Measurement of protein HC (α_1 microglobulin) and protein HC-IgA complex in different body fluids

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SUMMARY Protein HC and protein HC-IgA complex were measured in 18 different types of fluid sample from healthy subjects and patients with different illnesses to determine if the concentrations of protein HC and protein HC-IgA complexes could be used to monitor certain diseases, when measured separately. The normal values for HC ranged from between 0.30 mg/l in saliva and 11.7 mg/l in blood plasma. HC-IgA complex has a greater range, from undetectable concentrations (urine, colostrum, and cervical mucus) up to 59.2 mg/l in blood plasma. Undetectable concentrations of HC-IgA complex were also shown in serum from patients with IgA immune deficiency and in cerebrospinal fluid from patients with multiple sclerosis. Increased concentrations of HC were noted in bronchoalveolar fluid from a patient with pulmonary alveolar proteinosis, serum from patients with Behcet's syndrome, and in synovial fluid from patients with gout, chondrocalcinosis, and rheumatoid arthritis. On the other hand, the concentrations of HC-IgA complex were raised only in those patients with pulmonary alveolar proteinosis or rheumatoid arthritis.

Human complex forming glycoprotein heterogeneous in charge (protein HC), also called α_1 -microglobulin, is a low molecular weight glycoprotein originally isolated from normal urine samples.¹ The protein is widely distributed in body fluids as free form and complexed with IgA (HC-IgA).^{1,2} The free protein displays considerable charge heterogeneity¹ and carries an unidentified yellow-brown chromophore which does not correspond to any previously described human chromophore,³ but no chromophore was found to be associated with the IgA complexed with protein HC.⁴

Both HC and HC-IgA complex have been shown to inhibit neutrophil chemotaxis against endotoxin activated serum in *in vitro* assays, suggesting that protein HC may have a physiological role in the regulation of inflammatory response.⁵ It has recently been reported that HC-IgA complex carries antibody activity and that it comprises three types of chains: two light immunoglobulin chains, one regular IgA α -chain, and one chain carrying both α -chain and protein HC epitopes.⁶

Since the characterisation of protein HC 10 years ago, all the immunotechniques used to quantify the concentrations of protein HC in body fluids have allowed only total protein HC (free HC and HC-IgA complex) to be measured.⁷⁻¹⁵

We reported the use of a crossed immunoelectrophoresis method,² and more recently a combined competitive and sandwich enzyme immunoassay for the simultaneous quantitation of HC and HC-IgA complex.¹⁶ This paper reports the concentration of free HC and HC-IgA complex in 18 different human body fluids from healthy subjects and patients with different illnesses.

Material and methods

Eight different body fluids from healthy adults and 10 body fluids from patients with different illnesses were analysed. A detailed description of the patients is given in table 1.

Blood was collected from registered donors by venepuncture in sterile tubes. Plasma was obtained in the presence of sodium heparinate (14 IU/ml) by centrifugation at 1500 $\times g$ for 10 minutes at room temperature.

Human colostrum was collected from healthy women just after having given birth. The samples were centrifuged for 30 minutes at 5000 $\times g$ to remove fat and cellular pellet. The colostrum was then immediately frozen until required.

Synovial fluid was collected at the time of a clinically indicated diagnostic arthrocentesis. All samples were obtained aseptically from the knee in heparinised tubes and treated with hyaluronidase (5 IU/ml) for 15

Table 1 Clinical characteristics of patients studied

Diagnosis	No of patients	Age range (years)	Sex (F/M)	Treatment and clinical state
Selective IgA deficiency	6	11-26	4/2	None, four patients with recurrent upper respiratory infections
Behcet's disease	7	27-49	6/1	None; active disease
Multiple sclerosis	4	29-51	3/1	None; relapsing-remitting disease
Liver cirrhosis	4	49-62	0/4	Non-steroidal drugs
Pulmonary alveolar proteinosis	1	15	1/0	Antibiotics
Degenerative joint disease	6	58-84	3/3	None
Acute gouty arthritis	5	44-68	0/5	Two patients were taking colchicine
Rheumatoid arthritis	8	40-61	5/3	Non-steroid drugs
Chondrocalcinosis	4	60-67	2/2	None

minutes at 37°C to reduce viscosity. Finally, samples were centrifuged at 1500 $\times$ g for 15 minutes at 4°C and the supernatant collected. To avoid the possibility of peripheral blood contamination, blood stained specimens were excluded.

Control synovial fluids were collected from four patients with traumatic synovitis during meniscectomy.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture as a part of clinical diagnostic procedures.

Bronchoalveolar fluid was obtained from bronchoalveolar washing performed by transnasal fiberoptic bronchoscopy in a patient with pulmonary alveolar proteinosis (PAP) and in an age matched control. The recovery of instilled fluid was 62% in the patient with PAP and 50% in the control. HC and HC-IgA complex concentrations are given in relation to volume of bronchoalveolar fluid recovered.

Saliva, seminal fluid, and cervical mucus samples were obtained from normal subjects and used without any further treatment.

ENZYME IMMUNOASSAY

Quantitation of HC and HC-IgA complex was carried out using a sensitive enzyme linked immunosorbent assay (ELISA) recently described.¹⁶ Briefly, the total amount of HC (free plus IgA-complexed) was measured by a competitive procedure while the HC-IgA complex was quantitated by a sandwich enzyme immunoassay. The amount of free HC was then obtained as the difference between the two measured

values. The sensitivity of the immunoassay was 0.07 mg/l for the total amount of HC and 0.08 mg/l for the HC-IgA complex. Both enzyme immunoassays were used to identify HC and HC-IgA complex during their isolation.

Results

The concentrations of HC and HC-IgA complex in body fluids from healthy subjects are shown in table 2. The concentration ratio between these two molecular species of HC showed great differences, depending on the sample tested. In blood plasma the mean concentration of HC and HC-IgA complex were 11.7 and 59.2 mg/l (ratio 1:5), respectively. These results were not significantly different from those obtained in serum samples. On the other hand, the mean concentrations obtained in saliva (0.30 mg/l of HC and 0.16 mg/l of HC-IgA complex), seminal plasma (5.20 mg/l of HC and 0.92 mg/l of HC-IgA complex), and bronchoalveolar fluid (0.55 mg/l of HC and 0.091 mg/l of HC-IgA complex) corresponded to HC:HC-IgA concentration ratios of 2:1, 6:1, and 6:1, respectively. In the samples of urine, colostrum, and cervical mucus, the concentrations of HC-IgA complex were undetected by the sandwich ELISA, and the mean values of HC were 3.87, 2.27, and 0.76 mg/l, respectively.

Samples from patients with different illnesses were also studied (table 3). Sera from patients with IgA immune deficiency presented a mean value of

Table 2 Protein HC and protein HC-IgA complex concentrations in normal body fluids

Sample	(n=)	Protein HC (mg/l)			HC-IgA complex (mg/l)		
		Mean	SD	Range	Mean	SD	Range
Blood plasma	10	11.7	5.96	4.13-25.4	59.2	25.7	21.9-95.6
Serum	20	9.60	3.40	4.80-21.4	52.4	35.8	25.3-118
Urine	20	3.87	1.48	2.14-6.28	<0.08		
Colostrum	4	2.27	0.81	1.46-3.59	<0.08		
Saliva	4	0.30	0.07	0.23-0.37	0.16	0.02	0.14-0.19
Seminal fluid	4	5.20	1.28	3.07-6.53	0.92	0.52	0.50-1.80
Cervical mucus	1	0.76			<0.08		
Bronchoalveolar fluid	1	0.55			0.091		

Table 3 Protein HC and protein HC-IgA complex concentrations in body fluids from patients with different illnesses

Diagnosis	Sample	(n =)	Protein HC (mg/l)			HC-IgA complex (mg/l)		
			Mean	SD	Range	Mean	SD	Range
IgA immunodeficiency	Serum	6	7.58	1.83	5.49–9.95	<0.08		
Behcet's syndrome	Serum	7	26.9*	10.3	13.9–38.6	13.6†	5.28	11.5–23.0
Multiple sclerosis	Cerebrospinal fluid	4	0.095	0.019	0.074–0.133	<0.08		
Liver cirrhosis	Ascitic fluid	4	3.01	1.22	1.53–4.93	4.70	3.63	1.20–9.60
Pulmonary alveolar proteinosis	Bronchoalveolar fluid	1	27.2			0.55		

*p < 0.001; †p < 0.01. Statistical significance calculated in relation with normal values in table 2.

7.58 mg/l of HC and undetectable concentrations of HC-IgA complex. The mean concentration of HC in sera from patients with Behcet's syndrome was 26.9 mg/l, three times higher than the normal value, while the mean concentration of HC-IgA complex was 13.6 mg/l, four times less than the normal value. The HC:HC-IgA concentration ratio (2:1) was therefore inverted in these patients.

Cerebrospinal fluid from patients with multiple sclerosis presented undetectable concentrations of HC-IgA complex and very low concentrations of HC (0.095 mg/l). Ascitic fluid from patients with alcoholic liver cirrhosis showed a mean value of 3.01 mg/l of HC and 4.70 mg/l of HC-IgA complex.

The most striking increase in both proteins was observed in bronchoalveolar fluid from a patient with PAP—27.2 mg/l of HC and 0.55 mg/l of HC-IgA complex, with a concentration ratio of 49:1.

The concentrations of both HC and HC-IgA complex in synovial fluid from patients with inflammatory and non-inflammatory joint diseases were also studied (table 4). Those with traumatic synovitis showed a mean value of 6.90 mg/l of HC and 6.24 mg/l of HC-IgA complex (ratio 1:1). These concentrations were taken as normal reference values for synovial fluid. We also analysed the synovial fluid from patients with degenerative joint disease—values of 9.20 mg/l of HC and 5.82 mg/l of HC-IgA complex, with a concentration ratio of 2:1.

Of the inflammatory joint diseases studied, patients with gout and chondrocalcinosis showed an HC:HC-IgA concentration ratio of 3:1, while in the patients with rheumatoid arthritis this ratio was inverted (1:1.8). In all cases the mean value of HC was between two and three times higher than that in non-inflammatory processes.

Discussion

The concentrations of protein HC and protein HC-IgA complex in body fluids from healthy subjects and patients with clinical disorders were measured by using a combined competitive and sandwich ELISA.¹⁶ Of normal samples of blood plasma and serum, the mean values of HC-IgA complex were higher than those of HC, reaching an HC:HC-IgA concentration ratio of 1:5, in other normal body fluids the concentra-

tions of HC-IgA complex were much lower (saliva, seminal fluid, and bronchoalveolar fluid) or undetectable (urine, colostrum, and cervical mucus) (table 2).

Interestingly, HC-IgA complex was not detected in samples rich in IgA concentrations which was surprising. In the case of urine, undetectable HC-IgA complex may have been attributable to the selective exclusion of large molecules due to the renal filter, but we could find no explanation for our inability to detect HC-IgA complex in the other samples.

An interesting difference between the concentrations of the two proteins was observed in the group of patients with Behcet's syndrome. It is known that these patients have, among other abnormalities, increased neutrophil and monocytic chemotactic activity. Hypothetically, an increased chemotactic response could be due to a depletion in the concentrations of chemotaxis inhibitors. We recently reported that protein HC and its IgA complex inhibits *in vitro* the normal neutrophil chemotaxis.⁵ The results showed that concentrations of HC tended to be high in patients with Behcet's syndrome (mean of 26.9 mg/l) in contrast to those of HC-IgA complex (mean of 13.6 mg/l). The reason for this discrepancy is unclear, and a greater understanding of the function of HC may be necessary.

On the other hand, we did not detect the presence of HC-IgA complex in the CSF from patients with multiple sclerosis. The most striking increase in HC was found in bronchoalveolar fluid from a patient with PAP (table 3) as there is an accumulation of phospholipids, immunoglobulins, and other serum proteins in the alveoli and distal airways of the lungs.¹⁷ The HC:HC-IgA concentration ratio in this patient was 49:1, very different from that of other body fluids.

The concentrations of HC and HC-IgA complex were also measured in synovial fluid from patients with inflammatory and non-inflammatory joint diseases (table 4).

Because the self-amplifying character of the inflammatory response seems to be mediated in part through chemotactic factors, it has been suggested that a chemotactic inhibitor might serve as a component of a postulated regulatory mechanism.¹⁸ The results showed that protein HC concentrations in patients with gout and chondrocalcinosis were the most

Table 4 Protein HC and protein HC-IgA complex concentrations in synovial fluid from patients with joint disease

Diagnosis	(n =)	Protein HC (mg/l)			HC-IgA complex (mg/l)		
		Mean	SD	Range	Mean	SD	Range
Traumatic synovitis	4	6.90	1.46	5.44-8.37	6.24	1.44	4.80-7.68
Degenerative joint disease	6	9.20	2.05	5.70-12.1	5.82	3.08	1.44-9.60
Acute gouty arthritis	5	14.7*	4.14	9.09-19.3	4.79	2.18	2.88-8.96
Rheumatoid arthritis	8	10.8	9.50	4.56-30.0	19.9*	8.80	8.32-33.2
Chondrocalcinosis	4	19.2*	6.75	10.5-28.0	5.92	0.80	4.80-7.04

*p < 0.01. Statistical significance calculated in relation to values for traumatic synovitis.

increased (about two to three times higher than those of non-inflammatory processes, traumatic synovitis, and degenerative joint disease). On the other hand, protein HC-IgA complex concentrations were almost invariant, between 4.79 and 6.24 mg/l, except in rheumatoid arthritis where the HC-IgA complex concentration reached up to 19.9 (SD 8.8) mg/l. Interestingly, about half the patients with a chronic form of rheumatoid arthritis have serum IgA above the normal range and some of the serum IgA is complexed covalently to α -globulins.¹⁹ Measurement of the concentrations of HC and HC-IgA complex in synovial fluids may therefore be a useful tool for discriminating between joint disorders.

In accordance with the findings of this study, the fluids can be grouped into four different categories. In the first, both components are present in high amounts, but the concentration of HC-IgA complex is two to five times higher than that of free HC (normal blood plasma and serum, ascitic fluid from patients with liver cirrhosis, and in synovial fluids of patients with rheumatoid arthritis). The second group also has a high concentration, but the concentration of free HC is higher than HC-IgA complex (serum of patients with Behcet's syndrome, synovial fluids from those with chondrocalcinosis, acute gouty arthritis, traumatic synovitis, and degenerative joint disease, and bronchoalveolar fluid from a patient with pulmonary alveolar proteinosis and normal seminal fluid). In the third, both components are present at a very low concentration, but free HC is also higher than HC-IgA complex (cerebrospinal fluid from patients with multiple sclerosis and saliva from a control). In the fourth group free HC only is present (normal urine, normal colostrum, normal cervical mucus and sera from patients with IgA immune deficiency).

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