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Short communication

Histidine-rich glycoprotein and idiopathic pulmonary fibrosis

G. Ernst [a, b], E. Dantas [c], J. Sabatté [c], F. Caro [a], A. Salvado [b], P. Grynblat [a], J. Geffner [c, *]

[a] Hospital María Ferrer, Ciudad de Buenos Aires, Argentina
[b] Hospital Británico, Ciudad de Buenos Aires, Argentina
[c] Instituto de Investigaciones en Retrovirus y SIDA (INBIRS), UBA-CONICET, Ciudad de Buenos Aires, Argentina

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ABSTRACT

Histidine-rich glycoprotein (HRG) is an enigmatic glycoprotein able to interact with a variety of ligands such as IgG, complement components, heparan sulfate, thrombospondin, fibrinogen and plasminogen. HRG is present at high concentrations in plasma and there is evidence indicating that it is able to modulate the course of biological processes such as angiogenesis, fibroblast proliferation, complement activation, coagulation and fibrinolysis. Because these processes are involved in the pathogeneses of lung fibrosis we here analyzed a possible link between HRG and idiopathic pulmonary fibrosis (IPF). We found that plasma concentrations of HRG are significantly diminished in IPF patients compared to healthy subjects. Moreover, we found a positive correlation between HRG plasma levels and forced vital capacity (FVC) values, suggesting that plasma concentration of HRG would be a useful indicator of disease activity in IPF. HRG has been described as a negative acute phase reactant able to accumulate at sites of tissue injury. Hence, we also measured the concentrations of HRG in BAL samples from IPF patients. We found that the concentrations of HRG in samples from IPF patients were significantly higher compared to controls, suggesting that the reduced concentration of HRG in plasma from IPF patients could be due, at least in part, to an enhanced uptake of this protein in the lung.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease of unknown etiology, which accounts for 20%–30% of interstitial lung diseases. It has a poor prognosis with an average survival of 3–4 years from the time of diagnosis, but there is appreciable heterogeneity in the course of the disease among individual patients [1]. Our knowledge of the natural history of IPF and of the pathogenic mechanisms involved remains rudimentary, and the therapeutic options for IPF patients are very limited [2]. There are no previous studies aimed to analyze the participation of histidine-rich glycoprotein (HRG) in the pathogenesis of IPF or other interstitial lung diseases.

HRG is an abundant plasma glycoprotein (~100–150 μg/ml) synthesized in the liver, able to interact with a variety of molecules such as IgG, complement components, heparan sulfate, thrombospondin, fibrinogen and plasminogen [3]. The role of HRG has not been well defined. However, it is able to modulate different biological processes such as angiogenesis, cell adhesion, fibroblast proliferation, coagulation and fibrinolysis [3,4]. Because some of these processes are involved in the development of lung fibrosis [1,5], we speculate that HRG might be involved in the pathogenesis of IPF, and we here analyzed the plasma levels of HRG in IPF patients.

2. Methods

A total of 40 patients with IPF and 12 patients with subacute or chronic hypersensitivity pneumonitis, classified by histopathological features, were included in our study. IPF was diagnosed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) consensus criteria. Plasma samples from IPF and hypersensitivity pneumonitis patients were obtained at days 2–7 after forced vital capacity (FVC) measurement. Plasma samples obtained from healthy adult subjects (n = 25) were used as controls. The characteristics of patients with IPF and healthy volunteers are summarized in Table 1. In all cases, venous blood (10 ml) was obtained by standard venipuncture and collected in plastic Vacutainer tubes containing EDTA (K2E tube, Ref 367525, Becton Dickinson, Plymouth, England). Tubes were centrifuged for 20 min at

* Corresponding author. Instituto de Investigaciones en Retrovirus y SIDA (INBIRS), Paraguay 2155, Piso 11, C1121ABG, Ciudad de Buenos Aires, Argentina.
E-mail address: jorgegeffner@gmail.com (J. Geffner).

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Table 1
Characteristics of patients with IPF and healthy volunteers.

	IPF patients (n = 40)	Healthy volunteers (n = 25)
Age (mean ± SD)	67.0 ± 8.5	60.5 ± 6.2
Male/female	31/9	18/7
Smoking history		
Current	2	10
Ex-smoker	31	2
Never smoked	7	13
Histological diagnosis	18	–
FVC, % predicted (mean ± SD)	55.6 ± 18.2	ND
Current or previous pirfenidone use	1	–

room temperature and 1500 g within 1 h of blood collection. The plasma fraction was harvested and stored at −80 °C until the day of testing. BAL fluid was obtained using an Olympus flexible fiberoptic bronchoscopy. The lavage protocol was performed as described previously [6]. Briefly, five aliquots of 20 ml of sterile saline were sequentially instilled into the right middle lobe and immediately aspirated. The BAL fluid was placed on ice prior to centrifugation at 800× g for 5 min at 4 °C. Control BAL samples were obtained from 6 patients who underwent successful corrective surgery for traumatic tracheal stenosis. The mean ± SD age of these patients was 57.0 ± 6.0 years old. BAL samples were taken from 3 to 6 months after the surgical procedure in all cases. None of these subjects had a history of inflammatory lung disease and showed FVC values within the normal range, with a mean ± SD value of 90.2 ± 8.0 (% predicted). Levels of HRG in plasma and BAL samples were measured by ELISA (Sino Biol., Beijing, China). All participants provided informed consents, and the study was approved by the Ethics Committee of the "María Ferrer" Hospital, Buenos Aires.

3. Results and discussion

We found that the plasma levels of HRG were significantly lower in IPF patients compared to controls: 87.5 ± 4.4 *vs* 113.9 ± 4.6 μg/ml, respectively (mean ± SE, $p < 0.001$) (Fig. 1A). Values of HRG showed a heterogeneous distribution among IFP patients, ranging from 13 to 140 μg/ml. Taking this into account, we then analyzed whether the HRG plasma levels could be related to pulmonary function by evaluating FVC. We found a positive correlation between HRG plasma levels and FVC values ($r = 0.65$, $p < 0.001$) (Fig. 1B). This suggests that low HRG plasma levels in IPF are indicative of a poor pulmonary function.

No previous studies have analyzed the levels of HRG in the course of lung diseases. A reduction in the plasma levels of HRG has been described in different physiological and pathological conditions. The administration of estrogens has shown to reduce the concentration of HRG in a dose-dependent way [7]. Normal pregnancy is associated to low plasma levels of HRG. Interestingly, the fall in HRG levels was more pronounced in women who developed pre-eclampsia [8]. A reduction in the HRG plasma levels was also observed in patients with advanced liver cirrhosis [9], as well as in those undergoing some acute inflammatory conditions, suggesting that HRG might act as a negative acute phase reactant able to accumulate at sites of tissue injury [10]. Hence, we analyzed whether HRG might be detected at the sites of lung injury. To this aim, we measured the levels of HRG in BAL from IPF patients. Only a reduced number of BAL samples were available for these determinations. They correspond to 7 out of the 40 patients analyzed in Fig. 1. All these patients showed very low plasma levels of HRG compared to controls, with a mean of 59 ± 8 μg/ml. We found that the concentrations of HRG in BAL samples from IPF patients were

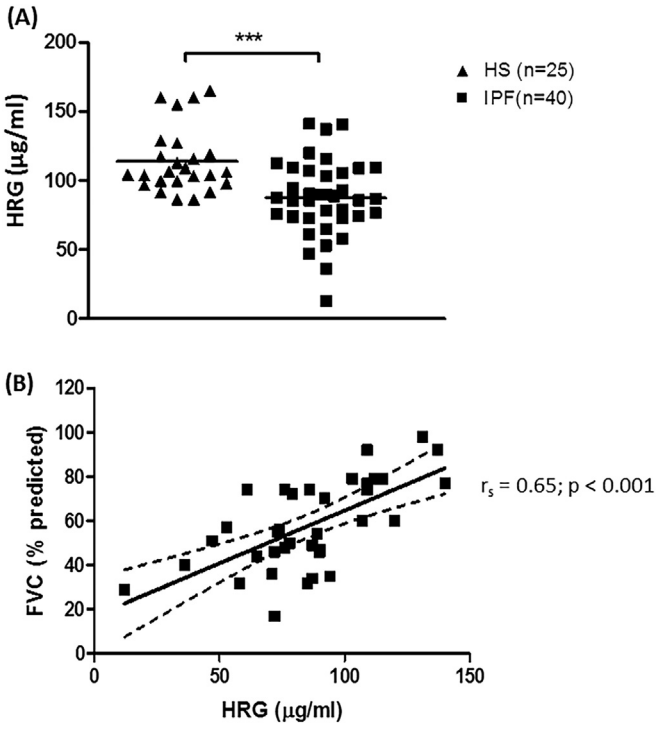

Fig. 1. Positive correlation between HRG plasma levels and FVC in IPF patients. A. HRG plasma levels in IPF patients. Differences between healthy subjects (HS) and IPF patients were evaluated by the non-parametric Mann Whitney test (*$p < 0.001$). B. Correlation between HRG plasma levels and FVC values in IPF patients was analyzed using the Spearman correlation test. All analyses were performed with the Prism 5 software (Graph Pad, La Jolla, CA).

significantly higher ($p < 0.01$) than controls: 5.3 ± 1.6 ng/ml (n = 7, range 3.8–7.5 ng/ml) vs. 1.7 ± 0.9 ng/ml (n = 6, range 0.4–2.7 ng/ml). However, a non-significant negative association between BAL levels of HRG and FVC in IPF patients was observed ($r_s = -0.56$, $p = 0.20$).

Cigarette smoking is not only one of the most important risk factors for development of IPF but also induces a detrimental effect on the survival of IPF patients [5]. Hence, we wonder whether HRG was actually a useful biomarker in both, smokers and nonsmokers IPF patients. Our cohort of IPF patients include 7 patients who had never smoked, and we found that smoker patients, but not nonsmokers patients, showed lower HRG plasma levels compared to controls: 76.8 ± 5.4, 109.6 ± 9.6, and 113.9 ± 4.6 μg/ml, for smokers with IPF (n = 33), nonsmokers with IPF (n = 7), and healthy controls (n = 25), respectively (mean ± SE, $p < 0.001$ smokers with IPF vs healthy controls). These data suggest that HRG, as a biomarker, would not be of value in non-smokers with IPF. This observation, however, should be validated using data collected from a larger number of non-smoker IPF patients.

To further analyze the utility of HRG as a specific biomarker for IPF patients, we determined the plasma levels of HRG in a group of patients with hypersensitivity pneumonitis, a form of diffuse interstitial lung disease which results from sensitization to inhaled antigens [11]. A total of 12 patients with diagnosis of subacute or chronic disease by histologic findings were included. Patients showed a mean ± SD age of 57 ± 9, a FVC (% predicted) of 54.1 ± 11.7 (mean ± SD), and included 4 males and 6 smokers. Interestingly, and contrasting with the observations made in the IPF cohort, these patients showed HRG plasma levels similar to healthy controls: 118.6 ± 13.5 μg/ml (mean ± SE, n = 12), suggesting that this biomarker could be useful to distinguish between patients with IPF

and hypersensitivity pneumonitis.

HRG is an enigmatic protein able to interact with a number of plasma proteins and modulate in vitro different biological processes, such as angiogenesis, cell adhesion, complement activation, coagulation and fibrinolysis. However, the role of HRG has not been well defined [3,4]. Our results reveal a strong correlation between HRG plasma levels and FVC (% predicted) suggesting the utility of HRG as a biomarker in IPF able to reflect disease activity.

So far, no studies have revealed the participation of HRG in the pathogenesis of IPF, and therefore we can only speculate about this. However, there are two areas where HRG might intersect with the molecular pathways involved in the development of pulmonary fibrosis. In contrast to "classically activated macrophages" (M1) which produce the inflammatory cytokines IL-1α, IL-6, TNF-α, IL-12p70, and IL-23, "alternatively-activated macrophages" (M2) produce profibrotic mediators, such as TGF-β and platelet-derived growth factor (PDGF) [12,13]. It is well established that the development of lung fibrosis is associated with the differentiation of monocytes and macrophages toward a M2 profile [1,14]. Interestingly, studies performed in the field of cancer immunity revealed that HRG strongly inhibits the development of M2 macrophages, promoting a M1-like phenotype [15]. Hence, we speculate that the diminished concentration of HRG found in the plasma of IPF patients might favor the differentiation of lung infiltrating monocytes into a pro-fibrotic M2 profile. On the other hand, it is well known that IPF is characterized by the accumulation of activated (myo)fibroblasts, a process that may be attributed, at least in part, to the phenomenon of epithelial–mesenchymal transition (EMT), a phenotypic switching of epithelial into fibroblast-like cells [1,16]. Interestingly, a recent study shows that HRG suppresses EMT in vivo [17]. We speculate that this suppressive effect might be compromised in IPF patients since they show low levels of plasma HRG. Future studies, however, are needed to define the possible participation of HRG in the pathogenesis of IPF.

A weakness of our study is the modest group of patients included. Our observations should be validated in future studies using a large cohort of patients, to better define the clinical utility of HRG as a biomarker in IPF.

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References

[1] P.J. Wolters, H.R. Collard, K.D. Jones, Pathogenesis of idiopathic pulmonary fibrosis, Annu. Rev. Pathol. 9 (2014) 157–179.
[2] W.A. Wuyts, K.M. Antoniou, K. Borensztajn, et al., Combination therapy: the future of management for idiopathic pulmonary fibrosis? Lancet Respir. Med. 2 (2014) 933–942.
[3] I.K.H. Poon, K.K. Patel, D.S. Davis, et al., Histidine-rich glycoprotein: the Swiss Army knife of mammalian plasma, Blood 117 (2011) 2093–2101.
[4] S. Wakabayashi, New insights into the functions of histidine-rich glycoprotein, Int. Rev. Cell Mol. Biol. 304 (2013) 467–493.
[5] M.P. Steele, D.A. Schwartz, Molecular mechanisms in progressive idiopathic pulmonary fibrosis, Annu. Rev. Med. 64 (2013) 265–276.
[6] R.P. Baughman, Technical aspects of bronchoalveolar lavage: recommendations for a standard procedure, Sem. Resp. Crit. Care Med. 28 (2007) 475–485.
[7] B.C. Hennis, D.I. Boomsma, K. Fijnvandraat, et al., Estrogens reduce plasma histidine-rich glycoprotein (HRG) levels in a dose-dependent way, Thromb. Haemost. 73 (1995) 484–487.
[8] M. Bolin, P. Akerud, A. Hansson, et al., Histidine-rich glycoprotein as an early biomarker of preeclampsia, Am. J. Hypertens. 24 (2011) 496–501.
[9] F.W. Leebeek, C. Kluft, E.A. Knot, et al., Histidine-rich glycoprotein is elevated in mild liver cirrhosis and decreased in moderate and severe liver cirrhosis, J. Lab. Clin. Med. 113 (1989) 493–497.
[10] K. Saigo, A. Yoshida, R. Ryo, et al., Histidine-rich glycoprotein as a negative acute phase reactant, Am. J. Hematol. 34 (1990) 149–150.
[11] F. Morell, A. Villar, M.A. Montero, X. Muñoz, T.V. Colby, S. Pipvath, M.J. Cruz, G. Raghu, Chronic hypersensitivity pneumonitis in patients diagnosed with idiopathic pulmonary fibrosis: a prospectivecase-vohort study, Lancet Resp. Med. 1 (2013) 685–694.
[12] S. Gordon, Alternative activation of macrophages, Nat. Rev. Immunol. 3 (2003) 23–35.
[13] C.D. Mills, Anatomy of a discovery: m1 and m2 macrophages, Front. Immunol. 6 (2015) 212–239.
[14] M. Stahl, J. Schupp, B. Jager, et al., Lung collagens perpetuate pulmonary fibrosis via CD204 and M2 macrophage activation, PLoS One 8 (2013) e81382.
[15] C. Rolny, M. Mazzone, S. Tugues, et al., HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF, Cancer Cell 19 (2011) 31–44.
[16] A. Gunther, M. Korfei, P. Mahavadi, et al., Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis, Eur. Resp. Rev. 21 (2012) 152–160.
[17] J. Cedervall, Y. Zhang, M. Ringvall, et al., HRG regulates tumor progression, epithelial to mesenchymal transition and metastasis via platelet-induced signaling in the pre-tumorigenic microenvironment, Angiogenesis 16 (2013) 889–902.