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Caeruloplasmin concentration and oxidase activity in polyarthritis

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Summary. Caeruloplasmin (Cp) concentration and oxidase activity have been shown to be elevated in rheumatoid arthritis (RA) and psoriatic arthritis, but normal in Reiter's syndrome, Behcet's syndrome and juvenile seronegative polyarthritis. Synovial fluid Cp was significantly depressed in comparison with serum Cp in RA. During second-line therapy in RA, Cp concentration and activity fell significantly ($P < 0.001$), but the change in Cp did not correlate with plasma viscosity.

Key words: Caeruloplasmin concentration – Oxidase activity – Polyarthritis

Introduction

Caeruloplasmin (Cp) is a single-chain copper-containing α_2-glycoprotein, the synthesis of which is induced by tumour necrosis factor and by female sex steroids. Various biochemical properties have been attributed to Cp, including the transport of copper, iron mobilisation, oxidase activity (acting on iron, aromatic amines, phenols and ascorbate), acute phase reactant and radical scavenger. Many workers have demonstrated elevated serum Cp concentrations in rheumatoid arthritis (RA) [1–3] and in ankylosing spondylitis (AS) [2–4], but Cp concentrations in other seronegative arthropathies have not been extensively investigated. Cp concentrations in synovial fluid (SF) from patients with RA are elevated relative to those seen in Osteoarthritis SF [5, 6] and this increase is greater than expected in relation to other proteins. Serum Cp concentrations also increase in normal women and female patients with RA who are taking oral contraceptives [7, 8].

There is less information on the enzymatic status of Cp in different arthropathies. Immunological methods are generally employed to determine Cp concentration, but various enzyme activities of Cp can also be determined. RA SF ferroxidase and ascorbate oxidase activities were thought to be markedly depressed relative to Cp concentration, the discrepancy in RA serum being much less apparent [9]. However, the results for ferroxidase activity have since been shown to be artifactual, there being a fall in activity during sample storage [10]. However, Cp has oxidase activity against other substrates, including aromatic amines and phenols. NADH, ascorbate and reducing agents are pseudosubstrates which can be oxidised indirectly by coupling to the oxidation of aromatic amines. Cp concentration in relation to oxidase activity against aromatic amines has not been investigated in RA or in seronegative arthropathies, nor has the change in any discrepancy been monitored serially, particularly in relation to the action of second-line therapy which may be expected to lower Cp concentration in RA. This may be of particular relevance if free radical generation or redox status of Cp Cu atoms affect oxidase activity.

This paper seeks to expand our knowledge of Cp in rheumatology by comparing Cp concentrations and oxidase activity in different arthropathies, in serum and SF in RA, and during serial change arising under the influence of second-line drugs. In addition, the relationship between Cp and the acute-phase response was investigated in RA by comparison with plasma viscosity, arguably the best global measure of the acute-phase response [11].

Materials and methods

Blood samples were collected from patients referred to an outpatient rheumatology clinic. All patients therefore had severe disease according to the various criteria listed below. Inevi-

tably this spectrum of diseases did not permit age and sex matching of patients. The control group comprised 72 normal volunteers free of all clinical symptoms and drug therapy. The disease groups were as follows:

1. Fifty-six patients with classical or definite RA (ARA criteria) with active disease as indicated by a Ritchie articular index > 20 and an ESR > 28 mm/h
2. Ten patients with psoriatic arthritis (PsA) as defined by undoubted psoriasis plus joint pain and inflammatory polyarthritis involving at least three joints
3. Seven patients with Reiter's syndrome (ARA criteria) involving an episode of peripheral arthritis lasting at least 1 month and occurring in conjunction with urethritis and/or cervicitis
4. Ten patients with Behcet's syndrome (Japanese criteria) with at least three of the following present: oral ulceration, skin lesions, eye lesions, genital ulceration; patients also had arthritic symptoms severe enough to require the attention of a rheumatologist
5. Thirteen patients with juvenile seronegative polyarthritis (JPA)

From each patient a 10-ml clotted blood sample was obtained during attendance at a morning clinic. Samples were centrifuged (550 g), and the serum separated and stored at −20 °C to await analysis. In addition, synovial fluid samples were obtained from the knees of 14 patients with RA, and these were also spun and stored at −20 °C. Furthermore, 32 of the 56 patients with RA were about to start second-line therapy and a further blood sample was obtained after 24 weeks therapy in these patients. Therapy included hydroxychloroquine (200 mg b.d.), sulphasalazine (2 g/day), D-penicillamine (125 mg/day rising to 500 mg/day after 8 weeks), and aurothiomalate (50 mg monthly).

Caeruloplasmin assays. Paired samples (i.e. serum and SF, or week 0 and week 24 for the same patient) were always analysed together to eliminate interrun variation. Cp concentration was determined by radial immunodiffusion [12] using plates prepared by the addition of 1 ml of human-Cp antiserum (Hoechst-Behring) to 14 ml of agar solution (1% agarose in barbiturate buffer, pH 8.6). A calibration curve was obtained using appropriate dilutions of a protein standard serum (Hoechst-Behring). Samples were diluted 50 : 50 with distilled water.

Cp oxidase activity against aromatic amines was determined by the action of Cp on p-phenylenediamine [13]. The reaction was allowed to proceed at pH 5.5 at 37 °C for 1 h, terminated by the addition of sodium azide, and read at 530 nm. Activity results were converted to apparent concentration units by a factor determined from the oxidase activity of a known concentration of normal human Cp standard.

The effect of one freeze/thaw cycle on the results obtained from both assays was determined by the analysis of 10 fresh patient samples followed by storage at −20 °C for 1 week and then re-analysis. The effect of storage on the oxidase activity was monitored for 6 months using pooled patient serum. The fresh serum was analysed in sextuplet and the mean result noted. Aliquots were then frozen at −20 °C. At four weekly intervals an aliquot was thawed and analysed in sextuplet.

Plasma viscosity. Plasma viscosity was determined for the 32 patients with RA proceeding on to second-line therapy using a Harkness capillary viscometer (precision < 1%).

Statistical analysis. Cp concentration and activity were compared with the mean results obtained for the normal controls using a one-sample *t*-test. Concentration was compared with activity in each disease using a two-sample *t*-test. Serum and SF results were compared in a similar manner. Serial changes in results during second-line therapy were also compared using a paired *t*-test. Pearson correlations were used to look for any relationship between plasma viscosity and Cp, and between Cp concentration and activity.

Results

The two assay methods for Cp were found to be reproducible and one freeze/thaw cycle resulted in negligible loss in Cp concentration and only a 2.0% loss of oxidase activity. However, samples stored at −20 °C for as little as 8 weeks were found to lose oxidase activity (Table 1). There was no further degradation up to 20 weeks of storage.

The serum concentration and activity of Cp in the different disease groups and the normal controls are shown in Table 2. Patients with RA showed increased Cp concentration and activity, and in addition the activity was significantly depressed in relation to the concentration by a mean of 9% in agreement with the loss incurred through storage. A similar significant elevation was seen in PsA. Surprisingly, patients with Reiter's disease had normal Cp concentrations but increased oxidase activity. Patients with Behcet's and JPA showed normal levels. However, the latter group strictly require a younger control group for comparison. In all disease groups the correlation between Cp concentration and activity was statistically significant.

The comparison of RA serum and SF results is shown in Table 3. In this subgroup there was no difference between concentration and activity in serum or in SF, though the SF levels were significantly lower than serum levels.

Table 4 illustrates a significant decrease in both Cp concentration and activity after 24 weeks of therapy with second-line drugs. The fall in Cp does not correlate with the concomitant fall in PV but the fall in Cp concentration correlates reason-

Table 1. Loss of oxidase activity upon storage of serum at −20 °C

Week	Mean	SD	CV (%)	Change from baseline (%)
0	0.519	0.014	2.7	0.0
4	0.564	0.021	3.7	+ 8.7
8	0.433	0.006	1.4	− 16.6
12	0.464	0.0075	1.6	− 10.6
16	0.470	0.013	2.8	− 9.4
20	0.455	0.010	2.2	− 12.3

Table 2. Caeruloplasmin concentration and oxidase activity in different arthritides. NS = not significant ($P > 0.05$)

Disease	n	Concentration (g/l)		"Activity" (g/l)		Concentration vs activity	
		Mean (SD)	P value[a]	Mean (SD)	P value[a]	Two-sample t-test P value	Correlation (P value)
Normals	72	0.39 (0.14)	–	0.37 (0.06)	–	NS	0.030 (NS)
RA	56	0.54 (0.12)	< 0.001	0.49 (0.09)	< 0.001	0.01	0.364 (0.01)
PsA	10	0.50 (0.11)	0.02	0.46 (0.11)	0.02	NS	0.706 (0.05)
Reiter's	7	0.39 (0.09)	NS	0.52 (0.10)	0.006	0.03	0.895 (0.01)
Behçet's	10	0.44 (0.13)	NS	0.40 (0.13)	NS	NS	0.937 (0.001)
JPA	13	0.44 (0.13)	NS	0.40 (0.09)	NS	NS	0.852 (0.001)

[a] One sample t-test against mean result for normal controls

Table 3. Caeruloplasmin concentration and oxidase activity in paired serum and synovial fluid samples in RA ($n = 14$). NS = not significant ($P > 0.05$)

	Concentration (g/l)	"Activity" (g/l)	$P <$
Serum	0.51	0.51	NS
SF	0.29	0.30	NS
$P <$	0.01	0.01	

Table 4a, b. Changes in serum caeruloplasmin activity and concentration in RA during treatment with second-line therapy. NS = not significant ($P > 0.05$)

a All drugs ($n = 32$)

Week	Concentration	Activity
0	0.54 (0.11)	0.48 (0.09)
24	0.47 (0.10)	0.43 (0.09)
$P <$	0.001	0.001

b D-penicillamine ($n = 8$)

Week	Concentration	Activity
0	0.59 (0.11)	0.43 (0.07)
24	0.50 (0.07)	0.39 (0.06)
$P <$	0.05	NS

ably with the fall in activity (Table 5). However, change in the activity is not entirely mirrored by change in concentration as only 31% of the variance is accounted for.

Discussion

The results presented here for serum Cp concentration in RA agree with those of previous workers. Levels were higher than those seen in normal controls or in seronegative arthropathies. The difference between serum concentration and oxidase activity in RA was accounted for by the loss of activity on sample storage. For the seronegative diseases it is interesting that PsA appears to have elevated concentrations while Reiter's syndrome, Behcet's syndrome, and juvenile polyarthritis have normal levels. Although the number of patients involved are small, these results are generally in agreement with another recent patient series [14].

Our results for SF demonstrate lower levels of CP compared with serum, but do not show a difference between concentration and activity in agreement with Gutteridge et al. [10] rather than Blake et al. [9]. Blake et al. investigated ferroxidase and ascorbate oxidase, while we have explored an aromatic amine as a substrate. Different oxidase activities may depend on the availability of different co-factors or may be affected by changes in the copper content of the Cp, or by changes in the redox status of crucial copper ions in the Cp molecule. The latter is perhaps the most likely in RA SF where there are few mechanisms for safely mopping up free radicals such as superoxide and hydroxyl radicals.

Table 5. Correlation between changes in caeruloplasmin and plasma viscosity during second-line therapy. NS = not significant ($P > 0.05$)

Parameters	r	P
Cp concentration (week 0–week 24) vs PV (week 0–week 24)	0.179	NS
Cp activity (week 0–week 24) vs PV (week 0–week 24)	0.021	NS
Cp concentration (week 0–wk 24) vs Cp activity (week 0–week 24)	0.560	0.001

Clearly a more in-depth study of RA serum and SF Cp is required to elucidate which enzyme activities are altered.

The serial fall in serum Cp concentration and activity during successful second-line therapy might have been expected since Cp behaves as an acute-phase protein. In the case of the eight D-penicillamine-treated patients there may be some indirect modification of Cp as a consequence of the copper-chelating properties of the drug, but the serial Cp results in the eight patients were similar to those for other drugs. Measurement of Cp as a routine objective index of disease activity cannot be recommended as the changes seen are small despite achieving statistical significance. PV is probably the method of choice for a global assessment of the acute-phase response [10] and CRP is probably the most useful individual protein to measure [15]. Nevertheless, the role of Cp in the disease process, particularly in RA, may be of crucial importance and hence the various enzymatic activities of Cp may be worthy of further study.

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