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PRACA ORYGINALNA

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Hepatocyte growth factor in exhaled breath and BAL fluid in sarcoidosis

Wątrobowy czynnik wzrostu w kondensacie powietrza wydechowego i popłuczynach oskrzelowo-pęcherzykowych w sarkoidozie

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Abstract

Introduction: Hepatocyte growth factor (HGF) is a strong mitogen stimulating lung epithelial cell growth. Elevated levels of HGF have been reported in various biological materials of patients with acute respiratory distress syndrome and in patients recovering from pneumonia or pneumonectomy. Sarcoidosis may be complicated by lung fibrosis. Consequently, HGF could be considered a new biomarker identifying patients with a higher risk of lung fibrosis.
The aim of the study was to verify whether: 1. HGF is measurable in bronchoalveolar lavage fluid (BALF) and exhaled breath condensate (EBC); 2. HGF in BALF or EBC is impaired in sarcoidosis; and 3. HGF correlates with chosen activity and prognostic markers.
Material and methods: Sixty-four EBC and 30 BALF of sarcoid patients, and 15 and 9 of healthy controls, respectively, were collected for the measurement of HGF using an ELISA test.
Results: HGF was detectable in 62% of EBC samples (56% sarcoidosis and 87% of controls) and in all the BALF samples. EBC and BALF concentrations were not different in comparison to the controls. Moreover, no correlation was found between EBC/BALF concentrations and radiological stage, lung function tests, duration of disease, number of relapses, BALF lymphocytes, serum ACE, or serum and urine calcium concentrations.
Conclusions: HGF is detectable in BAL and EBC. However, it does not distinguish sarcoidosis patients from healthy subjects. The above, as well as the lack of correlations with various parameters of disease activity and severity rule out EBC/BALF HGF as a biomarker for sarcoidosis monitoring.

Key words: sarcoidosis, exhaled breath condensate, bronchoalveolar lavage fluid, hepatocyte growth factor

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Streszczenie

Wstęp: Wątrobowy czynnik wzrostu (HGF) jest silnym mitogenem stymulującym wzrost komórek nabłonka pęcherzyków płucnych. Wyższe stężenia HGF w różnych materiałach biologicznych stwierdzono między innymi w zespole ostrej niewydolności oddechowej (ARDS), u chorych po przebytym zapaleniu płuc i po pneumonektomii. Niekorzystnym zejściem sarkoidozy jest włóknienie płuc. Wątrobowy czynnik wzrostu mógłby być przydatny jako marker pozwalający na rozpoznanie chorych obarczonych ryzykiem włóknienia płuc.
Celem pracy była ocena: 1) czy HGF jest wykrywalny w popłuczynach oskrzelowo-pęcherzykowych (BAL) i kondensacie powietrza wydechowego (EBC); 2) czy stężenia HGF w BAL-u i kondensacie chorych na sarkoidozę różnią się od stężeń osób zdrowych; 3) czy istnieją korelacje z parametrami aktywności i wybranymi czynnikami rokowniczymi.
Materiał i metody: Zebrano kondensat od 64 i popłuczyny oskrzelowo-pęcherzykowe od 30 chorych na sarkoidozę. Grupę kontrolną stanowiły osoby zdrowe (n = 15 dla EBC, n = 9 dla BAL). Wątrobowy czynnik wzrostu oznaczono immunoenzymatycznie.

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Wyniki: Stężenia HGF w kondensacie przekroczyły próg detekcji u 62% badanych (56% chorych i 87% zdrowych) i we wszystkich próbkach BAL. Nie stwierdzono różnic w stężeniach w EBC i BAL pomiędzy osobami chorymi i zdrowymi. Nie stwierdzono korelacji pomiędzy HGF w EBC/BALF a stopniem radiologicznym, parametrami czynności płuc, czasem trwania choroby, liczbą nawrotów, odsetkiem limfocytów w BAL, stężeniem enzymu konwertującego angiotensynę i wapnia w surowicy, utratą dobową wapnia z moczem.
Wnioski: Wątrobowy czynnik wzrostu jest wykrywalny w BAL i EBC. Jednak brak różnic pomiędzy chorymi na sarkoidozę i osobami zdrowymi oraz brak korelacji z markerami aktywności i czynnikami rokowniczymi uniemożliwiają jego zastosowanie w diagnostyce i monitorowaniu sarkoidozy.

Słowa kluczowe: sarkoidoza, kondensat powietrza wydechowego, popłuczyny oskrzelowo-pęcherzykowe, wątrobowy czynnik wzrostu

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Introduction

Although the majority of patients suffering from sarcoidosis have a good prognosis due to a high rate of spontaneous resolution [1], those suffering from a chronic disease have a significant risk of developing lung fibrosis [2].

Hepatocyte growth factor (HGF) is a strong mitogen for lung epithelial cells [3]. Bleomycin instillation to a rat's lung results in a 10-fold increase of HGF concentration in BALF [4]. HGF is protective in many animal models of lung injury [5]. Elevated levels of HGF have been found in the pulmonary oedema fluid of patients with acute lung injury, with higher concentrations in those with worse prognosis [6]. Production of this molecule by fibroblasts of patients suffering from emphysema is defective [7]. The role of HGF in lung regeneration may also be illustrated by increased serum concentrations in patients recovering from surgical lung resection [8]. In addition, elevated HGF concentrations in the exhaled breath condensates (EBC) of patients suffering from pneumonia have been reported [9]. HGF in BAL fluid has recently been reported to be higher in active, compared to inactive, sarcoidosis. The authors reported that HGF can prevent TGF-β-induced myofibroblast transformation *in vitro*. As a result, they propose a new mechanism of antifibrotic activity of HGF [10].

Bearing in mind all of the above data, we found it interesting to measure HGF concentrations in the EBC and bronchoalveolar lavage fluid (BALF) of patients suffering from sarcoidosis.

Material and methods

Exhaled breath condensates samples were collected from 64 patients and BALF samples were collected from 30 patients with active sarcoidosis. Sarcoidosis was confirmed by biopsy of the lung, mediastinal/hilar lymph nodes, or bronchial mucosa, with the exception of patients with typical radiological picture consistent with stage I/II with typical BAL pattern and Löfgren syndrome. None of the patients has been treated at the time of material collection. All patients were diagnosed with active sarcoidosis, based on clinical symptoms, radiological progression, increased BAL lymphocytes, increased SACE, or impaired calcium metabolism. There were 14 ex-smokers of cumulative cigarette consumption below 10 pack-years and the shortest time of abstinence was longer than 1 year. The rest of the group consisted of never-smokers.

A control group for EBC consisted of 15 healthy never-smoking volunteers. A control group for BALF comprised 9 healthy never-smokers. Bronchoscopy with BAL was performed in these subjects due to medical indications, but bronchoscopy, BAL, CT, and lung function tests were normal. There were 2 patients with episodes of blood-stained sputum, finally found to be caused by epistaxis, and 7 patients with suspected hilar/mediastinal mass based on chest X-ray, in whom this pathology was finally excluded on the basis of CT.

All the patients signed informed consent forms for participation in the study. In addition, all the patients undergoing bronchoscopy also signed a separate standard consent for the procedure. The study was approved by the Ethical Committee at the Medical University of Lodz (consent No. RNN/99/08/KE).

Exhaled breath condensate was collected according to the standards [11], using a commercial device (Ecoscreen, Jaeger, Germany), always before bronchoscopy. Briefly, the patients were asked to breathe out spontaneously through a mouthpiece equipped with a saliva trap for ten minutes. The respiratory rate ranged from 15 to 20 breaths/min. All the subjects wore a nose-clip and rinsed their mouths with distilled water just before and during the seventh minute of the collection in order to reduce nasal contamination. The samples were stored at –80 °C until analysis.

Bronchoalveolar lavage fluid was collected during a routine bronchoscopy, by instillation and

Table 1. Characteristics and results of measurements in sarcoidosis group (n = 64) depending on radiological stage

Measurement	Stage 1	Stage 2	Stage 3	Statistical analysis
EBC HGF [pg/ml]	42.9 ± 8.0	30.2 ± 5.3	56.5 ± 16.6	NS
BALF HGF [pg/ml]#	140.8 ± 8.5	141.4 ± 16.4	140.2 ± 37.5	NS
Age (years)	39 ± 2	42 ± 2	40 ± 3	NS
LS (%)	29	11	0	Not calculated
No. of episodes	1.13 ± 0.07	1.05 ± 0.05	1.27 ± 0.14	NS
Duration (weeks)	48 ± 25*	120 ± 41*	64 ± 26	$p < 0.05$
FEV_1 (% predicted)	102.0 ± 2.7*	78.8 ± 5.0*	84.2 ± 7.7*	$p < 0.001$ (1 *v.* 2) $p < 0.05$ (1 *v.* 3)
FVC (% predicted)	105.4 ± 3.2*	87.1 ± 4.5*	87.6 ± 7.6*	$p < 0.01$ (1 *v.* 2) $p < 0.05$ (1 *v.* 3)
FEV_1/FVC (%)	85.3 ± 1.6	78.2 ± 2.9	82.1 ± 3.5	NS
DLCOc (% predicted)	97.6 ± 3.9*	82.2 ± 3.0*	68.8 ± 6.1*	$p < 0.001$ (1 *v.*3) $p < 0.05$ (1 *v.* 2)
BALF lymphocytes (%)	57 ± 6	37 ± 6	42.4 ± 9.6	NS
SACE	57.0 ± 6.0	53.6 ± 10.8	92.7 ± 31.8	NS
CRP	20.6 ± 8.0	8.5 ± 1.4	3.7 ± 1.2	NS
Ca^{2+} serum [mmol/ml]	2.42 ± 0.03	2.44 ± 0.03	2.50 ± 0.03	NS
Ca^{2+} 24 hrs urine [mmol/24 hrs]	5.46 ± 0.61	6.70 ± 1.10	6.76 ± 1.11	NS

#BALF HGF was measured in 30 patients; *Significantly different
BALF — bronchoalveolar lavage fluid, CRP — C-reactive protein, DLCOc — diffusing capacity for CO corrected for haemoglobin concentration, EBC — exhaled breath condensate, FEV_1 — forced expiratory volume in 1st second, FVC — forced vital capacity, HGF — hepatocyte growth factor, NS — not significant, LS — Löfgren syndrome, SACE — serum angiotensin converting enzyme

subsequent withdrawal of 4 × 50 ml of 0.9% NaCl to the medial lobe or lingula. The fluid was filtered and cytospin slides were prepared and stained with May-Grünwald-Giemsa stain. The total number of cells and the percentage of particular cell types were calculated. The supernatants were stored at –80 °C until analysis.

The following tests were performed: chest X-ray (CXR), high resolution computed tomography (HRCT), lung function tests (FEV_1, FVC, FEV_1/FVC, DLCO), blood tests (serum angiotensin converting enzyme [SACE], C-reactive protein [CRP], and serum calcium concentrations) and 24-hour urine collection for calcium loss.

Human HGF was measured with the use of an immunoassay kit (BioSource Europe S.A., Belgium). Each sample was measured in duplicate, and a mean value was calculated. The detection limit was 20 pg/ml. The levels of the measured mediators below the detection limit were arbitrarily assumed to be half of the detection limit value. The intra-assay reproducibility (CV, coefficient of variation) for EBC was 20.1% and for BAL was 16.3%. According to the manufacturer, the intra-assay reproducibility for human serum was < 6.1%, the inter-assay reproducibility was < 7.0%, and recovery after addition of Hu HGF to human serum was 94–102%.

Statistical analysis

The data were presented as mean ± SEM. The Mann-Whitney test was used to compare sarcoidosis EBC HGF concentrations with the control group (non-Gaussian distribution of sarcoidosis EBC data), and Student's t-test was used to compare BALF HGF concentrations. The correlations were calculated with the Spearman test. One-way ANOVA and Bonferroni post-test (for data with Gaussian distribution) or Kruskall-Wallis followed by Dunn's Multiple Comparison Test (for data without normal distribution) were used to calculate differences between data characterizing sarcoidosis stages (see Table 1). $P < 0.05$ was assumed as statistically significant.

None of the authors has any conflict of interest.

Results

Hepatocyte growth factor was detectable in 62% of EBC samples (56% of the sarcoidosis and 87% of control samples). All the BALF HGF concentrations were above the detection limit of 20 pg/ml.

No differences were found between the sarcoid and control HGF EBC concentrations (40.9 ± 4.8 vs. 52.1 ± 8.1 pg/ml), or HGF BALF concentrations (141.0 ± 8.0 *v.* 121.4 ± 14.4 pg/ml) (Fig. 1).

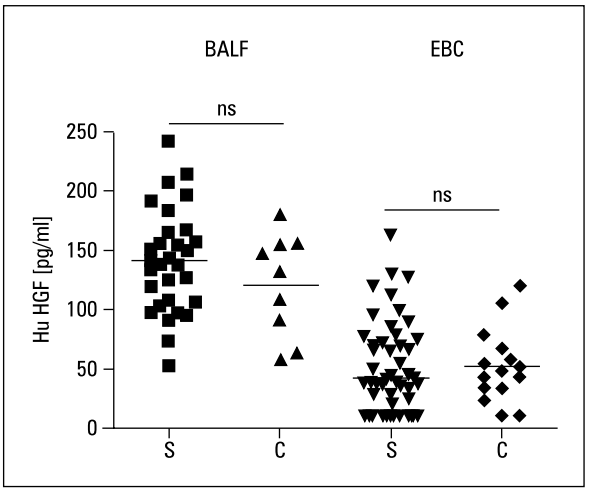

Figure 1. HGF concentration in bronchoalveolar lavage fluid (BALF) and exhaled breath condensate (EBC) in healthy controls (C) and patients with sarcoidosis (S)

Neither the EBC nor BALF concentrations in sarcoidosis patients correlated with radiological stage, the presence or absence of Löfgren syndrome, number of relapses and duration of disease, lung function parameters, BALF lymphocyte percentage/number, SACE, serum CRP, serum calcium concentrations, and 24-hour urinary calcium loss.

Table 1 shows the results and characteristics of the study group.

Discussion

One of most intriguing questions in sarcoidosis research is how to distinguish patients posing a risk of developing lung fibrosis, who could possibly benefit from long-term treatment, from those with good prognosis, in whom the disease will vanish eventually without any consequences. HGF is thought to be a promising molecule in this context, as its production was increased in several clinical situations characterized by lung epithelial damage with subsequent regeneration [6, 8, 9].

Despite its mitogenic activity towards epithelial cells, several additional mechanisms of antifibrotic action of HGF have been proposed:

— down-regulation of fibrogenic cytokines, such as transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), and connective tissue growth factor (CTGF) [11];
— inhibition of myofibroblast differentiation and overgrowth [12];
— induction of myofibroblast apoptosis [13];

An additional potential role of HGF in the pathogenesis of sarcoidosis may be a consequence of the suppression of antigens presenting activity of dendritic cells [14]. This may result in inhibition of further pathogenetic steps, leading through recognition of antigen-MHC complex linked to T-cell receptor and differentiation of Th0 to Th1 cells to oligoclonal proliferation of lymphocytes.

The following scenarios were taken into account: 1. providing HGF reflects the extent of epithelial damage, higher concentrations should have been expected in patients with extensive parenchymal involvement and worse LFT results; 2. assuming HGF is a molecule necessary for effective epithelial healing, the lack of its increase in response to damage may switch the regenerative pathway into extensive fibrosis. In this case, lower HGF in EBC or BALF could contribute to identifying patients with a worse prognosis. To strengthen the possibility of the latter, Marchand-Adam et al. [15, 16] suggested a severely defective secretion and activation of HGF by lung fibroblasts in idiopathic pulmonary fibrosis (IPF).

Our results, however, do not confirm any of these hypotheses. This might be accounted for by the following:

1. Even in very profound interstitial involvement in sarcoidosis, extensive epithelial damage rarely occurs. This is possible, considering the histopathology of sarcoid lung, overall good prognosis, and clinical observations showing a sporadic reversibility of sometimes very extensive interstitial changes.
2. The character of defects in HGF production may vary. The temporal lack of HGF increase (or decrease) in response to certain devastating stimuli may be difficult to capture in a clinical setting.

Regardless, of which of the above statements is true, HGF in BALF and EBC should not be recommended as a prognostic marker in sarcoidosis.

Conclusions

1. HGF is detectable in EBC and BALF, both in sarcoidosis patients and in healthy subjects.
2. HGF concentrations do not distinguish sarcoidosis patients from healthy subjects and do not correlate with chosen activity and prognostic markers. Thus, the HGF concentration level is unavailing as a biomarker in sarcoidosis.
3. Further studies are needed (comprising more patients with progressive disease, follow-up studies, etc) in order to definitely disqualify this biomarker for sarcoidosis monitoring.

References

1. Krychniak-Soszka A., Kuś J. Prognostic value of some clinical, radiological, laboratory and functional parameters in sarcoidosis. Pneumonol. Alergol. Pol. 2002; 70: 573–582.
2. American Thoracic Society. Statement on sarcoidosis. Am. J. Respir. Crit. Care. Med. 1999; 160: 736–755.
3. Yanagita K., Matsumoto K., Sekiguchi K., Ishibashi H., Nihoi Y., Nakamura T. Hepatocyte growth factor may act as a pulmotrophic factor on lung regeneration after acute lung injury. J. Biol. Chem. 1993; 268: 21212–21217.
4. Adamson I.Y.R., Bakowska J. Relationship of keratinocyte growth factor and hepatocyte growth factor levels in rat lung lavage fluid to epithelial cell regeneration after bleomycin. Am. J. Pathol. 1999; 155: 949–954.
5. Ware L.B., Matthay M.A. Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair. Am. J. Physiol. Lung. Cell. Mol. Physiol. 2002; 282: L924–L940.
6. Verghese G.M., McCormick-Shannon K., Mason R.J., Matthay M.A. Hepatocyte growth factor and keratinocyte growth factor in the pulmonary edema fluid of patients with acute lung injury. Am. J. Respir. Crit. Care Med. 1998; 158: 386–394.
7. Plantier L., Marchand-Adam S., Marchal-Somme J. et al. Defect of hepatocyte growth factor production by fibroblasts in human pulmonary emphysema. Am. J. Physiol. Lung Cell Mol. Physiol. 2005; 288: L641–L647.
8. Dikmen E., Kara M., Kisa U., Atinkaya C., Han S., Sakinci U. Human hepatocyte growth factor levels in patients undergoing thoracic operations. Eur. Respir. J. 2006; 27: 73–76.
9. Nayeri F., Millinger E., Nilsson I., Zetterstrom O., Brudin L., Forsberg P. Exhaled breath condensate and serum levels of hepatocyte growth factor in pneumonia. Respir. Med. 2002; 96: 115–119.
10. Faehling M., Anders D., Hetzel M. et al. HGF and IL-6 in BAL fluid of interstitial lung diseases: effect on human lung fibroblasts. Eur. Respir. J. 2008; 32 (Suppl. 52): 316S.
11. Inoue T., Okada H., Kobayashi T. et al. Hepatocyte growth factor counteracts transforming growth factor-beta1, through attenuation of connective tissue growth factor induction, and prevents renal fibrogenesis in 5/6-nephrectomized mice. FASEB J. 2003; 17: 268–270.
12. Yang J., Dai C., Liu Y. Hepatocyte growth factor suppresses renal interstitial myofibroblast activation and intercepts SMAD transduction. Am. J. Pathol. 2003; 163: 621–632.
13. Mizuno S., Matsumoto K., Li M.Y., Nakamura T. HGF reduces advancing lung fibrosis in mice: a potential role for MMP-dependent myofibroblast apoptosis. FASEB J. 2005; 19: 580–582.
14. Okunishi K., Dohi M., Nakagome K. et al. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. J. Immunol. 2005; 175: 4745–4753.
15. Marchand-Adam S., Marchal J., Cohen M. et al. Defect of hepatocyte growth factor secretion by fibroblasts in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 2003; 168: 1156––1161.
16. Marchand-Adam S., Fabre A., Mailleux A.A. et al. Defect of pro-hepatocyte growth factor activation by fibroblasts in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 2006; 174: 58–66.