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Hepatocyte growth factor levels are elevated in patients with Asthma

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Hepatocyte growth factor (HGF) is a multifunctional polypeptide with a broad range of functions. It acts as an endocrine, paracrine and autocrine factor and regulates a wide range of cellular processes such as cell survival, proliferation, migration and differentiation. Recently, some animal experiments suggest that HGF play important part role in the pathogenesis of asthma. On the basis of these results, the present study was designed to determine whether serum HGF levels are elevated in patients with asthma, and whether its level are associated with the severity of disease. We found that HGF levels were significantly higher in three asthmatic patient groups as compared with healthy control group [9.8 (6.3-14.5), 11.4 (8.5-16.2), 13.7 (9.9-18.3) and 2.3 (1.6-3.7) ng/mL, respectively, *P<0.01*]. The HGF levels were also elevated associated with the severity of disease, but this difference was not significant. The data suggest a role for HGF in the pathogenesis of asthma. Further studies are needed to clarify the involvement of HGF in various aspects of asthma.

Key Words: Asthma, HGF, serum, AHR , pathogenesis, airway remodeling.

INTRODUCTION

Hepatocyte growth factor (HGF), also called scatter factor, is a multifunctional polypeptide with a broad range of functions involved in embryo development, tissue repair and cancer growth. It is produced not only by hepatocytes but also by a wide variety of cell typesincluding mesothelial cells, platelets, monocytes, fibroblasts and certain tumour cells including mesothelioma. HGF is secreted as a single-chain propeptide which is cleaved extracellularly by serine proteases such as plasminogen activator and HGF activator, to give an active heterodimer (Naldini et al., 1992). HGF acts as an endocrine, paracrine and autocrine factor and regulates a wide range of   cellular

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processes such as cell survival, proliferation, migration and differentiation. For example, HGF prevented the onset and progression of hepatic fibrosis/cirrhosis as well as renal, lung and myocardial fibrosis in vivo (Yaekashiwa et al., 1997; Mizuno et al., 1998; Ueki et al., 1999; Taniyama et al., 2000). In addition, an immunosuppressive role of HGF has been recently highlighted. For example, in a mouse model of allogenic heart transplantation, HGF reduced acute and chronic rejection of the allograft with the increased expression of TGF-β and IL-10, indicating that HGF might induce allograft tolerance (Yamaura et al., 2004).

Asthma is an allergen-induced chronic inflammatory disease of the airways characterized by airway eosinophilia, hyperresponsiveness (AHR), and remodeling (Elias et al., 1999; Holt et al., 1999). The pathogenesis of asthma is complex, and many factors contribute to its development and progression. Asthma is also basically a Th2-type immune response in the airway, although a Th1-type response also plays some role. Recently, bronchoalveolar lavage (BAL) HGF levels were found increased in patients with acute alveolar injury Ito and colleagues reported that administration of exogenous HGF significantly prevented the development of AHR, inflammatory cell accumulation in the airways, and increases in Th2 cytokines in BAL fluid. Moreover, treatment with HGF significantly reduced growth factors TGF-β, platelet-derived growth factor (PDGF) and nerve growth factor (NGF) levels in BAL fluid and reduced the expression of TGF-β_1 in the lung tissue of sensitized and challenged mice. The development of goblet cell metaplasia, subepithelial collagen deposition/fibrosis and the thickness of the smooth muscle layer were also significantly reduced by recombinant HGF. In contrast, neutralization of endogenous HGF resulted in increased AHR as well as the number of eosinophils, levels of Th2 cytokines, and TGF-β_1 levels in BAL fluid (Stern et al., 2000; Ito et al., 2005). Although these findings were all found in animal experiments, they also suggest HGF potential involvement in the regulatory pathway of Th2-associated allergic responses, especially in the pathogenesis of asthma.

On the basis of these results, the present study was designed to determine whether serum HGF levels are elevated in patients with asthma, and whether its level are associated with the severity of disease.

SUBJEECTS AND METHODS

STUDY SUBJETCS

This was a cross-sectional case-control study. Adult subjects aged≥18 years were recruited from asthma clinic of the China-Japan Union Hospital of Ji Lin University. Detailed clinical history and physical examination were performed on all subjects. The diagnosis of asthma was based on the criteria of the American Thoracic Society (American Thoracic Society, 1987). Spirometric tests and assessment of asthma severity were in accordance with the National Institute of Health–World Health Organization guidelines (National Institutes of Health, 1997). Sex- and age-matched volunteers free from respiratory diseases were recruited as control subjects. The control subjects were all had a negative history of allergy, with no known respiratory diseases such as asthma, chronic obstructive pulmonary disease or bronchiectasis. All subjects were tested for atopy by skin prick testing and were free from respiratory tract infections for a minimum of 4 weeks before inclusion in the study. This study was approved by the Ethics Committee of the China-Japan Union Hospital of Ji Lin University and informed consent was obtained from all subjects.

PULMONARY FUNCTION TESTING

Spirometry was carried out using the spirometer Master screen (Jaeger, Hochberg, Germany), and the best value of 3 maneuvers was expressed as an absolute value and as a percentage of the predicted value.

ATOPIC STATUS

A positive skin prick test to any of 20 common aeroaller-

Table1. Characteristics of healthy controls and asthmatic patients.

	Healthy controls	mild asthma	moderate asthma	severe asthma
Sex (male/female)	24/17	23/18	28/20	23/16
Age (years)	40 (18-53)	38 (20-58)	34 (21-69)	41 (33-57)
Smoking	23	24	31	21
Asthma duration(years)	—	36 (13-54)	27 (10-64)	41 (23-51)
%FEV$_1$(%)	—	81.43(32.5-106.8)	75.8 (27.9-91.2)	63.5 (21.4-85.7)
Atopy	—	12	14	10
Asthma therapy				
LABA	—	25	34	37
Inhaled corticosteroid	—	41	48	39
Oral corticosteroid	—	—	—	16

gens (including mites, grasses, trees, fungus, domestic animals) was used to confirm atopy.

SAMPLE COLLECTION

Approximately 20ml of peripheral blood specimens were obtained from consenting healthy controls and asthmatic patients. Serum was isolated and stored in aliquots at -80°C.
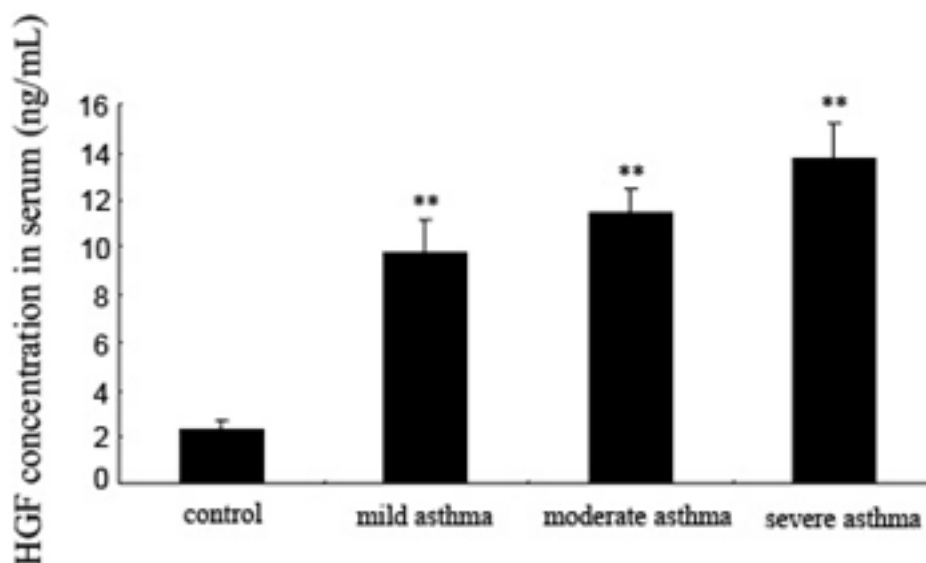
MEASUREMENT OF SERUM HGF CONCENTRATION

Serum was separated from peripheral blood by microcentrifuge at 3000 rpm at room temperature. Serum concentration of HGF was measured using the Quantikine Human HGF immunoassay kit (DOST00; R&D systems, Minneapolis, MN) according to the manufacturer's instructions. Briefly, 1:25 diluted samples were incubated in HGF antibody-precoated plates for 2 hours at room temperature. After washing, 200µl of labeled HGF conjugate solution was added to each well and incubated for 2 hours at room temperature. After washing, a substrate solution containing tetramethylbenzidine was used as a coloring agent. Measurements were taken on a plate reader (Model 550 Microplate Reader; Bio-Rad Laboratories, Hercules, CA) at 450 nm with wavelength correction at 550 nm.

STATISTICAL ANALYSES

Data were analyzed by the Statistical Package of the Social Science (SPSS) for Window version 11.5 (SPSS Inc., Chicago, IL, USA). Demographic data of the subjects were presented as mean ± SEM. The HGF concentration was expressed as the median and interquartile range (IQR), compared between the healthy control subjects and asthmatic patients by the Kruskal–Wallis test or the Mann–Whitney rank-sum test

FIGURE 1: Comparison of serum HGF concentrations in patients with mild, moderate or severe asthma and healthy controls, showing that HGF levels were significantly higher in asthmatic patients compared with healthy controls (**$P < 0.01$).

as appropriate. A probability P-value < 0.05 was considered as statistically significant.

RESULTS

CHARACTERISTICS OF SUBJECTS

Clinical characteristics of asthma patients and healthy controls are shown in Table 1. The four groups were comparable for age, gender distribution. Disease severity of asthma is also shown in Table 1.

LEVELS OF HGF CONCENTRATION IN SERUM FROM ASTHMA PATINETS AND HEALTHY CONTROLS

HGF levels were significantly higher in three asthmatic patient groups as compared with healthy control group [9.8 (6.3-14.5), 11.4 (8.5-16.2), 13.7 (9.9-18.3) and 2.3 (1.6-3.7) ng/mL, respectively, $P<0.01$]. The HGF levels

were also elevated associated with the severity of disease, but this difference was not significant (Fig.1).

DISCUSSION

HGF was originally identified and cloned as a potent mitogen for mature hepatocytes (Nakamura et al., 1986; Nakamura et al., 1987). It is composed of a 69-kDa α-chain and a 34-kDa β-chain linked by a disulfide bridge. The α-chain includes an N-terminal hairpin domain and a sequence of four kringle domains, while the β-chain has a serine protease-like domain (Nakamura et al., 1989; Miyazawa et al., 1989). HGF has many functions such as induction of angiogenesis, promotion of cell proliferation, migration and suppression of apoptosis. Therefore, HGF is now recognized as a multifunctional cytokine and/or a potent stimulator of a variety of cell types, and there is much support that HGF plays an essential part in parenchymal repair and protection in various organs (Nakamura et al., 1987; Matsumoto et al., 1996). For example, HGF prevents the onset and progr-

ession of hepatic fibrosis/cirrhosis, and renal, lung and myocardial fibrosis in vivo (Yaekashiwa et al., 1997; Mizuno et al., 1998; Ueki et al., 1999; Taniyama et al., 2000). However few studies have investigated the role of HGF during the course of asthma.

Asthma is a chronic inflammatory airway disease characterized by airway eosinophilia, mucus hypersecretion, airway AHR, and structural changes in the airway walls, those structural changes, referred to as airway remodeling, and has been recognized as a Th2-mediated disease (Holt et al., 1999; Kumar, 2001). Eosinophils play a pivotal role in the mechanism of allergic airway inflammation, and the chemotaxis of eosinophils is one of the most important events in the pathogenesis of allergic inflammation. Recently, Ito found that in a murine model of asthma, HGF attenuated AHR and allergen-induced airway remodeling and inflammation, involving eosinophils and lymphocytes (Ito et al., 2005). Moreover, they reported that HGF directly inhibited the factor-induced chemotaxis of human eosinophils in the absence of Th2 cytokines or granulocyte macrophage colonystimulating factor (Ito et al., 2007).They also found that administration of exogenous HGF significantly prevented the development of AHR, inflammatory cell accumulation in the airways, and increases in Th2 cytokines in BAL fluid. In contrast, neutralization of endogenous HGF resulted in increased AHR as well as the number of eosinophils, levels of Th2 cytokines levels in BAL fluid (Ito et al., 2005).

Another pathogenesis characteristic of asthma is airway remodeling. HGF, a pleiotropic factor, is known to regulate diverse biological responses, including cell proliferation, survival, migration, and differentiation in different organs. Evidence is increasing that HGF plays an essential role in parenchymal repair and protection. HGF has been shown to antagonize, in vitro, the profibrotic action of TGF-β, such as the expression of collagen type 1, and fibronectin in rat alveolar epithelial cells (Shukla et al., 2009). Recent studies suggest that both endogenous and exogenous HGF are protective against the onset and progression of different models of

chronic disease (e.g., renal, cardiac, and liver) (Mizuno et al., 1998; Ueki et al., 1999; Taniyama et al., 2000). Yaekashiwa and colleagues reported that exogenous HGF prevented the progression of lung fibrosis induced by bleomycin, and Ito and colleagues reported that the administration of exogenous HGF significantly decreased tissue fibrosis, remodeling, and dysfunction by suppressing the production of the PDGF, and NGF in a chronic asthma model (Yaekashiwa et al., 1997; Ito et al., 2005). In another study, Yamabayashi and colleagues found that ONO-1301, a synthetic prostacyclin agonist without the typical prostanoid structure of a five-member ring and allylic alcohol, attenuated the development of AHR and reversed the histological findings of airway remodeling in association with an increase in HGF production in the lungs. These antifibrotic effects of ONO-1301 were diminished by the administration of anti-HGF, suggesting that the effects of the compound were mediated, at least in part, through HGF induction (Yamabayashi et al., 2012). Although all these experiments were all done in animals, they suggest that HGF may play important part role in the pathogenesis of asthma, not only in airway eosinophilia and airway AHR but also in airway remodeling.

In the resent study, we demonstrated for the first time that asthmatic patients exhibited higher serum HGF levels (approximately five times) compared with the healthy control subjects, suggesting a peculiar role for this protein in asthma pathogenesis. But contrary to our hypothesis, we didn't find a positive correlation between HGF concentration and disease severity, a role for HGF in asthma therefore appears to be counterintuitive. This may be explained by the complicated pathogenesis of asthma, as we know, apart from HGF, many other factors contribute to its development and progression.

Our study did not include bronchial biopsies, which is a limitation because we did not have the opportunity to investigate by means of quantitative reverse transcriptase polymerase chain reaction for evaluation of HGF messenger RNA (mRNA) expression, and analyze the presence and location of HGF protein expression using immunohistochemistry.

In conclusion, our data suggest that HGF may play important part role in the pathogenesis of asthma, not only in airway eosinophilia and airway AHR but also in airway remodeling. However, the detailed mechanism of these negative actions of HGF remains unclear. Although HGF is also recognized as a homeostatic mediator that restores abnormal conditions in organisms, the biological potential of HGF is extremely obscure in allergic responses in particular. Therefore, it is necessary to clarify what kind of signal pathway plays an important role in the action of HGF against allergic response and further studies are needed to clarify the involvement of HGF in various aspects of asthma. Finally, this result may help to elucidate the mechanism of allergic diseases and suggests that HGF has potential in the treatment of asthma.

REFERENCES

American Thoracic Society (1987). Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am. Rev. Respir. Dis. 136(1): 225-244.

Elias JA, Zhu Z, Chupp G, Homer RJ (1999). Airway remodeling in asthma. J Clin Invest. 104(8): 1001–1006.

Holt PG, Macaubas C, Stumbles PA, Sly PD (1999).The role of allergy in the development of asthma. Nature. 402 (6760 Suppl): B12–B17.

Ito W, Chiba T, Kanehiro A, Kato H, Yamaguchi K, Ueki S, Kayaba H, Chihara J (2007). Hepatocyte growth factor attenuates eotaxin and PGD 2 -induced chemotaxis of human eosinophils. Allergy. 62(4): 415–422.

Ito W, Kanehiro A, Matsumoto K, Hirano A, Ono K, Maruyama H, Kataoka M, Nakamura T, Gelfand EW, Tanimoto M (2005). Hepatocyte growth factor attenuates airway hyperresponsiveness, inflammation and remodeling. Am. J. Respir. Cell. Mol. Biol. 32(4): 268–280.

Kumar RK (2001). Understanding airway wall remodeling in asthma: a basis for improvements in therapy? Pharmacol Ther. 91(2):93–104.

Matsumoto K, Nakamura T (1996). Emerging multipotent aspects of hepatocyte growth factor. J Biochem.119 (4): 591–600.

Miyazawa K, Tsubouchi H, Naka D, Takahashi K, Okigaki M, Arakaki N, Nakayama H, Hirono S, Sakiyama O, Takahashi K (1989). Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. Biochem Biophys Res Commun.163 (2): 967–973.

Mizuno S, Kurosawa T, Matsumoto K, Mizuno-Horikawa Y, Okamoto M, Nakamura T(1998). Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease. J. Clin. Invest. 101(9): 1827–1834.

Nakamura T, Nawa K, Ichihara A, Kaise N, Nishino T (1987).Purification and subunit structure of hepatocyte growth factor from rat platelets. FEBS Lett. 224(2): 311–316.

Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S (1989). Molecular cloning and expression of human hepatocyte growth factor. Nature. 342 (6248): 440–443.

Nakamura T, Teramoto H, Ichihara A (1986). Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. Proc Natl Acad Sci USA. 83(17): 6489–6493.

Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F, Comoglio PM (1992). Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. EMBO J. 11(33):4825-4833.

National Institutes of Health: National Heart, Lung, and Blood Institute. (1997). Guidelines for the diagnosis and management of asthma. Publication no.97-4051. Washington, DC: National Institutes of Health.

Shukla MN, Rose JL, Ray R, Lathrop KL, Ray A, Ray P (2009). Hepatocyte growth factor inhibits epithelial to myofibroblast transition in lung cells via SMAD7. Am

J Respir Cell Mol Biol. 40(6):643–653.

Stern JB, Fierobe L, Paugam C, Rolland C, Dehoux M, Petiet A, Dombret MC, Mantz J, Aubier M, Crestani B (2000). Keratinocyte growth factor and hepatocyte growth factor in bronchoalveolar lavage fluid in acute respiratory distress syndrome patients. Crit Care Med. 28(7): 2326–2333.

Taniyama Y, Morishita R, Nakagami H, Moriguchi A, Sakonjo H, Shokei-Kim, Matsumoto K, Nakamura T, Higaki J, Ogihara T(2000). Potential contribution of a novel antifibrotic factor, hepatocyte growth factor, to prevention of myocardial fibrosis by angiotensin II blockade in cardiomyopathic hamsters. Circulation. 102(2): 246–252.

Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J (1999). Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat. Med. 5(2): 226–230.

Yaekashiwa M, Nakayama S, Ohnuma K, Sakai T, Abe T, Satoh K, Matsumoto K, Nakamura T, Takahashi T, Nukiwa T(1997). Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin: a morphologic study. Am. J. Respir. Crit. Care. Med. 156(6):1937–1944.

Yamabayashi C, Koya T, Kagamu H, Kawakami H, Kimura Y, Furukawa T, Sakagami T, Hasegawa T, Sakai Y, Matsumoto K, Nakayama M, Gelfand EW, Suzuki E, Narita I (2012). A novel prostacyclin agonist protects against airway hyperresponsiveness and remodeling in mice. Am. J. Respir. Cell. Mol. Biol. 47(2):170-177.

Yamaura K, Ito K, Tsukioka K, Wada Y, Makiuchi A, Sakaguchi M, Akashima T, Fujimori M, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Suzuki J, Amano J, Isobe M (2004). Suppression of acute and chronic rejection by hepatocyte growth factor in a murine model of cardiac transplantation: induction of tolerance and prevention of cardiac allograft vasculopathy. Circulation. 110(12):1650–1657.