Changes in Serum Ceruloplasmin Activity after Whole Body Irradiation of Mammals

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Chronological changes in ceruloplasmin oxidase activity after γ-irradiation with semilethal doses of 3–5 Gy, were investigated in four mammalian species; rat, guinea pig, lamb and pig. The ceruloplasmin activity increased soon after irradiation but later decreased. Although the extent of the increase and its time-course varied among species, it was most remarkable in rats and least so in guinea pigs, with the highest activity generally attained at 12 h after irradiation. In contrast, erythrocyte and leukocyte counts decreased after irradiation, and showed minimum values when ceruloplasmin levels were maximum. These results suggest that ceruloplasmin is involved in the recovery from radiation disease.

INTRODUCTION

Ceruloplasmin (ferroxidase I; iron (II)-oxygen oxidoreductase, EC 1.16.3.1) is a copper binding α-glycoprotein present in the plasma of vertebrates, and plays an important role in tissue angiogenesis, copper transport and iron metabolism. It is thought to be a family of acute phase proteins which are produced in the liver in response to tissue inflammation and injury, and then released into circulation to serve in the defense against inflammation. Furthermore, ceruloplasmin is a prominent serum antioxidant that can scavenge a variety of oxygen-derived free radicals.

Oxygen radicals produced by radiation cause acute inflammation and tissue injuries, which result in radiation diseases. Ceruloplasmin is known to be induced during the course of inflammation, which is partially mediated by tumor necrosis factor (TNF) and interleukin 1 (IL-1). Recently, it has been shown that these cytokines are induced in mouse cells immediately after irradiation. Because ceruloplasmin is able to scavenge oxygen radicals and also promote hemopoiesis, it may contribute to the recovery process after irradiation.

In the present study, we have examined changes in serum ceruloplasmin levels in four mammalian species, rat, guinea pig, lamb and pig, at different periods after a semilethal dose of γ-irradiation. For lambs and pigs, we also analyzed post-irradiation changes in blood cell counts as well as concentrations of several

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serum proteins to elucidate a possible role for ceruloplasmin in the recovery from acute radiation effects.

MATERIALS AND METHODS

Experimental animals

The experiments were carried out with adult male white rats, 12 weeks old, weighing 203±11 g, separated into 10 groups with 10 animals in each. Experiments were also performed using 2 groups (n=10) of male guinea pigs, 15 weeks old, average weight 210±19 g, 5 male lambs of the same breed aged 3 months and weighing 21±3 kg, 5 male little pig siblings weighing 18±3 kg and 3 months old.

Irradiation

Groups of animals were irradiated in a perforated Plexiglas chamber using Radiotherapy cobalt-60 unit Rocus-M (Russia), at a dose rate of 0.68 Gy/min with the following semilethal doses: rats; 5.2 Gy, a source-to-target distance of 0.75 m, guinea pig; 3 Gy, source-to-target distance 0.75 m, lambs; 3.2 Gy, source-to-target distance 1.5 m, pigs; 4 Gy, source-to-target distance 1.5 m. For homogeneous irradiation of the whole body of larger animals (pigs and lambs) a dose field of 0.3x0.5 m was created, and a source-to-target distance of 1.5 m was chosen. This geometry allowed for homogeneity of the dose field with 10% deviation at 0.2 m from the beam center. Furthermore, the first half of the irradiation was delivered to one side of the body, c.g., the right flank, and then the other half delivered to the left flank, by rotating the animal.

For small animals the target distance was 0.75 m by which homogeneity of the irradiation was achieved in a field of 0.2x0.2 m.

Dosimetry was performed using a strontium calibrated ionization chamber. Lethal dose, LD50/30, for animals was determined experimentally, according to the method described by Urbakh11. The LD50/30 values of rats and guinea pigs were estimated in preliminary experiments using 50 and 30 animals, respectively. The applied doses were of 2, 4, 6, 8 and 10 Gy with dose rate of 0.68 Gy/min. The LD50/30 used for pigs and lambs were derived from previous studies18,19.

Blood samples of the lambs were taken from v. jugularis, of the pigs from v. opthalmica, and of rats and guinea pigs after decapitation. All experiments with animals were performed at Thracia University, Faculty of Veterinary Medicine, Dept. of Radiobiology, Stara Zagora, Bulgaria, according to the Institutional regulations for work with experimental animals and “Guiding Principles in the Care and Use of Animals”. Animals were given sodium phenobarbital at 40 mg/kg i.p.

Analytical methods

Oxidase activity of ceruloplasmin in blood serum was measured according to the method of Schosinski et al.20. The method is based on the ability of ceruloplasmin to oxidize substrates such as o-dianizidine (3,3',-dimethoxybenzidine) yielding a yellow product. Briefly, 0.75 ml of 0.1 M acetate buffer, pH 5, in 2 tubes was mixed with 0.5 ml of serum sample and kept for 5 min at 30°C. To both tubes 0.2 ml of 0.25% o-dianizidine dihydrochloride was added and one mixture was incubated at 30°C for 5 min, and the other for 15 min. The reaction was stopped by adding 2 ml of 9 M sulfuric acid. The optical density was determined at 540 nm using Ultraspec III UV/Visible Spectrophotometer (Pharmacia LKB, Sweden).

The oxidase activity of ceruloplasmin was calculated as:
The concentration of ceruloplasmin in mg/l was estimated by a standard curve, based on the oxidase activity of pure human ceruloplasmin protein (Sigma Chemical Co, St. Louis, MO).

The serum protein fractions were separated by agarose gel electrophoresis and their percentages were determined by densitometrical measurement with CORNING Densitometer Type 710 (San Antonio, CA). The number of erythrocytes or leucocytes was counted using a standard haemocytometer chamber (Burker). The copper concentration in serum was measured by atomic absorption spectrophotometry with AAS-1M (Carl-Zeiss, Jena, Germany).

All reagents used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO), Merck (Darmstadt, Germany) and Fluka Chemie AG (Buchs, Switzerland).

**Statistical Analysis**

The data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using t-test with \( p \leq 0.05 \).

**RESULTS**

The results obtained with rats showed a chronological change in ceruloplasmin oxidase activity after γ-irradiation with LD50/30 of 5.2 Gy. Twelve hours after irradiation we observed a maximum oxidase activity of 74.3 U/l, compared to the control level of 40.9 U/l. Copper serum concentration showed a similar change: 16×10⁻⁶ M in control groups and 33×10⁻⁶ M at 12 h after irradiation. Later, these values slowly decreased and at 120 h they were 48.6 U/l and 16.9×10⁻⁶ M, respectively (Table 1).

Different and interesting results were obtained after irradiation of lambs with LD50/30 of 3 Gy. Soon after exposure oxidase activity of ceruloplasmin increased significantly from 22.7 U/l to 33.5 U/l at 12 h. This value remained unchanged for 10 days, and then decreased, reaching 29.4 U/l on the 30th day. The concentration of hemoglobin, erythrocytes and leucocytes decreased and were at minimum levels at around day 10, although the ceruloplasmin activity was maximum (Table 2).

In pigs exposed to LD50/30 of 4 Gy, acute primary radiation reactions occurred, namely, strong

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**Table 1.** Changes in ceruloplasmin oxidase activity after irradiation of rats with a semilethal dose LD50/30 of 5.2 Gy.

| Time after irradiation (h) | Control | 1 | 3 | 6 | 12 | 24 | 48 | 72 | 96 | 120 |
|---------------------------|---------|---|---|---|----|----|----|----|----|-----|
| n=10                      | 40.9±1.2| 56.5±2.4*| 57.8±2.4*| 56.2±2.3*| 74.3±3.3*| 63.8±2.6*| 62.6±1.4*| 61.2±2.3*| 50.7±2.1*| 48.6±2.2*|
| Ceruloplasmin (U/l)       |         |   |   |   |    |    |    |    |    |     |
| Serum copper (μmol/l)     | 16.0±0.1| 25.5±0.1*| 27.5±0.1*| 25.2±0.2*| 32.7±0.3*| 29.3±0.2*| 28.8±0.1*| 28.0±0.1*| 27.8±0.1*| 26.9±0.1*|

* n: number of animals  
* \( p \leq 0.05 \)
depression, tremor, rejection of food, strong thirst and fever. Oxidase activity of ceruloplasmin increased in a time dependent manner, from 44.4 U/l before irradiation to 63.4 U/l at 12 h after irradiation and decreased at day 3 and 14, although they were well above the control level. Erythrocyte and leukocyte counts decreased after irradiation, and the levels of some serum proteins showed similar changes to those in lambs (Table 3). The results with lambs and pigs obtained in the current study were similar to our previous observations of changes in glycoproteins after irradiation.

**Table 2.** Changes in ceruloplasmin oxidase activity and some serum criteria of lambs irradiated with a semilethal dose LD$_{50/30}$ of 3.2 Gy.

| Time after irradiation | Control | 30 min | 12 h | 40 h | 10 days | 17 days | 24 days | 30 days |
|------------------------|---------|--------|------|------|---------|---------|---------|---------|
| n=5                    | n=5     | n=5    | n=5  | n=5  | n=5     | n=5     | n=5     | n=5     |
| Ceruloplasmin, (U/l)   | 22.7 ±0.3 | 31.1 ±0.9* | 33.5 ±0.8* | 34.8 ±0.7* | 33.3 ±0.3* | 29.1 ±0.8* | 29.2 ±0.9* | 29.4 ±0.8* |
| Serum copper, (µ mol/l) | 18.7 ±0.3 | 20.0 ±0.2* | 27.6 ±0.7* | 26.7 ±0.3* | 27.8 ±0.8* | 23.4 ±1.1* | 23.0 ±0.8* | 24.4 ±0.7* |
| Serum protein, (g/l)   | 66.0 ±1.4 | 67.0 ±0.9 | 67.1 ±1.2 | 62.0 ±0.7 | 72.9 ±2.3 | 67.8 ±1.8 | 66.8 ±0.9 | 72.5 ±1.1 |
| Haemoglobin, (mmol/l)  | 5.04±0.08 | 4.96±0.07 | 3.94±0.11* | 3.92±0.09* | 3.94±0.06* | 3.71±0.10* | 4.81±0.08* | 5.13±0.11 |
| Erythrocytes, (10^12/l) | 10.36±0.27 | 10.02±0.09 | 5.64±0.10* | 5.35±0.17* | 5.64±0.20* | 8.13±0.19* | 9.01±0.11* | 10.11±0.21 |
| Leukocytes, (10^9/l)   | 8.40±0.09 | 8.20±0.11 | 2.32±0.29 | 2.32±0.18 | 2.32±0.09* | 2.70±0.51* | 3.60±0.32 | 5.25±0.19* |
| Albumins, (%)          | 52.3 ±0.9 | 50.5 ±1.1 | 50.3 ±1.0 | 47.7 ±2.1 | 43.2 ±1.7 | nd² | nd | nd |
| α-1 Globulins, (%)     | 8.5 ±0.4 | 8.7 ±0.7 | 9.7 ±0.5 | 9.8 ±0.8 | 9.7 ±0.6 | nd | nd | nd |
| α-2 Globulins, (%)     | 13.0 ±0.7 | 13.3 ±0.7 | 14.3 ±1.1 | 17.7 ±1.4* | 13.0 ±0.7 | nd | nd | nd |
| β Globulins, (%)       | 6.0 ±0.3 | 16.9 ±0.7* | 13.0 ±0.9* | 15.1 ±1.1* | 12.0 ±0.6* | nd | nd | nd |
| γ Globulins, (%)       | 20.1 ±0.4 | 10.1 ±0.1* | 12.7 ±0.7* | 13.1 ±0.7* | 22.0 ±0.5* | nd | nd | nd |

³n; number of animals

*nd: not done

* p ≤ 0.05

**Table 3.** Changes in ceruloplasmin oxidase activity and some serum criteria of pigs irradiated with a semilethal dose LD$_{50/30}$ of 4 Gy.

| Time after irradiation | Control | 12 h | 3 days | 14 days |
|------------------------|---------|------|--------|--------|
| n=5                    | n=5    | n=5  | n=5    | n=5    |
| Ceruloplasmin, (U/l)   | 44.4 ±2.3 | 63.4 ±3.5* | 53.0 ±0.3* | 53.8 ±6.5 |
| Serum copper, (µ mol/l) | 25.0 ±3.2 | 42.2 ±3.7* | 34.1 ±2.0* | 32.5 ±3.6 |
| Serum protein, (g/l)   | 48.0±2.56 | 45.5±1.45 | 47.4±0.91 | 47.8±0.99 |
| Erythrocytes, (10^12/l) | 5.83±0.49 | 4.57±0.10* | 3.76±0.13* | 2.47±0.63* |
| Leukocytes, (10^9/l)   | 19.9±1.10 | 7.70±0.12* | 3.20±0.10* | 2.00±0.32* |
| Albumins, (%)          | 35.0 ±0.6 | 34.0 ±0.9 | 34.3 ±1.2 | 25.1 ±1.2* |
| α-1 Globulins, (%)     | 8.2 ±0.6 | 9.3 ±0.4 | 6.5 ±1.0 | 6.7 ±0.7 |
| α-2 Globulins, (%)     | 21.3 ±0.5 | 21.1 ±0.3 | 25.4 ±0.4* | 24.2 ±0.6* |
| β Globulins, (%)       | 14.4 ±0.3 | 15.9 ±0.2* | 16.5 ±0.5* | 19.8 ±0.6* |
| γ Globulins, (%)       | 20.4 ±0.2 | 20.6 ±0.8 | 17.2 ±0.2* | 24.1 ±0.3* |

³n; number of animals

* p ≤ 0.05
In the experiment on dose-response in guinea pigs, we found that the dose of 3 Gy, corresponding to LD_{50,30}, caused a slight increase in serum ceruloplasmin activity at 12 h after irradiation (Table 4). Although this value was significantly higher than the control level, it is clear that guinea pigs were resistant to radiation in terms of induction of serum ceruloplasmin.

**DISCUSSION**

After exposure of animals to semilethal doses of ionizing radiation, radiation disease, i.e., an acute inflammatory process occurred. The results of the present experiments revealed increased levels of oxidase activity of ceruloplasmin soon after irradiation, suggesting that it is a radiation inducible protein at least in rats, lambs and pigs. So far as we are aware, this is the first report to show the induction of ceruloplasmin by radiation in mammals. It has been reported that superoxide dismutase activity in rat tissues increased after irradiation\(^{23}\) or exposure to radon\(^{24}\). These two antioxidant enzymes are thought to serve in the defense against oxidative stress in acute phase response.

In radiation exposed animals, generation and accumulation of oxygen radicals, peroxides and their toxic products appear to result in radiation injury. Ceruloplasmin is a circulating serum antioxidant, capable of scavenging a variety of oxygen-derived free radicals including •OH, \(\text{O}_2^-\) and • \(\text{O}_2\)\(^{\Delta}\). The mechanism by which electrons are transferred from these radicals to ceruloplasmin is not yet identified. It has been suggested that ceruloplasmin catalyses the conversion of • \(\text{O}_2\) to \(\text{H}_2\text{O}_2\) in a manner similar to superoxide dismutase\(^{8}\). It is also reported that ceruloplasmin inhibits lipid autooxidation, thus limiting the production of lipid peroxides\(^{22}\). Because ceruloplasmin possesses ferroxidase activity\(^{16}\), oxidation of \(\text{Fe}^{2+}\) to \(\text{Fe}^{3+}\) occurs with simultaneous reduction of \(\text{O}_2\) to \(\text{H}_2\text{O}\).

The present results clearly show that ceruloplasmin levels in rats and lambs increased within one hour after irradiation. This suggests that increased levels of ceruloplasmin function to eliminate oxygen radicals and their toxic products both in the early and later phases after irradiation. Because of its ferroxidase activity, this protein may also contribute to the erythropoiesis during recovery after irradiation. Ceruloplasmin molecules are thought to be released from the liver\(^{21,16,20}\), or result from de novo synthesis induced by cytokines like interleukin-1\(\beta\) (IL-1\(\beta\)), which is able to activate the synthesis of acute phase proteins in liver and other organs. Recently it has been reported that ionizing radiation caused induction of IL-1\(\beta\) gene in mouse macrophages\(^{15}\).

The experiments with bigger animals (lambs and pigs) were of particular interest, because we measured not only the induction of ceruloplasmin, but also the changes in hematological and biochemical data in

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**Table 4.** Changes of ceruloplasmin oxidase activity with radiation dose (dose-response dependence) in guinea pigs at 12 hour after irradiation.

| Dose, (Gy) | 0   | 1   | 2   | 3   | 4   | 5   | 7   | 10  |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|
| No. of animals | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |
| Ceruloplasmin (U/l) | 23.8±0.2 | 23.3±0.4 | 23.9±0.3 | 25.8±0.4* | 25.0±0.3* | 24.4±0.3 | 24.3±0.3 | 22.7±0.3* |

\(^{*}p \leq 0.05\)
serum samples. In both species, following irradiation with LD_{100}, oxidase activity of ceruloplasmin increased immediately, reached a maximum at about 12 h, and remained high for at least 10 days. Copper serum concentration increased in the same manner. In contrast, blood values of hemoglobin, erythrocytes and leucocytes decreased after irradiation, and showed minimum values at the time when ceruloplasmin level was maximum.

Such a rapid increase after irradiation resembled the response of typical acute phase proteins, i.e., serum globulin fractions, suggesting that ceruloplasmin is involved in preventing the deterioration of inflammation and other acute diseases caused by radiation. In this regard, it is worth noting that ceruloplasmin suppressed the production of active oxygen species by macrophages during the inflammation process. The present study was unable to reveal the direct participation of ceruloplasmin in the recovery from radiation disease.

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