非侵襲性方法研究氣喘病患呼吸道發炎反應與腺核甘單磷酸呼吸道高反應性

Noninvasive Assessment of the Relation Between Airway Inflammation and Hyperresponsiveness to Adenosine Monophosphate in Asthmatic Patients

計畫編號：NSC 89-2314-B-002 -063
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
主 持 人：郭炳宏　　國立台灣大學醫學院內科

一、中文摘要

關鍵詞：氣喘，呼吸道發炎反應，腺核甘單磷酸，呼吸道高反應性吐氣一氧化氮濃度，吐氣濃縮液，過氧化氫

呼吸道高反應性為氣喘病患重要表徵之一。吸入性腺核甘 (Adenosine) 在 1980 年代就被發現具有支氣管縮作用， 一般認為其乃透過間接的方式於過敏性發炎呼吸道中可經由其受體促進肥胖細胞釋出發炎介質等引起支氣管收縮。近年來有學者發現吸入性 AMP 對氣喘病患之特異性優於 methacholine，但臨床應用仍未普遍。究竟氣喘病患呼吸道對 AMP 之高反應性與呼吸道發炎有無相關仍未有定論。非侵襲性呼吸道發炎反應之評估方面，吐氣中一氧化氮(NO)的濃度已被認為可作為氣喘病患呼吸道發炎反應之指標，且可監傳測各種抗發炎藥物治療後之療效。本計畫之目的在研究呼吸道對 AMP 之高反應性與氣喘病患呼吸道發炎之相關。首先使用脈衝振動法測量吸入性腺核甘單磷酸對 25 位具有氣典型哮鳴發作之病患以及 12 位咳嗽變異型氣喘病人之影響。並測量試驗前後吐氣一氧化氣(NO)以及吐氣濃液中過氧化氫的含量變化。我們發現氣喘病人之 AMP 支氣管高反應性明顯高於咳嗽變異型氣喘病患，但吐氣一氧化氮及吐氣濃縮液中 H_2O_2 之濃度則無差異。在吸入性類固醇治療後。有 16 位氣喘病患及 8 位咳嗽變異性病患再次接受 AMP 支氣管激發試驗。氣喘病患及咳嗽變異性病患之 AMP 高反應性有下降且兩組呈現無差異。吐氣濃縮液 H_2O_2 有下但吐氣中 NO 無法看出有變動之跡象。這些結果顯示 AMP 支氣管反應性及吐氣濃縮液 H_2O_2 似乎可反映氣喘病患呼吸道發炎反應及敏感度之差異。但吐氣 NO 則無法反映此一現象。其臨床用途值得懷疑。

二 、英文摘要

Keywords: Asthma, Airway inflammation, Adenosine monophosphate airway hyperresponsiveness, nitric oxide

Recently, airway hyperresponsiveness (AHR) to adenosine monophosphate (AMP) may be a better marker of airway inflammation than methacholine or histamine. This study was undertaken to assess the usefulness of noninvasive measurements of AHR to AMP in the assessment of airway inflammation in asthmatics. First, we compared AHR to AMP in 25 typical asthmatics (Group A) and in 12 patients with cough-variant asthma (Group B). Bronchoprovocation was monitored by impulse oscillometry (IOS) until respiratory resistance at 5 Hz

(R5) increased for > 50% (PC50R5). Exhaled nitric oxide (NO) was determined using a real-time chemiluminence analyzer. Hydrogen peroxides (H_2O_2) in the breath condensate was measured by a determined before and after AMP inhalation challenge. The PC50R5 values were higher in Group B (150.2 ± 70.2 mg/ml) than those in Group A (86.3 ± 60.7 mg/ml) ($P < 0.001$). In Group A, there was a significant correlation ($R = 0.48$, $p < 0.05$) between AHR to AMP and exhaled H_2O_2 concentrations but not to the levels of expired NO. After 1 month of asthma therapy, 16 patients in Group A and 8 patients in Group B underwent AMP bronchoprovocation again and the PC50R5 increased to 291.3 ± 95.2 and 313.6 ± 118.2 mg/ml, respectively) (P=NS). The levels of expired H_2O_2 also decreased after steroid therapy in both groups but we could not find a significant difference in the levels of expired NO. These data indicate that AHR to AMP and expired H_2O_2, but not expired NO, may provide noninvasive markers that may be helpful in the monitoring of disease activity in asthmatics.

三、緣由與目的

Some studies showed that AHR to methacholine could not predict the child's response to these asthma medications (Chang 1998). Recently, AHR to AMP has been considered to be a better marker of airway inflammation. Increased oxidative stress is implicated in asthma (7) and this may be reflected by expired H2O2. An increased concentration of exhaled H2O2 may represent an increased production of oxidants and/or a reduced free radical scavenging capacity in asthmatic airways. Exhaled NO is another noninvasive marker of airway inflammation that has been shown to be dose-dependently reduced following asthma treatment. The objectives of the study are (1) To explore the changes of expired NO and H2O2 before ands after AMP inhalation challenge; and (2) to evaluated whether AHR to AMP can be used as a marker of disease activity in typical and cough-variant asthmatics.

四、材料及方法:

Selection of patients: Group A consisted of 45 patients with typical asthmatic symptoms (18 male and 27 female nonsmokers, age 18-66 years old). All patients in this group had at least one episode of dyspnea and nocturnal wheeze in the previous three months. Each patient also demonstrated reversible airway obstruction by a 12% or greater increase in 1-s forced expiratory volume (FEV1) after inhaled β2-agonist, and the FEV1 after bronchodilator use was at least 75% of predicted at screening. No patient had regular use of oral corticosteroids. Group B: 12 patients with cough-variant asthma who were selected from 80 chronic coughers with cough lasting for over three weeks without wheezing but with AHR to AMP or Mch. Before pulmonary function testing, subjects were instructed to abstain from the use of inhaled steroid, bronchodilators, antihistamines for as long as 24 hours. IOS parameters were measured during normal breathing for approximately 20 sec.

AMP bronchoprovocation test: Baseline values were obtained after inhalation of an aerosol of 0.9% sodium chloride solution. Then quadrupling concentrations of AMP (0.097, 0.39, 1.56, 6.25, 25, 100 and 400 mg/ml) (Sigma)

were inhaled every 3 min until the respiratory resistance at 5 Hz (R5) increased for more than 50% of its baseline values.

Monitoring of Exhaled Nitric Oxide: Expired NO was determined using a sensitive chemiluminescence analyzer (Logan) with a detection limit as low as 0.1 ppb. The subjects were inspire to total lung capacity (TLC) and, with no breath holding, exhaled at a constant rate to maintain a constant mouth pressure of 4-5 cm H2O by observing a visual display of this pressure. NO levels were taken from the plateau at the end of exhalation and the mean of triplicate measurements was used as the representative value.

Assay of H_2O_2 in Breath Condensate: Breath condensate was collected using a plastic condensing device containing ice and water. After rinsing their mouth, subjects were instructed to breathe tidally through a mouthpiece connected to the inlet for 10 min while wearing a nose-clip. Approximately 0.75-1 ml of condensate was collected and stored at -70° C. 100 µl of condensate was mixed with 100 µl of 420 µM 3'3'5'5'-tetramethylbenzidine in 0.42 M citrate buffer pH 3.8 and 10 µl of horseradish peroxidase (52.5 U/ml). The samples were incubated at room temperature for 20 min and the reaction stopped by the addition of 10 µl of 2 N sulfuric acid. The product was measured spectrophotometrically (ELISA reader) at 450 nm for each assay.

四、結果與討論

The $PC_{50}R5$ values were higher in Group B (150.2 ± 70.2 mg/ml) than those in Group A (86.3 ± 60.7 mg/ml) ($P < 0.001$). In Group A, there was a significant correlation ($R = 0.48$, $p < 0.05$) between AHR to AMP and exhaled H2O2 concentrations but not to the levels of expired NO. After 1 month of asthma therapy, 16 patients in Group A and 8 patients in Group B underwent AMP bronchoprovocation again and the $PC_{50}R5$ increased to 291.3 ± 95.2 and 313.6 ± 118.2 mg/ml, respectively) (P=NS). The levels of expired H_2O_2 also decreased after steroid therapy in both groups (from 0.69 ± 0.10 to 0.57 ± 0.09 µM in group A and from 0.59 ± 0.11 to 0.41 ± 0.08 µM, both $p < 0.05$). However, we could not find a significant difference in the levels of expired NO (from 14.5 ± 4.4 to 12.7 ± 4.1 ppb in Group A and from 12.2 ± 3.4 to 11.3 ± 3.9 ppb in Group B, both P=NS). Data from this study suggest that AHR to AMP and expired H_2O_2 can reflect and monitor disease activity in asthmatics, but the levels of expired NO were neither sensitive nor predictive of a response to asthma therapy.

Methacholine is a direct stimulus that acts mainly via muscarinic receptors on smooth muscle. It has been suggested that indirect challenges such as isocapnic dry air hyperventilation, exercise, or inhalation of AMP would illustrate the inflammatory state of the airways better than methacholine challenges (Polosa R, 1995). The IOS has potential advantages for challenge testing, as multiple tests at many dose levels will be easier to tolerate for the patient. (Bohadana, 1999). On the other hand, challenge testing without deep inspiration may reduce the difference between patients with asthma and healthy subjects (Skloot, 1995). Whether this effect occurs in patients with chronic cough remains unclear.

The degree of airway inflammation in cough-variant asthma has long been regarded to be less severe as compared to

that in typical asthma, although differences exist in factors eliciting the inflammatory processes, as well as in the site of airway inflammation and type of infiltrating cells. Niimi A et al found that there were no differences in the AHR to methacholine between patients with classic and cough-variant asthma. (Numi, 1999). Some studies showed that AHR to methacholine could not predict the child's response to these asthma medications (Chang 1998). Rutgers et al reported that short-term treatment with budesonide does not improve AHR to AMP in COPD (Rutgars, 1998). Results from this study, however, indicate that AHR to AMP could reflect the differences of AHR between typical and cough-variant asthmatics, and might be used to monitor the effect of steroid therapy.

Some previous studies have shown that expired NO and H_2O_2 could monitor the changes in airway inflammation. A recent study by Horvath et al evaluated the relationship between exhaled NO and H2O2 in the exhaled air of asthmatics and also evaluated AHR to Methacholine and cellular counts of induced sputum. These results suggested for the first time that H_2O_2 might be a more sensitive and reliable marker of airway inflammation than exhaled NO in this population. Data from our study showed a significant correlation between exhaled H_2O_2 and AHR to AMP. Our results also support the use of AHR to AMP and expired H_2O_2, but not expired NO, in the assessment of airway inflammation in asthmatic patients. Nevertheless, measurements of expired H2O2 are time-consuming and may be affected by contamination from upper airway airway and saliva. In this regard, AHR to AMP is easier to be determined and perhaps more sensitive to monitor allergic inflammation in asthma. Further studies are required to evaluate the validity of AMP bronchosensivity and its standard method of measurement.

五、參考文獻 (References)

Chang AB, Phelan PD, Carlin JB, Sawyer SM, Robertson CF. 1998. Randomized controlled trial of inhaled salbutamol and beclomethasone for recurrent cough. Arch Dis Child 79:6-11

Dohlman AW, Black HR, Royall JA. 1993. Expired breath hydrogen peroxide is a marker of acute airway inflammation in pediatric patients with asthma. Am J Respir Crit Care Med 148:955-6

Gallati H, Pracht I. 1985. Horseradish peroxidase: kinetic studies and optimization of peroxidase activity determination using the substrates H_2O_2 and 3,3¢,5,5¢-tetramethylbenzidine. J Clin Chem Clin Bioch 23:453-6

Horvath I, Donnely LE, Kiss A, et al. 1998. Combined use of exhaled hydrogen peroxide and nitric oxide in monitoring asthma. Am J Respir Crit Care Med 158:1042-6.