1450}$ 3.0 µg/mL n = 6		After ADT
	Mean±SD	Mean±SD	*p*	Mean±SD	Mean±SD	*p*
Immunogenic						
TNF- α	20.33±3.01	15.00±2.10	0.005	21.67±1.63	16.33±1.03	0.001
Neurogenic						
CGRP	11.50±1.38	7.33±3.44	0.020	12.33±1.37	8.67±1.97	0.004
VIP	5.00±1.26	12.33±1.86	0.001	8.00±1.41	23.00±1.79	0.001

RESULTS

The immunopositive staining of neurogenic and immunogenic biomarkers on nasal and bronchus tissue samples using were counted per view using the Olympus™ CX-31 light microscope. The CGRP expression in nasal and bronchus was shown in Figure 2. This study showed that the effect of oral infection via *Pg*LPS$_{1435/1450}$ injection that disseminate to nose and bronchus resulted in increasing trend of pro-inflammatory mediators expression, however the result was not always significant (Table 1 and Table 2).

In nose tissue, significant decrease of TNF-α expression was seen after performing ADT on *Pg*LPS$_{1435/1450}$ injected rats (p=0.001 and p=0.014) and only slight decrease of CRGP expression was noted (p>0.05). On the other hand, VIP expression in nose tissue after ADT on low dose injected of *Pg*LPS$_{1435/1450}$ was significantly increased after ADT (p=0.004) (Table 3).

Performing ADT on the two experimental groups resulted in significant decrease of pro-inflammatory mediators (CGRP and TNF-α) in bronchus tissue. There were significant decrease on the number of cells with CGRP expression between low (p=0.002) and high

(p=0.004) dose of PgLPS$_{1435/1450}$ injection in bronchus compared to control before and after ADT. While the CGRP expression decreased almost half of control after ADT, the TNF-α expression was only slightly decreased within the groups, however the results were still statistically significant (p=0.005 vs p=0.001 respectively) (Table 4). On the contrary, the expression of VIP on bronchus tissue after ADT of PgLPS$_{1435/1450}$ injection showed two fold increase in the low dose group (p=0.001) and almost three fold increase in the high dose group (p=0.001) (Table 4).

DISCUSSION

During the past decades the relationship between dentistry and internal medicine, especially the concept of focal infection theory has long been a debatable matter.[11] The pathogenesis of focal diseases has been classically attributed to dental pulp pathologies and periapical infections.[2] Nonetheless, in recent years, their roles are being dismissed, while increasing interest is being devoted to the possible association between periodontal infection and systemic diseases.[3,12] In fact, periodontal pathogens and their products, as well as inflammatory mediators produced in periodontal tissues, might enter the bloodstream, causing systemic effects and/or contributing to systemic diseases. Chronic periodontitis has been suggested as a risk factor for cardiovascular diseases, diabetes mellitus, preterm delivery, etc.[12] Many hypotheses, including common susceptibility, systemic inflammation, direct bacterial infection and cross-reactivity, or molecular mimicry, between bacterial antigens and self-antigens, have been postulated to explain the mechanism.[12]

Mast cells, best known for their role in allergic reactions, are also involved in immunity and inflammation. They are located at strategic point, that close to small blood vessels and nerve fibers and often containing substance P (SP) and CGRP.[13] The pain models of reversible or irreversible pulpitis simply suggest the complexity of neural-inflammatory interactions within the dental pulp.[7] Nevertheless, interestingly, in periodontal inflamation, chronic periodontitis does not elicit pain.[14] In the pulp and periapical area, neuropeptides and cytokines modulate vascular responses and increase permeability. Immunoreactive nerve fibers and TNF-positive MCs were found localized around blood vessels in periapical granulomas.[7] By generating a profound number of potent mediators, MCs may serve as a link between the immune, endocrine and nervous systems in pulp and periodontal inflammation.[5]

Mast cells and nerves interaction have been proven to be responsible for flare reaction to noxious stimuli, as seen in the skin. Local injury and/or antidromic stimulation of neurons sensitizes local C fibers which then release chemical mediators during the axon-reflex. The local C fibers are SP, CGRP and other neuropeptides.[7] Substance P has an important role in acute inflammation, whereas CGRP in chronic inflammation.[13] In this study, the effect of oral infection via PgLPS$_{1435/1450}$ injection to distant organ (nose and bronchus) was described in Table 1 and Table 2. There was increased of proinflammatory mediators even not always significant. It was in accordance previous study that revealed that PgLPS was "weaker" than *E. coli* LPS, and organ-dependent.[14] In that study, PgLPS only stimulated scalp but not heart, resulted a different biomarkers modulation from bronchus.

In patient with migraine and asthma, CGRP,[15,16] VIP[15] and TNF-α[16] were considered as valid diagnostic biomarkers. Therefore, recent drug findings were antagonists and inhibitors, such as CGRP-receptor antagonist for migraine[17] and TNF-α inhibitor for asthma.[18] However, drug-dependent have deleterious effect i.e. cardiovascular side effects (CGRP antagonist)[17] and more for TNF-α inhibitor (heart failure, infections, neutropenia etc),[18]. Despite of the idea of drug-therapy, our interesting finding was a non-drug therapy which is the ADT. It has the ability to reduce migraine and asthma symptoms within minutes. In this animal study, after ADT there was significant decrease of pro-inflammatory mediators both in low and high dose PgLPS injections in bronchus CGRP (p=0.020 and p=0.004) and TNF-α (p=0.005 and p=0.001) expressions; concomitantly with the significant increase of antiinflammatory mediators in bronchus, that was VIP (p=0.001 and p=0.001) within minutes. Subsequently, euthanasia was done 30 mins after ADT (Table 3 and Table 4). These results verified the rapid relief after ADT towards migraine and asthma. Decrease of pro-inflammatory mediators that accompanied by increasing of anti-inflammatory mediators should lead the body to homeostasis, thus cure the illness.

It was interesting that in nasal tissue, the results of ADT towards neurogenic inflammation was not as remarkable as in bronchus tissue. Decrease of nasal CGRP were insignificant (p=0.577 and p=0.035). The non-significant decrease of CGRP may caused by the stimulated sensory CGRP- receptor in maxillary nerves after ADT, which is a mechanical massage therapy with manual scaler. It was in accordance with previous study which used several rotary endodontic instruments. The study reported that the severity of periodontal ligament inflammation was directly proportional to the degree the mechanical stress exerted on the tooth.[19] This mechanical stress then stimulates the release SP and CGRP.[19] In our study, the increase of periodontal CGRP propagated to maxillary nerve in the nose which nearer to the mouth than bronchus. Thus, increase of CGRP expression in nasal tissue after ADT was logical.

The increase of VIP after ADT even it was a biomarker for migraine and sinusitis[15] was not regarded as a

negative effect, owing to its anti-inflammatory effect by acting as macrophage-deactivating factors to prevent the excessive production of pro-inflammatory cytokines i.e. inhibits LPS-induced TNF-α, IL-6, and IL-12 production in activated macrophage.[8] It was reported that VIP is a potent vasodilator of airway smooth muscle in vitro and in vivo.[20] In isolated tracheal or bronchial segments, VIP attenuates the constrictor effect of histamine, leukotriene D4, kallikrein and neurokinin A. The bronchodilatory effect of VIP in human bronchi is almost 100 times more potent than adrenergic dilatation by isoproterenol, and VIP is the most potent endogenous bronchodilator described so far. The problem was owing to very short half-life of 2 to 5 mins that makes difficult for application. However, it was invented intranasal VIP that was considered beneficial for correcting chronic inflammatory response syndrome (CIRS) which is a common illness nowadays.[21] As the result, increasing VIP level is beneficial for maintaining systemic health.

The presence of MCs-nerve interaction was verified based on the simultaneous increase of both pro-inflammatory biomarkers (CGRP and TNF-α) after injection which followed by simultaneous decrease after ADT. In this study, ADT stimulate anti-inflammatory mediators VIP. VIP was considered to have important role in rapid relief of ADT.

CONCLUSION

In conclusion, oral focal infection theory could be explained as an interaction of immunogenic and neurogenic inflammation from the oral tisssue that amplified and propagate to the whole body. The ADT is able to downregulate immunogenic and neurogenic inflammation by increasing VIP in distant organs, thus recreates homeostasis within minutes. However, further multidisciplinary research with medical researchers should be performed to give more understanding on the mechanism.

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