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Ceruloplasmin Reduces the Adhesion and Scavenges Superoxide During the Interaction of Activated Polymorphonuclear Leukocytes with Endothelial Cells

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The plasma protein, ceruloplasmin, has been implicated as an anti-inflammatory agent, although this property has not been demonstrated unequivocally* in vivo. *The role of this protein in an* in vitro *system of cultured endothelial cells and polymorphonuclear leukocytes (PMNs) was investigated. One of the initial steps in an inflammatory response is increased adhesion between PMNs and the endothelial lining of the blood vessels. The results showed that ceruloplasmin interferes with this process and reduces the number of phorbol myristate acetate-activated leukocytes that adhere to endothelium. Preincubation of either the activated PMNs or the endothelium with ceruloplasmin did not produce the same results, suggesting that the continuous presence of ceruloplasmin is required. During attachment PMNs become activated and release a variety of substances, including toxic oxygen species such as superoxide and hydrogen peroxide. In the* in vitro *system used in this study no injury occurred to the endothelial cells, as measured by ^{51}Cr release, when activated PMNs were added with ceruloplasmin. The data show that ceruloplasmin reduced, in a dose dependent manner, the levels of superoxide produced by the activated PMNs, further supporting ceruloplasmin's previously reported role as a scavenger of superoxide. Ceruloplasmin also reduced the levels of superoxide when activated PMNs were in contact with endothelial cells. Although ceruloplasmin interfered with the copper-dependent scavenger enzyme, superoxide dismutase (SOD), in a cell-free system, ceruloplasmin had no effect on SOD in intact endothelial cells. These results suggest that ceruloplasmin may act as an anti-inflammatory agent by reducing the number of PMNs attaching to endothelium and by acting as an extracellular scavenger of superoxide. (Am J Pathol 1989, 135:647–655)

Ceruloplasmin was first described in 1948 by Holmberg and Laurell[1] as the blue plasma protein. This α-2 glycoprotein (mw, 130 kd) is the main copper metal binding protein in blood, binding upwards of 90% of total circulating copper.[2] Low levels of ceruloplasmin in blood can lead to abnormal copper metabolism, and the resulting excessive absorption of dietary copper, in particular by the liver and the brain, leads to Wilson's disease.[3,4] Elevated levels of ceruloplasmin are associated with increased serum and synovial fluid copper[5–7] and have been associated with inflammatory disease.[8]

Ceruloplasmin is normally present in blood at approximately 150 to 300 μg/ml[8] and can increase to as much as 900 μg/ml during inflammation.[9,10] The corresponding elevation in copper levels observed was believed to play a protective role,[5,6,11] a view supported by an enhanced inflammatory response in copper-deficient rats.[12] This protective role was believed to be due partly to the ability of copper to affect prostaglandin production.[13] Ceruloplasmin itself was recently described as an anti-inflammatory agent due to its ability to act as a scavenger of superoxide anion radicals, (O_2^-)[14,15] which can be generated by PMNs during inflammation and lead to tissue damage.[16]

Direct effects of copper were described *in vivo;* for example, it is required for angiogenesis.[17–19] Similarly, ceruloplasmin depleted of copper ions does not exhibit angiogenic activity, whereas fractions of ceruloplasmin with copper bound do.[20]

Increased polymorphonuclear leukocyte adhesion to the endothelial cells of the blood vessel wall is an early event in inflammation and is necessary for subsequent PMN migration between the endothelial cells into the connective tissue and towards the focus of injury. The adhesion event can be influenced by inflammatory mediators such as leukotriene B_4 and interleukin-1.[21–24] In this study

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we examined how ceruloplasmin affects PMN adhesion to endothelial cells and the superoxide scavenging capacity of ceruloplasmin during the interaction of activated PMNs with endothelial cells. Our results suggest that elevated levels of ceruloplasmin reduce the adhesion of activated PMNs to endothelial cells and reduce the levels of superoxide surrounding the cells.

Materials and Methods

Isolation of Neutrophils

Human PMNs were obtained by drawing 50 ml of venous blood into a syringe containing 10 U/ml heparin. The blood was mixed with 6% dextran (mw, 500 kd) at a ratio of 1:3 (dextran to blood) and allowed to stand for 30 to 45 minutes or until all the RBCs had settled. The supernatant was centrifuged at 7.5*g* for 5 minutes. The pellet was resuspended in 1 ml of distilled water for 30 seconds to lyse the remaining RBCs, followed by the addition of 10 ml of Hank's balanced salt solution, buffered with 10 mM HEPES, pH 7.4 (buffered HBSS). The suspension was centrifuged at 7.5*g* for 5 minutes, and the resulting pellet of PMNs was resuspended at the required concentration in buffered HBSS or test medium. The cell suspension contained more than 90% PMNs with less than one platelet per PMN. PMNs were activated by the addition of phorbol myristate acetate (PMA; 15 ng/ml[25]; Sigma Chemical Co., St. Louis, MO).

^{51}Cr Labeling of Neutrophils

Human PMNs (8×10^7/ml) were incubated with 100 uCi of ^{51}Cr (sodium chromate solution, 1 mCi/ml, New England Nuclear, Boston, MA) per ml of cells for 1 hour at 4 C. Buffered HBSS was added to the suspension and centrifuged at 7.5*g* for 5 minutes. The pellet was resuspended in buffered HBSS or test media at the desired concentration.

Culture of Endothelial Cells

Bovine pulmonary artery endothelial cells were isolated as described previously.[26] The cells were grown to confluence in DMEM + 5% FCS + 5% Nu Serum (Collaborative Research Inc, Bedford, MA) and split into 24-well cluster dishes (Gibco, Grand Island, NY). Cultures were incubated at 37 C in a humidified atmosphere in 5% CO_2 with twice weekly changes of culture media. Endothelial cells were characterized by their cobblestone morphology and by the presence of angiotensin-converting enzyme activity.

Adhesion Assay

Adhesion of PMNs to various substrata was assessed using a monolayer collection assay developed by Walther et al.[27] ^{51}Cr-labeled PMNs were suspended in test medium at 10^6 cells/ml. To each 16 mm diameter well of a 24-well cluster dish was added 0.5 ml, which was incubated at 37 C for 30 minutes. After incubation, the substratum was rinsed with buffered HBSS and attached PMNs were lysed with 1 N NH_4OH. Scintillation fluid (Aquasol, New England Nuclear, Boston, MA) was added to the samples and counted in a Beckman beta scintillation counter (Model LS-250). The percentage of adherent cells was calculated as a ratio of the radioactive counts initially added to each well. Incubation of PMNs with ceruloplasmin (Sigma Chemical Co., St. Louis, MO) did not decrease cell viability as determined by trypan blue exclusion. (The ceruloplasmin preparation was free from superoxide dismutase [SOD] contamination as confirmed by SDS polyacrylamide gel electrophoresis and Western blotting using a polyclonal anti-SOD antibody provided by Dr. Ian Burr, Department of Pediatrics, Vanderbilt University.) In some cases, PMNs, endothelial cells, or both were preincubated with ceruloplasmin for 30 minutes and rinsed twice with buffered HBSS before the adhesion assay. All materials used in the preparation of cells and in subsequent assays were endotoxin-free.

Cytotoxicity Assay

The effect of PMNs on endothelial cell integrity was determined with a ^{51}Cr release assay as described by Sacks *et al.*[16] Confluent cultures of endothelial cells were grown in dishes and incubated with ^{51}Cr for 2 hours at 37 C. Approximately 1% of the total label was incorporated by the endothelial cells. The PMNs were rinsed twice with buffered HBSS and then 0.5×10^6 PMNs were added to the cultures in 0.5 ml of the appropriate test medium and in the presence of PMA (15 ng/ml). The medium was removed after 90 minutes, and the endothelial cells were rinsed with buffered HBSS. The rinses were combined with the incubation medium and the amount of ^{51}Cr released from the endothelium was determined by liquid scintillation counting. In all experiments the monolayers were observed before and after the incubation periods to check visually for disruption of the monolayer that could account for an increase in ^{51}Cr release. In all cases monolayers remained intact throughout the experiment.

Superoxide Production

Superoxide (O_2^-) production was determined by measuring the reduction of ferricytochrome c as described by

Babior et al.[28] The cells were incubated in ferricytochrome c (0.9 mg/ml in buffered HBSS) for 90 minutes at 37 C. To stop the activity the assay dishes were placed on ice. The optical densities (550 nm) of the samples were compared with identical samples containing superoxide dismutase (EC 1.15.1.1) (Sigma) at 10 μg/ml, and the quantities of superoxide were determined using an extinction coefficient of 19.8 mM^{-1} cm^{-1}. PMA (Sigma) was added at 15 ng/ml to stimulate O_2^- release from the PMNs. Superoxide levels were also determined in the supernatants of cultures of endothelial cells to which PMA-stimulated PMNs were added and incubated with or without ceruloplasmin for 90 minutes.

Measurement of Superoxide Scavenging Activity

The superoxide scavenging activity of ceruloplasmin was measured in the manner described by Crapo et al[29] for superoxide dismutase. Ferricytochrome c (Cyt c Fe^{3+}) was reduced to ferrocytochrome c (Cyt c Fe^{2+}) by O_2^- generated from the action of xanthine oxidase on xanthine and measured spectrophotometrically at 550 nm. Inhibition of the reduction of cyt c was expressed as a percentage and compared with the inhibition caused by superoxide dismutase.

Analysis of Data

Multiple Student's *t*-tests were performed on the results, comparing data (adhesion and superoxide levels) at each concentration of ceruloplasmin to that in buffered HBSS alone. A Bonferroni correction was employed that considered the multiple comparisons performed. The results were significantly different from those obtained with the control ($P < 0.05$) for 600 μg/ml and 900 μg/ml ceruloplasmin.

In all figures, except Figure 2, a single comparison was made of adhesion and superoxide levels within the normal range of ceruloplasmin (300 μg/ml) to those within the elevated range of ceruloplasmin (900 μg/ml), as this is more physiologically relevant. The results of these analyses are shown in the figure legends.

Adhesion data in Figure 2 were pooled, using all the values in the normal range (100 μg/ml and 300 μg/ml ceruloplasmin, values not significantly different from one another) and all those in the elevated range (600 μg/ml and 900 μg/ml ceruloplasmin, values not significantly different from one another), and compared.

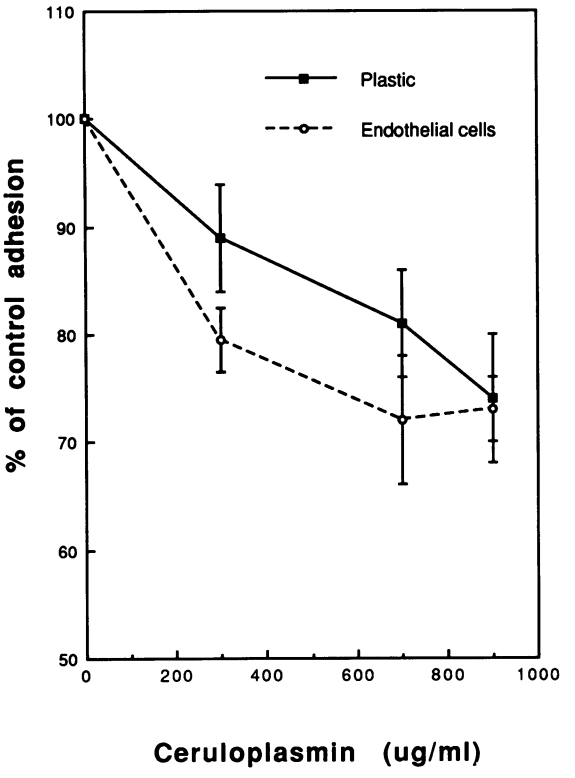

Figure 1. *Adhesion of PMNs to plastic and endothelial cells in ceruloplasmin expressed as a percentage of control. The bars represent standard errors. Absolute control adhesion: 27.5% ± 3.0% on endothelial cells (N = 9), 31.4% ± 3.7% on plastic (N = 9). Adhesion to plastic was significantly different in concentrations of ceruloplasmin of 300 and 900 μg/ml, compared by the Student's t-test, P < 0.01. Adhesion to endothelial cells was not significantly different at these concentrations.*

Results

Adhesion of PMNs

Adhesion of unstimulated PMNs to both endothelial monolayers and to tissue culture plastic was reduced in the presence of ceruloplasmin. Adhesion to tissue culture plastic decreased with increasing concentrations of ceruloplasmin up to 900 μg/ml. The adhesion of PMNs to endothelial monolayers was reduced in ceruloplasmin concentrations up to 700 μg/ml but did not appear to be reduced further at 900 μg/ml (Figure 1). Figure 2 shows that adhesion of PMA-stimulated PMNs to endothelial cells was significantly reduced in elevated ceruloplasmin concentrations compared with that in normal ceruloplasmin concentrations.

Preincubation of PMNs or Endothelial Cells with Ceruloplasmin

To determine whether ceruloplasmin must be present for inhibition of adhesion to occur and also to determine through which cell type the inhibition was acting, either or

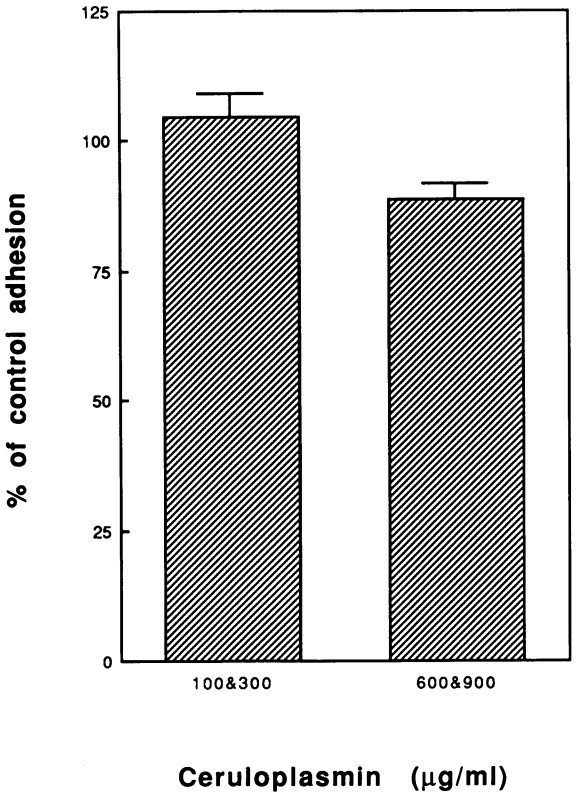

Figure 2. *The effect of ceruloplasmin on adhesion of PMA-stimulated PMNs to endothelial cells. The bars represent standard errors. Control adhesion = 50.8% ± 11.3% (N = 9). Adhesion data pooled for the normal range of ceruloplasmin, ie, 100 and 300 μg/ml (not significantly different from each other) were compared with those pooled in the elevated range ie, 600 and 900 μg/ml (not significantly different from each other) by the Student's t-test, P < 0.01. Regression analysis showed a significant negative correlation between ceruloplasmin concentration and adhesion; r = 0.80, P < 0.05.*

both cell types were preincubated with ceruloplasmin (0 to 900 μg/ml) for 30 minutes before the adhesion assay. In contrast to the decrease observed with ceruloplasmin present (Figure 1), when PMNs were preincubated with ceruloplasmin there was an increase in adhesion to tissue culture plastic and to endothelial cells compared with control levels (Figure 3). When endothelial cells were preincubated with ceruloplasmin in the same concentration range, a similar increase in PMN adhesion was observed. Incubating both cell types with ceruloplasmin before the adhesion assay resulted in an increase in adhesion of PMNs to endothelial cells. This increase in adhesion could be abolished by including ceruloplasmin (300 μg/ml) in the adhesion assay after preincubation. Under these conditions, the extent of adhesion was not different from that observed with controls ($P > 0.1$).

The Effect of Ceruloplasmin on Endothelial Cell Integrity in the Presence of Stimulated PMNs

Endothelial cell damage, as assessed by ^{51}Cr release, was measured in the presence of PMA-stimulated PMNs and increasing concentrations of ceruloplasmin. Stimulated PMNs alone caused no appreciable damage to the endothelial monolayers when measured after 90 minutes. Figure 4 shows that no damage occurred to the endothelial cell monolayers when ceruloplasmin was present. Cellular integrity was also monitored after an incubation period of 4 hours. Compared with spontaneous release (endothelial cells alone), the addition of PMNs and ceruloplasmin did not increase ^{51}Cr release (PMNs alone, 110.9% ± 4.9%; PMNs with ceruloplasmin [300 μg/ml], 112.2% ± 10.3%; PMNs with ceruloplasmin [900 μg/ml], 110.2% ± 5.3%).

Superoxide Production by Activated PMNs in the Presence of Ceruloplasmin

When PMA-stimulated PMNs alone were incubated with ceruloplasmin for 90 minutes and the supernatants measured for superoxide, ceruloplasmin appeared to act as a scavenger of O_2^- in that the levels of O_2^- were decreased compared with control levels, and increasing ceruloplasmin concentrations resulted in increased scavenging of O_2^- (Figure 5). In another set of experiments, when catalase (10 μg/ml) was added to the incubation mixture, there were no significant differences in the amounts of O_2^- detected at the end of the incubation period (300 μg/ml ceruloplasmin, 4.52 ± 0.2 nmols superoxide produced without catalase compared with 4.33 ± 0.2 nmols produced in the presence of catalase; correspondingly, 900 μg/ml ceruloplasmin, 3.74 ± 0.4 nmols produced without catalase compared with 3.61 nmols produced in the presence of catalase, $P > 0.7$).

Further evidence for the role of ceruloplasmin as a superoxide scavenger came from data showing the extent

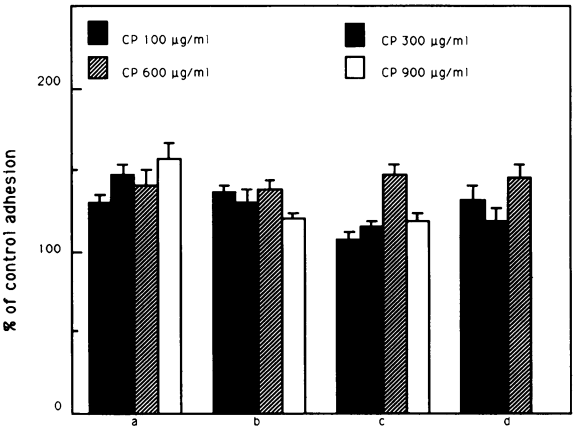

Figure 3. *The effects of preincubation of PMNs or endothelial cells in ceruloplasmin before an adhesion assay. The bars represent standard errors.* **a**: *PMNs preincubated then added to plastic;* **b**: *PMNs preincubated then added to endothelial cells;* **c**: *PMNs added to preincubated endothelial cells;* **d**: *Both PMNs and endothelial cells preincubated.*

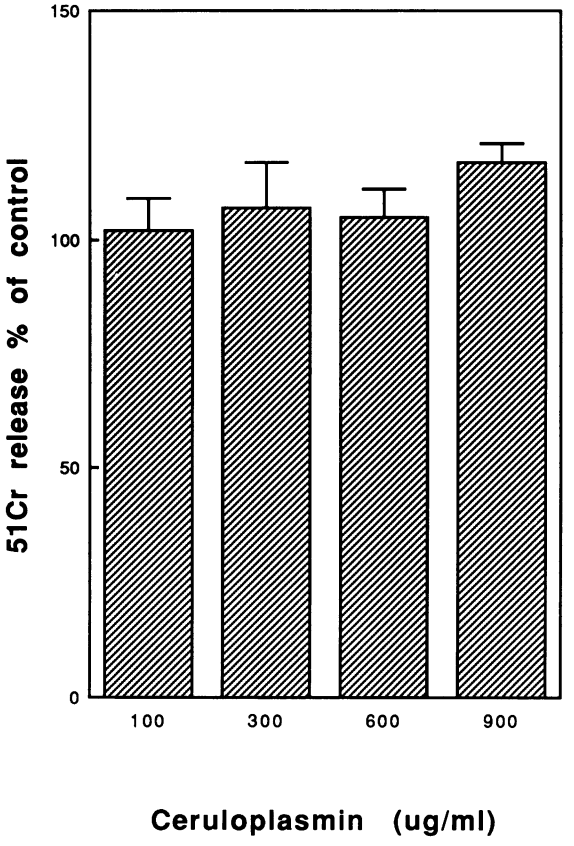

Figure 4. *^{51}Cr release from endothelial cells incubated with PMA-stimulated PMNs and ceruloplasmin. Results are expressed as a percentage of control (control release 7.8% ± 0.4%). The bars represent standard errors (N = 12). There was no difference in ^{51}Cr release in 900 μg/ml of ceruloplasmin and that in HBSS,* P > *0.1.*

of reduction of ferricytochrome c by ceruloplasmin compared with that of superoxide dismutase (Figure 6). Ceruloplasmin inhibited cyt c reduction in a dose-dependent manner.

Increased amounts of SOD (>200 ng) resulted in the complete inhibition of cyt c reduction. However, when ceruloplasmin and SOD were added together in a cell free system, their inhibitory activities were not additive (Figure 7). In a similar assay, preincubation of intact endothelial cells with ceruloplasmin did not affect endothelial cell SOD activity (inhibition of cyt c reduction was 34% after preincubation with ceruloplasmin *versus* 30% without ceruloplasmin; 100% inhibition was caused by 200 ng SOD).

Superoxide Levels During PMN: Endothelial Cell Interactions

PMA-stimulated PMNs were added to endothelial monolayers and incubated in the presence of ceruloplasmin. Figure 8 shows the levels of superoxide measured in the supernatants under these conditions. The results indicated that ceruloplasmin reduces the amount of superoxide present when activated PMNs interact with endothelial cells. Although ceruloplasmin appeared to be capable of scavenging O_2^- in this interaction, it is possible that the SOD activity of the endothelial cells contributed to reduced O_2^- levels. Whether ceruloplasmin can provide additional scavenging during the interaction of stimulated PMNs with endothelial cells, therefore, was investigated. PMNs were allowed to attach to the endothelium for 20 minutes at which point unattached PMNs were removed. This provided comparable situations in which equivalent numbers of PMNs were interacting with endothelial cells. After a further incubation for 90 minutes, levels of superoxide measured in the supernatant were decreased in the presence of ceruloplasmin (Figure 9) suggesting that any superoxide liberated into the medium was scavenged by ceruloplasmin.

Discussion

Ceruloplasmin levels increase *in vivo* under conditions of inflammation, and it has been suggested that this protein

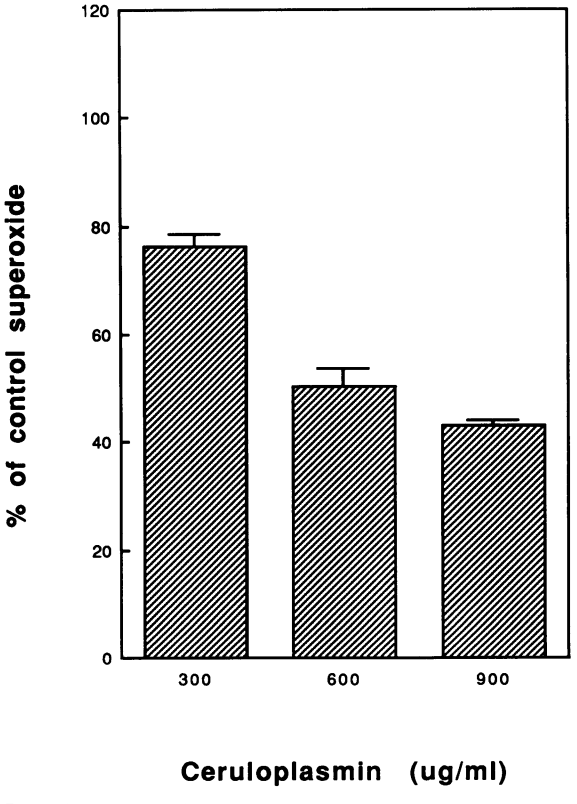

Figure 5. *Superoxide produced by PMA-activated PMNs incubated with ceruloplasmin for 90 minutes. Results are expressed as a percentage of control. Superoxide produced in control: 11.9 ± 0.3 nmols (N = 8). The bars represent standard errors. Superoxide production was decreased in elevated levels of ceruloplasmin comparing superoxide production in 300 μg/ml and 900 μg/ml,* P < *0.001.*

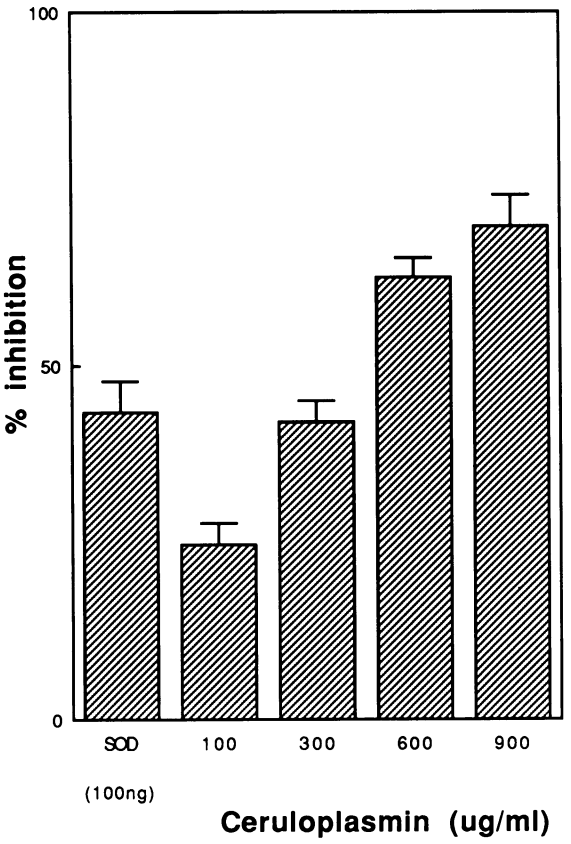

Figure 6. *Inhibition of cytochrome c reduction by ceruloplasmin compared with superoxide dismutase (100 ng). The bars represent standard errors. Inhibition increased with increasing concentrations of ceruloplasmin. Comparison of inhibitory activity of ceruloplasmin at concentrations of 300 and 900 μg/ml,* $P < 0.001$.

has anti-inflammatory properties. During inflammation, PMN:endothelial cell interactions are increased and activated PMNs produce toxic oxygen metabolites that not only kill invading bacteria but also contribute to the damage of surrounding tissue. We determined whether ceruloplasmin had any effect on PMN:endothelial cell adhesion and whether ceruloplasmin exhibited antioxidant activity during the interaction between PMNs and endothelial cells.

Ceruloplasmin reduced the amount of adhesion of both activated and nonactivated PMNs to endothelial cells. These findings agreed with previous work by Curtis and Forrester[30] who also showed that ceruloplasmin reduced adhesion of PMNs to tissue culture plastic. Receptors for ceruloplasmin were described on endothelial cells[31] from liver, but not on any other endothelial cells or thus far on PMNs. The inhibition of adhesion of PMNs to endothelial cells in the presence of ceruloplasmin does not appear to be receptor mediated, as preincubation of either cell type with ceruloplasmin did not result in a reduction of adhesion. The inhibitory effect of ceruloplasmin may simply be due to steric hindrance, changes in cell surface charge, or both.

Stimulated-PMN adhesion to endothelial cells was reduced in the presence of ceruloplasmin, and thus more superoxide was likely to be present in the supernatant. However, there was no apparent damage to the endothelial cells. Previous work by Hoover et al[32] showed that less superoxide is detectable in the media of activated PMNs when endothelial cells are present, a decrease that was attributed to the action of endothelial cell SOD. The same report also described how superoxide dismutase is more efficient in the detoxification of oxygen radicals when PMNs are attached to the endothelium as opposed to when they are in suspension. The results shown in Figure 4, therefore, may suggest that ceruloplasmin had an additional superoxide scavenging effect, resulting in protection of the cells from damage by superoxide in the supernatant. However, the same report by Hoover et al, stated that even when superoxide levels were increased, in this case with the addition of an SOD inhibitor, no damage occurred to the endothelial cells until inhibitors of catalase or glutathione peroxidase were added. In our study, therefore, although PMN adhesion was reduced in the presence of ceruloplasmin, the resulting increase in superoxide levels in the medium did not necessarily cause damage to the endothelium. Other workers showed that it is not superoxide as such that causes damage but its dismutated products.[33-35]

We, therefore, measured the effect of ceruloplasmin on the production of superoxide by PMNs under various conditions to determine whether ceruloplasmin scavenges superoxide *in vivo*. Measurements of superoxide produced by stimulated PMNs in the presence of cerulo-

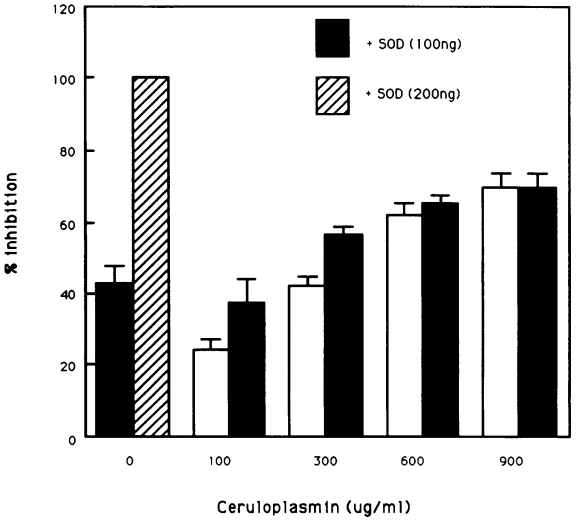

Figure 7. *Effect of ceruloplasmin combined with superoxide dismutase on inhibition of cytochrome c reduction. The bars represent standard errors (N = 4). At ceruloplasmin concentrations of 100 and 300 μg/ml, inhibitory activity of ceruloplasmin was significantly less than when ceruloplasmin was combined with 100 ng of SOD,* $P < 0.05$. *However, at ceruloplasmin concentrations of 600 and 900 μg/ml, the combined effect of ceruloplasmin and SOD was no greater than with ceruloplasmin alone,* $P > 0.1$.

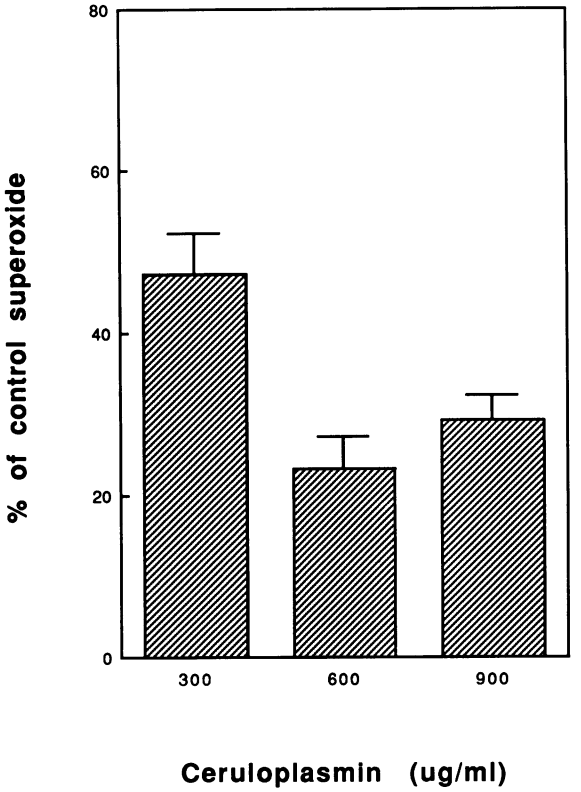

Figure 8. *Superoxide levels after incubation of activated PMNs with endothelial cells for 90 minutes in the presence of ceruloplasmin. The bars represent standard errors (N = 6). Results are expressed as a percentage of control. Superoxide produced in control: 6.3 ± 0.2 nmols. Production of superoxide was reduced in 900 μg/ml ceruloplasmin compared with that in 300 μg/ml ceruloplasmin,* $P < 0.01$.

plasmin suggested that this protein does indeed act as a scavenger of superoxide. Previous studies concerning the action of ceruloplasmin as a scavenger were carried out under cell free conditions.[14,15] Our results agreed with those in that ceruloplasmin reduced the levels of superoxide produced by activated PMNs. Results from the inhibition of cyt c reduction by ceruloplasmin also supported its role as a superoxide scavenger. Although ceruloplasmin was found to have SOD-like activity,[14,15] Bannister et al[36] reported that this inhibition of cyt c reduction is not a true dismutation as there is no increase in hydrogen peroxide levels. If this is true, then *in vivo* ceruloplasmin would not generate toxic dismutated products of O_2^- and its role as an anti-inflammatory agent would be enhanced. If, however, dismutated products are produced, they have to be dealt with by other intracellular enzymes such as catalase or glutathione peroxidase.

The results of the present study also indicated that ceruloplasmin reduces the levels of superoxide generated during the interaction between activated PMNs and endothelial cells. Thus, the superoxide scavenging activity of ceruloplasmin, which has been demonstrated previously in cell free systems, can be exhibited during PMN:endothelial cell interactions.

The increase in ceruloplasmin during inflammation may have two consequences: 1) to reduce the adhesion of activated PMNs to endothelial cells and 2) to act as an extracellular scavenger of superoxide. As a result of this, endothelial cell SOD does not become overloaded when a massive infiltration of PMNs occurs, as may be the case in an inflammatory response. During inflammation ceruloplasmin can rise to a level of up to 900 μg/ml. In our study, maximal reduction of adhesion occurred at 600 to 700 μg/ml, with maximum superoxide scavenging activity at 600 μg/ml, and was not significantly increased at higher concentrations. Perhaps *in vivo* this concentration is sufficiently high for ceruloplasmin to exert its beneficial effects.

From numerous reports of clinical cases in which inflammation is a factor, ceruloplasmin has been implicated as an anti-inflammatory agent due to the beneficial effects of copper.[13] In addition, administration of copper complexes has proved beneficial in the treatment of rheumatoid arthritis and in experimental inflammation.[37] Studies carried out on inflammation in copper-deficient animals supported this idea in that the inflammatory response was greatly increased in animals fed a copper-deficient diet.[12]

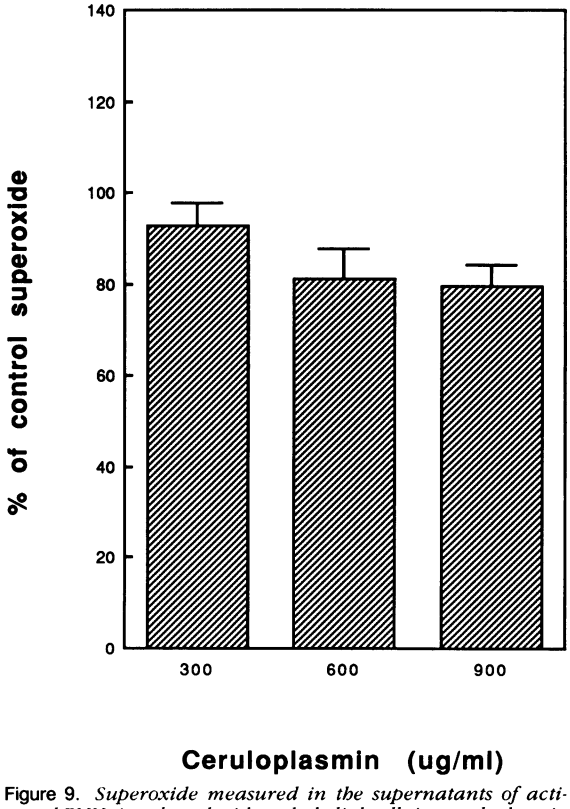

Figure 9. *Superoxide measured in the supernatants of activated PMNs incubated with endothelial cells in ceruloplasmin for 90 minutes after the removal of unattached PMNs. The bars represent standard errors (N = 9). Results are expressed as a percentage of control. Superoxide produced in control: 4.8 ± 0.3 nmols. Production of superoxide was significantly reduced in 900 μg/ml ceruloplasmin compared with that in 300 μg/ml ceruloplasmin,* $P < 0.05$.

Copper is also required by endothelial cell SOD, and increased inflammation in copper deficient animals has also been shown to be attributable to decreased SOD activity.[38] That there may be some competition for copper for the activity of both ceruloplasmin and endothelial cell SOD might be suggested from the results shown in Figure 7, where there appears to be some interference between SOD and ceruloplasmin activities. Preincubation of intact endothelial cells with ceruloplasmin, however, did not affect endothelial cell SOD activity under the same assay system.

Other mechanisms in which ceruloplasmin may be involved have been described. For example, there is evidence that hydroxyl radicals can be generated by protein-bound copper and that these hydroxyl radicals will react at their site of generation to cause site-specific protein damage;[39,40] however, intracellular catalase and glutathione peroxidase should reduce the amount of H_2O_2 so that this does not occur. The antioxidant activity of ceruloplasmin, as others showed,[41,42] was ascribed to its ferroxidase activity, which can inhibit ferrous ion-stimulated lipid peroxidation and ferrous ion-dependent formation of hydroxyl radicals in the Fenton reaction. This effect of ceruloplasmin was independent of its O_2^- scavenging activity. Ceruloplasmin has also been reported to prevent copper ions from stimulating lipid peroxidation.[41] Reports suggested that the antioxidant activity of serum from patients with rheumatoid arthritis is greater than that from normal controls,[5,43] whereas others have indicated that the ferroxidase activity and the ability to inhibit copper-stimulated lipid peroxidation are decreased in synovial fluid[44] and serum[45] taken from rheumatoid arthritis patients. Another report[46] suggested that rheumatoid arthritis serum has a higher antioxidant activity than normal serum for iron-induced damage, although it did not increase protection against copper-induced damage.

The precise role of ceruloplasmin in the inflammatory response remains unclear; however, the results presented here provide *in vitro* evidence for the ability of ceruloplasmin to reduce PMN:endothelial cell adhesion, which is increased in an inflammatory lesion, and to reduce the levels of the superoxide anion generated during this interaction.

References

1. Holmberg CG, Laurell CB: Investigations in serum copper II: Isolation of the copper containing protein and the description of some of its properties. Acta Chem Scand [B] 1948, 2:550–556
2. Poulick MD, Weiss ML: In The Plasma Proteins. Vol. 2. Structure and Function and Genetic Control. Edited by FW Putnam. 1975, pp. 51–508
3. Scheinberg IH, Gitlin D: Deficiency of ceruloplasmin in patients with Hepatolenticular degeneration (Wilson's disease). Science 1952, 114:484–485
4. Scheinberg IH, Morell AG: Exchange of ceruloplasmin copper with ionic Cu with reference to Wilson's disease. J Clin Invest 1957, 36:1193–1201
5. Scudder PR, Al-Timini D, McMurray W, White AG, Zocb BC, Dormandy TL: Serum copper and related variables in rheumatoid arthritis. Ann Rheum Dis 1978, 37:67–70
6. Scudder PR, McMurray W, White GA, Dormandy TL: Synovial fluid copper and related variables in rheumatoid and degenerative arthritis. Ann Rheum Dis 1978, 37:71–72
7. Jayson MIV, Davis P, Whicker JT, Walters G: Serum copper and ceruloplasmin in ankylosing spondylitis, systemic sclerosis and morphea. Ann Rheum Dis 1976, 35:443–445
8. Scheinberg IH, Morell AG: Exchange of ceruloplasmin copper with ionic Cu^{64} with reference to Wolson's disease. J Clin Invest 1957, 36:1193–1201
9. Conforti A, Franco L, Milanino R, Velo GP: Copper and ceruloplasmin concentrations during the acute inflammatory response in the rat. Agents Actions 1982, 12:303–307
10. Deshmuck VK, Raman PH, Dhuley JN, Naik SR: Role of ceruloplasmin in inflammation: Increased ceruloplasmin levels during inflammatory conditions and its possible relationship with anti-inflammatory agents. Pharmacol Res Commun 1985, 17:633–642
11. Sorenson JRJ: Evaluation of copper complexes as potential anti-arthritic drugs. J Pharm Pharmacol 1977, 29:450–452
12. Milanino R, Mazzoli S, Passarella E, Tarter G, Velo GP: Carageenan oedema in copper deficient rats. Agents Actions 1978, 8:618–622
13. Maddox IS: The role of copper in prostaglandin synthesis. Biochem Biophys Acta 1973, 306:74–82
14. Goldstein IM, Kaplan HB, Edelson HS, Weissman G: A new function for ceruloplasmin as an acute phase reactant in inflammation: A scavenger of superoxide anion radicals. Trans Assoc Am Physicians 1979, 92:360–369
15. Goldstein IM, Kaplan HB, Edelson HS, Weissman G: Ceruloplasmin: An acute phase reactant that scavenges oxygen derived free radicals. Ann NY Acad Sci 1982, 389:368–379
16. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacobs HS: Oxygen radicals mediate endothelial cell damage by complement stimulated granulocytes. J Clin Invest 1978, 61: 1161–1167
17. McAuslan BR: A new theory of neovascularisation based on identification of an angiogenic factor and its effects on cultured endothelial cells. EMBO Workshop on Specific Growth Factors, Rome 1979
18. McAuslan BR, Gole GA: Cellular and molecular mechanisms in angiogenesis. Trans Ophthalmol Soc UK 1980, 100:354–358
19. McAuslan BR, Reilly WG, Hannan GN, Gole GA: Angiogenic factors and their assay: Activity of formyl methionyl leucyl phenylalanine, adenosine diphosphate, heparin, copper and bovine endothelium stimulating factor. Microvasc Res 1983, 26:323–338
20. Raju KS, Alessandri G, Ziche M, Gullino PM: Ceruloplasmin, copper ions and angiogenesis. JNCI 1978, 69:1183–1188
21. Boxer LA, Allen JM, Baehner RL: Diminished polymorphonuclear leukocyte adherence: Function dependent on release

of cAMP by endothelial cells after stimulation of B-receptors by epinephrine. J Clin Invest 1980, 66:268–274

22. Hoover RL, Karnovsky MJ, Austen KF, Corey EJ, Lewis RA: Leukotriene B_4 action on endothelium mediates augmented neutrophil/endothelial adhesion. Proc Natl Acad Sci USA 1984, 81:2191–2195
23. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA: Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related cell lines. J Clin Invest 1985, 76:2003–2011
24. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA: Interleukin-1 activation of vascular endothelium: Effects on procoagulant activity and leukocyte adhesion. Am J Pathol 1985, 121:393–403
25. Lehrer RI, Cohen L: Receptor-mediated regulation of superoxide production in human neutrophils stimulated by phorbol myrisitate acetate. J Clin Invest 1981, 68:1314–1320
26. Bowman CM, Butler EN, Repine JE: Hyperoxia damages cultured endothelial cells causing increased neutrophils adherence. Am Rev Respir Dis 1983, 128:469–472
27. Walther BT, Ohman R, Roseman S: A quantitative assay for intercellular adhesion. Proc Natl Acad Sci USA 1973, 70: 1569–1573
28. Babior B, Kipnes R, Curmitte J: Biological defence mechanisms: The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 1973, 52:741–744
29. Crapo JD, McCord JM, Fridovich I: Preparation and assay of superoxide dismutases. Methods Enzymol 1978, 53:382–393
30. Curtis ASG, Forrester JV: The competitive effects of serum proteins on cell adhesion. J Cell Sci 1984, 71:17–35
31. Kataoka M, Tavassoli M: The role of liver endothelium in the binding and uptake of ceruloplasmin: Studies with colloidal gold probe. J Ultrastruct Mol Struct Res 1985, 90:194–202
32. Hoover RL, Robinson JM, Karnovsky MJ: Adhesion of polymorphonuclear leukocytes to endothelium enhances the efficiency of detoxification of oxygen free radicals. Am J Pathol 1987, 126:258–268
33. Weiss S, Young J, LoBuglio A, Slivka A, Nimeh N: Role of hydrogen peroxide in neutrophil mediated destruction of cultured endothelial cells. J Clin Invest 1981, 68:714–721
34. Harlan J, Levine J, Callahan K, Schwartz B, Harker L: Glutathione reductase cycle protects cultured endothelial cells against lysis by extracellular generated hydrogen peroxide. J Clin Invest 1984, 73:706–713
35. Hyslop PA, Hinshaw DB, Halsey WA et al: Mechanisms of oxidant mediated cell injury: The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. J Biol Chem 1983, 258:14778–14783
36. Bannister JV, Bannister WH, Hill HAO, Mahood JF, Willson RL, Wolfenden BS: Does caeruloplasmin dismute superoxide? No. FEBS Letters 1980, 118:127–129
37. Sorenson JRJ: Copper complexes: A unique class of antiarthritic drugs, Progress of Medicinal Chemistry, vol. 15. Edited by GP Ellis, GB West. Amsterdam, Elsevier/North Holland 1978, pp 221–260
38. Dameron CT, Harris ED: "Copper and the regulation of superoxide dismutase activity in aortic tissue," Biology of Copper Complexes. Edited by JRJ Sorenson. Clifton, New Jersey, Humana Press 1987, pp 43–55
39. Steveninck JV, Van der Zee J, Dubbleman TMAR: Site specific and bulk phase generation of hydroxyl radicals in the presence of cupric ions and thiol compounds. Biochem J 1985, 232:309–311
40. Samuni A, Chevion M, Czapski G: Unusual copper induced sensitization of the biological damage due to superoxide radicals. J Biol Chem 1981, 256:1665–1675
41. Gutteridge JMC: Antioxidant properties of caeruloplasmin towards iron- and copper-dependent oxygen radical formation. FEBS Letters 1983, 157:37–40
42. Yamashoji S, Kajimoto G: Antioxidant effect of caeruloplasmin on microsomal lipid preoxidation. FEBS Letters 1983, 152:168–170
43. Gutteridge JMC, Stocks J: Caeruloplasmin: Physiological and pathological perspectives. CRC Crit Rev Clin Lab Sci 1981, 14:257–329
44. Blake DR, Blann A, Bacon PA, Farr M, Gutteridge JMC, Halliwell B: Ferroxidase and ascorbate oxidase activities of caeruloplasmin in synovial fluid from rheumatoid arthritis patients. Clin Sci 1983, 64:551–553
45. Gutteridge JMC, Hill C, Blake DR: Copper stimulated phospholipid membrane peroxidation: antioxidant activity of serum and synovial fluid from patients with rheumatoid arthritis. Clin Chim Acta 1984, 139:85–90
46. Gutteridge JMC: Antioxidant properties of the proteins caeruloplasmin, albumin and transferrin: A study of their activity in serum and synovial fluid from patients with rheumatoid arthritis. Biochim Biophys Acta 1986, 869:119–127