日本財団補助金による
1997 年度日中医学協力事業助成報告書
－在留中国人研究者研究助成－

1998 年 3 月 10 日

財団法人 日中医学協会
理事長 中島章殿

I. 研究者氏名 賈 玉霞

研究機関 東北大学医学部老人科 研究指導者 佐々木英忠 職名 教授

所在地 〒980 仙台市青葉区星陵町 1-1 電話 022-717-7180 内線 (教授室)

II. 過去の研究歴

私は、1987年に上海医科大学を卒業後、北京医院に内科医として勤務しました。1995年に東北大学と北京医院間の交換研究者に選出されました。これにより東北大学医学部老人科に研究を行っています。

III. 過去の研究実績

- Altering airway tone influences the sensitivity of cough reflex in awake guinea pigs Journal of Autonomic Pharmacology (manuscript in revising)
- Effects of adrenomedullin and calcitonin gene-related peptide on airway and pulmonary vascular smooth muscle in guinea pigs British J. of Pharmacology 1996; 119: 1477-83.

IV. 本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

American Thoracic Society 1998 International Conference in Chicago.
Role of Heme Oxygenase-1 (HO-1) In Airway Microvascular Leakage Caused By Antigen Challenge In Sensitized Rats

(2) 学会誌等に発表した論文 (無)・有 （雑誌名・論文名）

V. 今後の研究計画及び希望

モルモットにドーパミン受容体阻害剤の投与下でサブスタンスPの低下と嚥下反射の低下を観察し、ドーパミン補充療法は嚥下反射を正常化し、不顕性誤嚥を防ぐ手段であることを証明する。これからもよろしくお願いいたします。

VI. 研　　究　　報　　告（日本語、又は英語で書いて下さい。4,000字以上で記載して下さい。別紙可）

研究報告は別紙に添付いたします。

VII. 指導教官の意見

賈医師は当科にて多くの仕事をしている。そのいくつかは現在 英文誌に掲載されはじめている。又、現在も教室員と共同研究をしており、その論理的考え方より、実績が上がっている。これまで、多くの中国人留学生が当教室を訪れたが、最も優秀である。現在大学院生であり、これからも支援していきたいと考えている。

VI：研究報告.

Role of HO-1 in pulmonary vascular leakage caused by antigen challenge in sensitized rats

Jia YX, Sekizawa K, Okinaga S, Lee L, and Sasaki H
Department of Geriatric Medicine, Tohoku University School of Medicine, Sendai 980 Japan

Introduction

Reactive oxygen species (ROS) been increasingly implicated in the pathogenesis of a variety of diseases and important biologic processes, including inflammatory disorders[1,2]. Toxic effects of these oxidants, including the superoxide and hydroxyl radicals and hydrogen peroxide, commonly referred to as oxidative stress, can cause cellular damage[3,4]. The lung is one major target organ for injury by these ROS, generated endogenously by inflammatory cells and by exogenous oxidants[5]. One major inflammatory lung disease is asthma. Asthma is now viewed as a chronic inflammatory process[6,7]. Edema of the airway wall as a result of microvascular leakage, and luminal obstruction due to exuded plasma and airway secretions are important mechanisms of asthmatic airway obstruction[8,9]. Recently, much interest has been stimulated by observations that HO-1 plays important roles in cellular protection against oxidant injury[10-14]. Otterbein and colleagues observed that hemoglobin induced HO-1 induction plays a key role in providing protection against oxidant-mediated endotoxic shock and lung injury[14]. In view of the important functional role of HO-1 against oxidative lung injury, we employed an established allergic asthmatic model of ovalbumin-sensitized animal to investigate the role of HO-1 in pulmonary vascular leakage caused by antigen challenge.

Materials and methods

Ovalbumin sensitization

Animals were sensitized as previously described[15]. Adult male Sprague-Dawley rats weighing 200-300g were injected subcutaneously with 1.0ml of a suspension containing 1.0mg of ovalbumin (OA) and 200mg of $Al(OH)_3$ in physiologic saline and intraperitoneally with 0.5ml of a Bordetella pertussis antigen solution containing $1.0x10^9$ organisms/ml.

Antigen provocation and measurement of PIP

Ten to fourteen days following active sensitization to OA, rats were anesthesized intraperitoneally with pentobaribital sodium (100-120mg/kg, 50mg/ml saline). A tracheal

cannula was inserted via a tracheostomy into the lumen of the trachea and connected to a constant-volume ventilator (Model 607;). A tidal volume of 5ml/kg and a frequency of 90 breaths/min were used to ventilate the animals. Pulmonary insufflation pressure (PIP) was measured by means of a differential pressure transducer (MP45; Validyne Engineering Corp. Northridge, CA) connected to a side arm in the expiratory limb of the ventilation tubing, and was recorded on a pen recorder (RECTI-HORIZ-8K; San-ei, Tokyo, Japan). To evaluate validation of the methods of measuring PIP, known resistances (from 0.1 to 1.0cmH2O/ml/s) were connected to the tracheal cannula. Linear increases in PIP were recorded with increases in resistance.

Measurement of airway plasma extravasation

The magnitude of vascular permeability was quantified with a modification of the method described by Saria and Lundberg[16], which correlates well with extravasation of radiolabeled albumin[17]. Evans blue (EB) dye (30mg/kg, 30mg/ml), dissolved in saline solution, was injected into a jugular vein. To determine the tissue content of EB dye after stimulation, the systemic circulation was perfused with saline to wash the dye out of the vasculature. The left ventricle was incised, a blunt-ended needle was inserted into the aorta, and the ventricles were cross-clamped. Blood was expelled from the incised right atrium at a pressure of 100mmH_2O until the perfusate was clear (about 200ml infused). The lungs were then removed. The connective tissues, vasculature, and parenchyma were gently scraped, and airways were divided into three components: lower part of the trachea, main bronchi, and segmental bronchi. The tissues were blotted dry and weighed. To quantify the amount of extravasated EB dye, each tissue sample was immersed in 2ml of 100% formamide for 24 hours at 50°C to extract the dye, and concentrations of dye in the solutions were determined spectrophotometrically by measuring the absorbance at 620nm (220A Spectrophotometer; Hitachi Ltd., Tokyo, Japan). Tissue content (nanograms of dye per milligram of wet tissue weight) was calculated from a standard curve of dye concentrations ranging from 0.5 to 10ug/ml.

Effects of Hb and SnPP on antigen-induced vascular leakage

Male Sprague-Dawley rats, weighing 200-300g, were sensitized to ovalbumin, as described above. These sensitized animals were challenged and subsequently studied after 10 to 14 days when they weighed 300-420g. The EB dye (30mg/kg) was injected into a jugular vein, followed 1 minute later by intravenously administered ovalbumin (200ug/kg, 200ug/ml in saline). Animals were divided into three groups, control (n=11), Hb group (n=10), and SnPP group (n=15). Hb group animals received rat hemoglobin (150mg/kg, 75mg/ml in saline), which was injected into the rat tail vein 16h before antigen-challenge, while in SnPP group, SnPP (50umol/kg) was administered subcutanously 21h before

antigen-challenge.

Drugs

The following drugs were used: Evans blue dye, Ovalbumin,rat hemoglobin (Sigma chemical co.), $Al(OH)_3$, Bordetella pertussis (Wako pure chemical industries LTD.), and SnPP (Porphyrin products, Inc.).

Statistical analysis

Results are reported as means+/- SEM. For statistical analysis, we used one-way analysis of variance. Significance was accepted at $p<0.05$.

Results

Intravenous injection of ovalbumin (200ug/mg) caused a significant leakage of EB dye in the trachea (138.7+/-20.0ng/mg), main bronchi (100.1+/-14.1ng/mg), and segmental bronchi (47.1+/-8.1ng/mg). The EB dye leakage is highest in the trachea, followed by main bronchi and segmental bronchi. 16h pretreatment with Hb (150mg/kg) significantly reduced the vascular leakage in the trachea ($p<0.05$) by 42.2% and main bronchi ($p<0.05$) by 42.5%, but not in segmental bronchi ($p>0.05$). In contrast, 21h pretreatment with SnPP (50umol/kg) further potentiated ovalbumin-induced vascular leakage in all tissues, increased 31.5% in the trachea, 36.7% in the main bronchi and 57.0% in the segmental bronchi (Figure 1).

PIP was without obvious change after ovalbumin challenge in all groups (data not shown).

Discussion

Our results show that pretreatment of Hb decreases the pulmonary vascular permeability that is produced by antigen-challenge, whereas SnPP increases it. Hb is a potent inducer of HO-1, and SnPP is a competitive inhibitor of HO. HO is the rate limiting enzyme in the catabolism of heme molecules. Two isoforms of HO have been characterised, the constitutive form heme oxygenase-2 and an inducible form heme oxygenase-1[18]. HO is induced not only by the substrate heme but also by a variety of non-heme inducers such as heavy metals, cytokines, hormones, endotoxins, sulfhydrgl reagents, and heat shock[19-20]. Recent studies suggest that HO-1 plays important roles in cellular protection against oxidant injury[10-14]. Our results suggest that HO-1 is also involved in the pulmonary vascular leakage induced by antigen-challenge.

Several of the inflammatory cells which are believed to participate in the inflammatory

response of asthmatic airways have been shown to release reactive oxygen species after activation by a variety of stimuli. Activation of mast cells, macrophages[21,22], eosinophils[23], and neutrophils[24] generates O_2^-, which are rapidly converted to H_2O_2 by superoxide dismutase (SOD). These metabolites may have effects on the various target cells of the airway. Oxygen radicals cause increased vascular permeability, possibly via a direct damaging effect on vascular endothelial cells[25]. Antigen-challenge activates eosinophils. It is likely that HO-1 effects as a scanvenger of oxygen radicals, consequently inhibits the increase in vascular leakage induced by antigen-challenge. However, in the present study, Hb pretreatment did not completely inhibit the vascular leakage if it is referred that the EB dye extravasation is lower than 30ng/mg tissue in trachea, main bronchi and segmental bronchi in OA-sensitized animals without OA challenge[26]. Hb (75mg/kg) pretreatment reduced the vascular leakage of EB dye by 42.2% in trachea, 42.5% in main bronchi.This study suggests the involvement of HO-1 in the rat model of increased vascular permeability induced by antigen challenge. Other mediators are also significant contributors to increased vascular permeability during antigen challenge[27,28].

One of our previous studies showed that the vascular permeability is low in OA-sensitized guinea pigs without antigen-challenge. The leakage of EB dye is lower than 30ng/mg tissue in the trachea, main bronchi, and nasal mucosa[28]. We found that intravenous injection of ovalbumin (200mg/kg) significantly increased the leakage of EB dye compared with saline control in the trachea (41.3+/-8.7ng/mg), main bronchi (56.3+/-8.8ng/mg), and nasal mucosa (80.1+/-10.0ng/mg) in sensitized guinea pigs. The present study is partly in accord with the previous one. In OA-sensitized rats, antigen-challenge (OA 200mg/kg, iv) also induced a significant leakage of EB dye in the trachea (138.7+/-20.0ng/mg), main bronchi (100.1+/-10.1ng/mg), and segmental bronchi (47.1+/-8.1ng/mg) . But the absolute value of EB dye leakage is much higher in rats than that in guinea pigs. Furthermore, in guinea pigs, the EB dye leakage is highest in segmental bronchi, followed by main bronchi and trachea[26]. But our study shows that it is highest in trachea, followed by main bronchi and segmental bronchi. This difference may be due to different species.

Otterbein et al emphasized a crucial pretreatment period for the functional effect of HO-1 inducer[14]. They observed that experiments in which rats receiving simultanous administration of hemoglobin and a lethal dose of LPS all died within 8 to 10 h, comparable to rats receiving a lethal dose of LPS alone. Additionally, a shorter pretreatment period (4h) of hemoglobin administration also failed to provide a protection against a lethal dose of LPS. Importantly, HO enzyme activity was not induced at 4h after

hemoglobin injection in rat lungs. They conclude that for protection against lethal endotoxemia, pretreatment with hemoglobin to induce HO-1 induction before administration of a lethal dose of LPS is essential. In the present experiment, we chose a 16h pretreatment because previous reports have observed that 16h after hemoglobin was the time point at which peak HO enzyme activity is observed in rat tissue[29]. Increased HO-1 mRNA induction was highest in the rat lung, followed by heart, liver, spleen, and kidney 16h after hemoglobin administration.

Plasma exudation is a feature of the inflamed airways of asthmatics and may contribute significantly to the pathogenesis of asthma and bronchial hyperresponsiveness. Reduction of plasma leakage may, thus, be useful in asthma therapy. The present study shows that OA antigen-induced increases in vascular permeability were inhibited in part by pretreatment of HO-inducer, hemoglobin, and potentiated by pretreatment of HO inhibitor, SnPP. Thus HO-1 may play an important role in regulating allergic reactions. HO-1 or HO-1 inducers may be of value in the treatment of bronchial asthma.

Acknowledgment

This work was supported partly by 日中医学協会.

References

1.Halliwell B. and Gutteridge MC. 1985. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, England.

2.Fridovich I. 1978. The biology of oxygen radicals. Science. 201:875-880.

3.Kimball RE., Reddy K, and Pierce TH. 1976. Oxygen toxicity: augmentation of anti-oxidant defense mechanisms in rat lung. Am.J.Physiol.230:1425-1431.

4.Freeman B, and Crapo JD. 1981. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. J.Biol.Chem. 256:10986-10092.

5.Clark JM, and Lambertson CJ. 1971. Pulmonary oxygen toxicity: a review. Pharmacol.Rev. 23:37-133.

6.Barnes PJ. 1989. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. J. Allergy Clin. Immunol. 83:1013-1026.

7.Barnes PJ. 1989. A new approach to asthma therapy. N. Engl. J.Med. 321:1517-1527.

8.Barnes,P.J. 1990. Reactive oxygen species and airway inflammation. Free Radical bilolgy& Medicine 9:235-243.

9.Djukanovic R, Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, and Holgate ST. 1990. Mucosal inflammation in asthma. Am Rev Respir Dis 142:434-457.

10.Vile GF, Basu-Modak S, Waltner C, and Tyrrell RM. 1994. Heme oxygenase I mediates an adaptive response to oxidative stress in human skin fibroblasts. Proc. Natl. Acad. Sci. USA 91:2607-2610.
11.Vile GF, Tyrrell RM. 1994. Oxidative stress resulting from ultraviolet A irradiation of human skin fibroblasts leads to a heme oxygenase-dependent increase in ferritin. J. Biol. Chem. 268:14678-14681.
12.Abraham NG, Lavrovsky Y, Schwartzman L, Stoltz RA, Levere RD, Gerritsen ME, Shibahara S, and Kappas A. 1995. Transfection of the human heme oxygenase gene into rabbit coronary microvessel endothelial cells: protective effect against heme and hemoglobin toxicity. Proc. Natl. Acad. Sci. USA 92:6798-6802.
13.Nath DA, Balla G, Vercelotti GM, Balla J, Jacob HS, Levitt MD, and Rosenberg ME. 1992. Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat. J. Clin. Invest. 90:267-270.
14.Otterbein L, Sylvester SL, and Choi AMK. 1995. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. Am. J. Respir. Cell Mol. Biol. 13:595-601.
15.Blythe S, England D, Esser B, Junk P, and Lemanske RF. 1986. IgE antibody mediated inflammation of rat lung: histologic and bronchoalveolar lavage assessment. Am Rev Respir Dis. 134:1246-1251.
16.Saria A, Lundberg JM. 1983. Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissues. J Neurosci Methods.8:41-49.
17.Udake K, Takeuchi Y, Movat HZ. 1970. Simple method for quantitation of enhanced vascular permeability. Proc Soc Exp Biol Med. 133:1384-1387.
18.Willis D. 1995. Expression and modulatory effects of heme oxygenase in acute inflammation in the rat. Inflamm Res 44, Suppl 2: S218-S220.
19.Abraham NG, Lin JHC, Schartzman L,Levere RD, and Shibahara S. 1988. The physiological significance of heme oxygenase. Int. J. Biochem. 20:543-558.
20. Lautier D, Luscher P, and Tyrell RM. 1992. Endogenous glutathione levels modulate both constitutive and UVA radiation/hydrogen peroxide inducible expression of the human heme oxygenase gene. Carcinogenesis. 13:227-232.
21.Drath DB, Karnovsky ML. 1975. Superoxide production by phagocytic leukocytes. J. Exp. Med. 141:257-262.
22.Babior BM. 1984. The respiratory burst of phagocytes. J. Clin. Invest. 73:599-601.
23.De Chatelet LR, Shirley PS, McPhail LC, Huntley CC, Muss HB, and Bass DA. 1977. Oxidation metabolism of the human eosinophil. Blood 50:526-535.
24.Babior BM, Kipnes RS, Curnutte JT. 1973. Theproduction by leukocytes of

superoxide, a potential bactericidal agent. J. Clin. Invest. 52.741-744.

25. Del Maestro RF, Bjork J, and Arfors KE. 1981. Increase in microvascular permeability induced by enzymatically generated free radicals. I. In vivo study. Microvasc. Res. 22:239-254.

26. Miura M, Ichinose M, Kageyama N, Tomaki M, Tsuneyuki T, Ishikawa J, Ohuchi Y, Oyake T, Endoh N, and Shirato K. 1996. Endogenous nitric oxide modifies antigen-induced microvasclar leakage in sensitized guinea pig airways. J Allergy Clin Immunol. 98:144-151.

27. Evans TW, Rogers DF, Aursudkij B, Chung KF, Barnes PJ. 1988. Inflammatory mediators involved in antigen-induced airway microvascular leakage in guinea pigs. Am Rev Respir Dis. 138:395-399.

28. Sekizawa K, Nakazawa H, Morikawa M, Yamauchi K, Maeyama K, Watanabe T, and Sasaki H. 1995. Allergens, IgE, Mediators, inflammatory mechanisms: Gustanube B-methyltransferase inhibitor potentiates histamine- and antigen-induced airway microvascular leakage in guinea pigs. J Allergy Clin Immunol. 96:910-916.

29. Pimstone NR, Engel P, Tenhunen R. Seitz PT, Marver HS, and Schmid R. 1971. Inducible heme oxygenase in the kidney: a model for the homeostatic control of hemoglobin catabolism. J. Clin. Invest. 50:2042-2050.