Effect of Vernonia Cinerea in Improvement of Respiratory Tissue in Chronic Nicotine Treatment

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Objective: To demonstrate the effect of Vernonia cinerea (VC) on rat respiratory tissue in chronic nicotine condition.

Material and Method: Pathology of rat respiratory tissue was induced by intraperitoneally injection with 1 mg/kg BW of rat. Male Wistar rats were divided into three groups, control group (C), nicotine treated group (N) and nicotine treated with Vernonia cinerea (VC) supplementation (NV, 100 mg/kg BW of rat) for 3 and 6 months. The animals were sacrificed and the respiratory tissues were removed and further processed for paraffin embedment and stained with Hematoxylin & Eosin (H&E), Periodic Acid Schiff (PAS), and Masson’s trichrome techniques.

Results: The histopathology of lung tissue and trachea occurred in a chronic nicotine treatment. The thickness of alveolar walls and proliferation of alveolar type 2 cell were found. There was remarkable increasing of various inflammatory cells, alveolar macrophages, lymphocytes and plasma cells after nicotine treatment for 6 months. A large number of small blood vessels appeared in the alveolar wall. Nicotine also caused fibrosis which dispersed throughout the lung parenchyma in perivascular, peribronchiole and alveolar wall regions. Moreover, there was the appearance of epithelial cell injury and goblet cell hyperplasia in the trachea. Regarding the VC supplementation, the result of a recovery of alveolar walls, i.e. decreasing of various inflammatory cells and alveolar type 2 cells was clearly demonstrated. In addition, the fibrosis and goblet cell hyperplasia were almost disappeared in the lung tissue after VC treatment.

Conclusion: Administration of VC in a chronic nicotine treatment resulted in an improvement of respiratory tissue. The recovery of the respiratory tract, especially trachea and lung tissue was characterized by the remarkable decrease of various inflammatory cells, fibrotic areas, and goblet cell hyperplasia. The VC, therefore shows the potential effect to be a new herbal therapeutic agent for alleviate the symptoms of the respiratory tract caused by nicotine from heavy cigarette smoke.

Keywords: Nicotine, Cigarette smoke, Vernonia cinerea, Respiratory tissue, Fibrosis, Goblet cell hyperplasia

Cigarette smoke is the most prominent factor for severe health problems, determining the morbidity and mortality(1). It is a major risk factor for a number of diseases, including chronic obstructive pulmonary disease, lung cancer, goblet cell metaplasia, lung fibrosis and emphysema(2,3). The epithelial mucosa of lung is the primary target for initial exposure to cigarette smoke. Therefore, the repeated cycles of tissue damage, inflammation and mucosal repair of lung tissue after response to chronic cigarette smoke exposure can result in epithelial squamous metaplasia of bronchus, alveolar wall hypertrophy and hyperplasia, lung fibrosis, mucus hypersecretion and goblet cell metaplasia, etc(3,4).

The major component of cigarette smoke is nicotine (N), which has a major effect on respiratory, cardiovascular, endocrinology and central nervous
systems. Their effects are cell inflammation, proliferation, angiogenesis and promoting carcinogenic cellular transformation\textsuperscript{4,5}. Therefore, chronic nicotine exposure causes a mitogenic stimulation which leads to activate the growth factor and anti-apoptotic signaling pathways e.g. phosphatidylinositol-3-kinase (PI3K), serine/threonine kinase (Akt), nuclear factor-kappaB (NF-kB), cyclooxygenase-2 (COX-2), B-cell lymphoma 2 (Bcl2), mitogen-activated protein kinases (MAPKs) and etc\textsuperscript{6-8}.

The production of Th2 cytokines also affects the functions of other cell types, e.g. goblet cells for an overproduction and secretion of mucus as well as there is a proliferation of goblet cells in a bronchial epithelium. Moreover, there is an increase of smooth muscle contraction in the airway\textsuperscript{9,10}. The hyperplasia and hypertrophy of alveolar type 2 cell are also reported as one of the prominent responses to the pulmonary toxicants. The alveolar type 2 cell population is rather sensitive to the deposition of toxicants in the epithelial lining of the alveolar wall whereas the alveolar macrophages are sensitive to pulmonary toxicants and able to release various inflammatory mediators\textsuperscript{11,12}. Moreover, there are recent evidence suggested that eosinophils, mast cells, neutrophils and alveolar macrophages also play a critical role in Th2 cell trafficking in animal models of lung damage\textsuperscript{13}.

\textit{Vernonia cinerea} is Thai traditional herbal medicine which is locally used for remedy many diseases, \textit{i.e.} asthma, stomachache, peptic ulcer, complications during menstruation, painful urination, rheumatoid arthritis, back and flank pains, including reduced addiction for smoking cessation\textsuperscript{14,15}. There are some evidence indicated the effects of VC having an anti-inflammatory activity in pyrexia and paw edema, analgesic, antipyretic, adjuvant arthritic in rats\textsuperscript{16,17}. Recently, VC extract has notably been demonstrated to inhibit lung metastasis by regulating the activities of matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), prolyl hydroxylase, lysyl oxidase, extracellular-signal-regulated kinases (ERK-1, ERK-2), tissue inhibitors of metalloproteinases (TIMP) and proinflammatory cytokine gene expression\textsuperscript{18}. Although many people have used VC as the herbal medicine for treating a person who want to stop smoking, but there were no scientific data concerning the anti-inflammatory function of this plant to the pulmonary function.

Therefore, the aim of the present study is to evaluate the effect of VC supplementation in chronic nicotine induced animal model, emphasizing on the morphological changes of the respiratory organs.

**Material and Method**

**Animals**

Male Wistar rats (body weight; BW, 200-250 g), were obtained from National Laboratory Animal Center of Mahidol University, Thailand. The animals were maintained in controlled animal laboratory with 12:12 hour light-dark cycle. The present study was approved by the Institutional Animal Care and Use Committee of Faculty of Medicine, Srinakharinwirot University (SWU), Thailand.

**Vernonia cinerea extract**

Whole plants of VC were collected from the agricultural field at Nakhon Nayok, Thailand whereas the VC extract was prepared at Pharmacology department, Faculty of Medicine, SWU. The plants were collected, washed and pre-drying under the sun shine, and further drying in the oven. The 500 g dried powdered herbal plant was mixed and squeezed with 2,000 ml of distilled water, followed by shaking at 40°C for 2 h. The supernatant was filtrated through Buchner funnel which was connected with vacuum pump. The clear supernatant was collected and kept in freezer-80°C immediately, for overnight. Finally, the supernatant was further lyophilized and completely removed under reduced pressure. Then, the semisolid crude extract from this supernatant was kept in dessicator until use. The percentage yield of semisolid mass of the crude extract was 10.09%.

**Experimental designs**

The experiments were designed into three experimental groups, 6 animals in each group: Control (C), nicotine treated (N) and nicotine treated with VC supplementation groups (NV). Control group was daily injected normal saline (1 mg/kg BW/day) whereas the N and NV groups were daily intraperitoneally injected with dose of nicotine free base 1 mg/kg BW/day\textsuperscript{19} which was dissolved in normal saline (Sigma, St. Louis, MO; i.p.). Moreover, NV group also received daily dose of VC extract orally (100 mg/kg BW/day) by forced catheterization. All groups were treated for 3 and 6 months time interval\textsuperscript{20}.

**Histological study**

At the end of experiment, the lung and tracheal tissues were removed and immediately fixed with 5% Bouin’s fluid (Sigma, USA) for 24 h at room temperature, followed by the standard paraffin technique of dehydration, clearing and mounting with permount. The embedded tissues were then cut into five micron-thick-
serial sections for staining. The lung sections were stained with hematoxylin-eosin (H&E) and Masson’s Trichrome (detection Kit from Sigma, USA) whereas the tracheal sections were stained with Periodic Acid Schiff (PAS) (detection Kit from Sigma, USA) and counter stained with hematoxylin. The sections were observed the pathological alterations and photographed by an Olympus light microscope (BX50, Japan).

**Fibrotic lesion**

Masson’s Trichrome staining was used in the present study for revealing the collagen fibers in the connective and muscular tissues. The evaluation of fibrosis was due to the density of dark blue staining of collagen fibers with the presence of an inflammatory cell infiltration within an extracellular matrix.

**Goblet cell hyperplasia**

Periodic Acid Schiff (PAS) staining was used for the demonstration of mucopolysaccharide in the tracheal tissues.

**Determination of plasma cotinine**

In order to compare the amounts of nicotine in blood circulation among three animal groups, cotinine which is nicotine’s metabolite, was used as a representative of nicotine in blood serum. Animals were sacrificed at 3 and 6 months under pentobarbital sodium anesthesia, blood serum samples were collected (5 ml/case) after nicotine exposure for 4 h. Blood serum was transferred to Toxicology Department, Faculty of Medicine, Ramathibodi Hospital, Mahidol University for analysis of basic drugs and drugs abuse (Limit of Quantitative = 0.05 ng/ml), by using standard gas chromatography mass spectrometry (GCMS).

**Evaluation of inflammatory cells and alveolar sac area**

The numbers of plasma cells, macrophages, and lymphocytes in lung sections were counted per area (2 x 10^3 micron^2) whereas the area of each alveolar sac was measured in the total area of 5 x 10^3 micron^2 each time. All data of three experimental groups were recorded and analyzed by using program CellSens Dimensions version 1.4 from Olympus.

**Statistical analysis**

Data were analyzed by Graph Pad Prism software 5.0 (GraphPad Software) using ANOVA, followed by Tukey’s test. For all the tests, the results were expressed as mean ± standard error (SE). The value of p < 0.05 was considered as a statistically significant difference.

**Results**

**Histopathological study of lung tissues**

Interestingly, the sign of chronic inflammation were noticed in the respiratory tissues of the 6 month-nicotine treated groups when compared with the control group (Fig. 1A, B). It was found that there were morphological changes of lung tissues in N groups of both 3 and 6 month exposure (Fig. 1C, D), especially more severity is present in the 6-month group as shown in Fig. 1D. The lung parenchyma, especially in respiratory portion in the nicotine group showed destructive changes of epithelial lining of alveolar duct, alveolar sac and alveoli. The submucosal layer of the bronchus and bronchiole contained several kinds of the inflammatory cells, as resulted in a marked...
increasing the thickness of the submucosal layer (Fig. 1C, D). In nicotine treated with VC group, the morphology of lung structures could be recovered as the control group (Fig. 1E,F).

In addition, there were significantly decreased in alveolar sac areas after 3- and 6-months nicotine treatment to 370.1 ± 94.04 and 248.2 ± 41.37 micron², respectively when compared to the control group. Surprisingly, it was found that the alveolar sac areas of 3- and 6-months NV group were 647.3 ± 50.43 and 639.2 ± 44.86 micron², respectively which were not significantly different from the control group (Table 1).

**Evaluation of numbers of inflammatory cells**

After 6 months of nicotine exposure, histopathological changes were demonstrated by proliferation of alveolar type 2 cells at alveolar walls, together with increased numbers of alveolar macrophages (Fig. 2A). Inflammatory cell infiltration at submucosal layer of the bronchus and bronchioles of 6 month-nicotine treated group was predominantly lymphocytes and plasma cells (Fig. 2C, E). Nevertheless, the improvement of lung tissues was found after VC administration. The sings of inflammation were significantly reduced, as indicated by the decreased numbers of alveolar macrophages, infiltrated lymphocytes, and plasma cells when compared to N group (Fig. 2B,D, F and Fig. 3).

**Effect of nicotine and VC on level of plasma cotinine**

Cotinine, nicotine’s metabolite, was used as a representative of nicotine in blood serum. The blood cotinine level in nicotine treated rats increased significantly up to 0.58 ± 0.03 ng/ml when compared with the control group of 0.04 ± 0.01 ng/ml. Interestingly, blood cotinine level of nicotine treated with VC supplementation decreased significantly to 0.07 ± 0.01 ng/ml when compared with nicotine treated rats.

**Effect of nicotine and VC on fibrosis**

Fibrosis was detected at 6 months of nicotine exposure throughout the lung parenchyma. Concerning to fibrosis investigation, the related same types and sizes of blood vessels and bronchiole among control, N, and NV groups were compared respectively. Fibrotic lesions were mainly located at perivascular, peribronchiole regions, and alveolar wall areas. These

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**Table 1.** Comparisons in alveolar sac area (micron²) at 3 months and 6 months of control, nicotine and treated nicotine fed with VC (100 mg/kg BW). The measurements of alveolar sacs were performed in the vicinity of 5 x 10³ micron² of lung section

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Area of alveolar sac at 3 months (micron²)</th>
<th>Area of alveolar sac at 6 months (micron²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>697.4 ± 53.77</td>
<td>690.3 ± 32.62</td>
</tr>
<tr>
<td>Nicotine (N)</td>
<td>370.1 ± 94.04*</td>
<td>248.2 ± 41.36*</td>
</tr>
<tr>
<td>Nicotine+VC (NV)</td>
<td>647.3 ± 50.43*</td>
<td>639.2 ± 44.86*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE *p<0.05 compared with control group in each time
morphological changes were verified by Masson’s trichrome method. The condensed dark blue staining areas of huge bundles of collagen fibers were the fibrotic area present in Fig. 4A, C of the N group. After supplementation with VC, the fibrosis was markedly decreased. The lung parenchyma showed normal collagen fibers surrounding the peribronchiole, perivascular regions, and alveolar wall (Fig. 4B, D).

**Effect of nicotine and VC on goblet cell**

With regard to the goblet cell hyperplasia, it was indicated that the tracheal epithelium showed the increase of positive PAS goblet cells of both 3 and 6 months N conditions when compared with those in C group (Fig. 5A-D). There were significant increases of PAS positive cells, especially 6 month-N group more than that of the 3 month-N group (Fig. 5C, D). In contrast, there was less goblet cell hyperplasia in the tracheal epithelium which found in both 3 and 6 month NV groups (Fig. 5E, F).

**Discussion**

The present study demonstrates that VC can stimulate the recovery of lung tissue of chronic nicotine treated animal. It has been reported that the nicotine causes the inflammation of the lung tissue. It is
indicated by hypertrophy of an alveolar type 2 cell, increased microvascular structures in alveolar wall. The movement of leukocytes and alveolar macrophages was also occurred into the inflamed regions\(^6\). The authors’ result of lung parenchyma showed a significant increase in number of plasma cells, alveolar macrophages and lymphocytes in the nicotine exposure rats. Thus, the authors finding is in agreement with the previous works of Iwalewa et al\(^{16}\) and Mazumder et al\(^{17}\). In addition, significant increase of plasma cells which is known to participate in immune response, reported by Lapperre et al\(^{22}\) is supported our finding in nicotine treated rat. Moreover, the severity of cell infiltration was increased with the long duration of nicotine exposure as shown in the 6-month group compared with the 3-month treatment\(^{22}\).

The authors experiment of VC supplementation can attenuate the inflammation caused by nicotine as shown by having less numbers of inflammatory cells present in the lung tissue when compared with nicotine treatment alone. Our finding of anti-inflammation of VC is confirmed by the previous works of Iwalewa et al\(^{16}\), Mazumder\(^{17}\) and Latha et al\(^{20}\). This result, therefore, suggests that VC may have a putative function in anti-inflammation process, since recently there is an evidence of anti-inflammatory activity reported in acute and chronic models in rat paw edema\(^{16}\). In addition, there are some evidence reported about the active chemical compounds of VC having the anti-inflammatory effect in rats, such as steroid, saponin, alkaloid and flavonoid\(^{20,23}\). The function of these compounds was reported to act through various pathways, i.e. COX-2\(^6\), suppress Bcl2 to stimulate cell survival\(^7\) and reduce over-expressed genes involved in MAPKs pathways\(^8\). Therefore, this putative anti-inflammatory function of VC will be possible to act through one of these above mechanisms. However, the anti-inflammatory function from which chemical compounds should be needed a further investigation. Generally, the main function of the alveolar type 2 cell is to produce a surfactant, a phospholipid for reducing a surface tension of the alveolar sac and alveoli. The authors finding in the 6 month N treated group showed the thicker alveolar wall as well as decrease of alveolar sac area with the high numbers of alveolar type 2 cell. Thus, a thick coat of surfactant producing from the proliferation of alveolar type 2 cells may be the mechanism to protect the alveolar sac areas for maintaining their respiratory function in gas exchange. After VC supplementation, the alveolar type 2 cells proliferation as well as the alveolar sac areas has been recovered closely to normal. To date, the mechanism of how VC can improve the lung tissues from the effect of chronic nicotine exposure is still obscured. The further study in this area will provide the new insight of therapeutic remedies in using our local herbs, VC to alleviate the heavy cigarette smokers who suffer from a chronic inflammation of the respiratory system.

The authors finding of increased fibrotic areas at peribronchiole, perivascular and alveolar walls in 6 months nicotine treated condition is in agreement with the previous works of Dasgupta et al\(^9\). This fibrosis was present next to a smooth muscle layer of bronchiole and also occurred next to an elastic layer of muscular pulmonary vessels. It has been proposed previously that alveolar macrophages are able to produce a transforming growth factor (TGF) which activates the proliferation of fibroblast which in turn stimulates collagen fiber synthesis\(^{25}\). Moreover, the presence of platelet-derived growth factor (PDGF) from macrophage has also been reported to associate with fibrotic lung\(^{26}\). Therefore, the formation of fibrosis in lung parenchyma after nicotine administration found in our experiments is probably due to the increased number of alveolar macrophages whose function could act through either TGF or PDGF. The VC supplementation demonstrated a marked diminished pulmonary lung fibrosis; therefore, VC might have its positive effect in improvement of fibrosis.

The previous works of Ye et al\(^2\) and Bartalesi et al\(^3\) demonstrated the goblet cell metaplasia in bronchioles of the cigarette smoker. Their findings were also related to the function of endogenous platelet-activating factor (PAF) that was proposed to play a key role in goblet cell metaplasia induced by cigarette smoke\(^{27}\). However, the appearance of goblet cell hyperplasia was found in the nicotine treated group and the recovery was seen after VC administration, especially at 6 month administration. Therefore, it is expected that the some chemical gradients compounds of VC might decrease disturb the effect reinforcement of nicotine by effecting through the pathway using PAF and its receptor.

Moreover, the analyses of cotinine in blood serum would demonstrate the effect of VC supplementation in nicotine treated animal. Cotinine, which was a metabolite of nicotine in blood serum, was analyzed and compared among the control, N and NV groups. The significantly high number of blood cotinine level in nicotine treated rat was demonstrated. However, blood cotinine level of nicotine treated rat with VC supplementation was decreased and turned out to be
close to the control rats. Base on standard value, the data of blood cotinine level is referenced to 0.05 ng/ml by EPA United States Environmental Protection Agency, in 1999-2000. It might be implied that VC would have effect to decline the level of nicotine level in blood serum.

Conclusion
In conclusion, the chronic nicotine-treated animal, especially at 6 months, demonstrated histopathological damages of respiratory tissues. The supplementation of VC extract demonstrated the improvement of the respiratory tissue, in remarkably decreasing of inflammatory cells, fibrotic areas and goblet cell hyperplasia. With regarded the present study of VC supplementation in nicotine exposure, this plant has a potential to be a choice of remedy for a heavy smoker. The ongoing researches of this plant are expected to provide either a new herbal medicine in curing lung diseases of both smokers and passive smokers.

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Potential conflicts of interest
None.

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ผลของสมุนไพรหญ้าดอกขาวที่ช่วยฟื้นฟูเนื้อเยื่อทางเดินหายใจในภาวะที่ได้รับสารนิโคตินเรื้อรัง

ชโมพร พระพุทธยา, วิภัตตา อนุพันธศิริ, นุษยา เปย์ยาชาน, สารินี สถาปัตยานันท์, ภัทรศิริ ภัณฑ์ชัยรัตน์, ตันทิยา นBORบุษบา ปันยารชุน, ธาริณี สวัสดิ์พาณิชย์, ฤทธิ์ วัฒนชัยยิ่งเจริญ, อรลักษณา แพรัตกุล, นริศา คำแก่น, หัทยา เฟื่องพิบูลไทย

วัตถุประสงค์: เพื่อดำเนินการทดลองสารสกัดหญ้าดอกขาว ต่อการฟื้นฟูเนื้อเยื่อทางเดินหายใจในภาวะที่ได้รับสารนิโคตินเรื้อรัง

วัสดุและวิธีการ: หนูทดลองเพศผู้ถูกเหนี่ยวนำให้เกิดภาวะที่ได้รับสารนิโคตินเรื้อรัง โดยการฉีดสารนิโคติน (1 มก./น้ำหนักหนู 1 กก.) แบ่งหนูเป็น 3 กลุ่ม คือ กลุ่มควบคุม กลุ่มฉีดนิโคติน กลุ่มฉีดนิโคตินและป้อนสารสกัดจากหญ้าดอกขาว (100 มก./น้ำหนักหนู 1 กก.) เบื้องต้น 3 และ 6 เดือน เก็บเนื้อเยื่อปอดและหลอดลม เพื่อนำมาทำการศึกษาทางเนื้อเยื่อวิทยา ด้วยเทคนิคการย้อมสี Hematoxylin and Eosin (H & E), Periodic Acid Schiff และ Masson’s trichrome พร้อมวิเคราะห์ผลด้วยซอฟต์แวร์รูปทรงรวม

ผลการศึกษา: เนื้อเยื่อปอดของหนูกลุ่มที่ได้รับนิโคตินมีการเกิดพยาธิสภาพของเนื้อเยื่อในบริเวณ alveolar wall โดยหนาตัวมากขึ้น และมีจำนวนเซลล์หลากหลายชนิดมากขึ้น รวมถึงจำนวน alveolar type 2 โดยเฉพาะที่ระยะ 6 เดือน พบว่ามีการเพิ่มจำนวนเซลล์ในบริเวณ alveolar wall อย่างมากของเซลล์ alveolar type 2 โดยเฉพาะที่ระยะ 6 เดือน พบว่ามีการเพิ่มจำนวน alveolar macrophage, lymphocytes และ plasma cell รวมถึงการเกิด fibrosis พบกระจายตาม alveolar wall บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริเวณ alveolar wall พบการเกิด fibrosis ที่บริว