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SYMPOSIUM

# Free radical properties of metal complexes+

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**ABSTRACT** Since metals are cable of catalysing the free radical reactions, different metal (Mg, Mn, Zn) compounds were studied to determine their hydroxyl radical generating and/or scavenging abilities depending on ligand in a $H_2O_2$/•OH -luminol system with or without microperoxidase. Magnesium oxide, magnesium gluconate and manganese gluconate increase the free radical reactions in the $H_2O_2$/•OH -luminol system. Most of examined metal compounds have different hydroxyl radical scavenging activity in $H_2O_2$/•OH- microperoxidase -luminol system. Magnesium citrate has the highest antioxidant effect, while manganese compounds seem to be prooxidant. Vitamin E and C elevate the free radical level in this experimental system.
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**KEY WORDS**

chemiluminescence
free radical generating ability
scavenging activity
magnesium, manganese and zinc compounds

In living organisms free radical formation and the free radical defense system are closely related to metal ions and complexes (Tampo and Yonaha 1992; Sigel and Sigel 1999). Fenton reaction describes Fe(II) induced hydroxyl radical formation (Fenton 1894, Haber and Willstätter 1931, Haber and Weiss 1934) and other transition metal ions (*e.g.* V, Cr) take part in Fenton-like reactions (Halliwell and Gutteridge 1990; Goldstein et al. 1993). Nevertheless, none transition metal ions also play a significant role, directly or indirectly, in free radical reactions. For example manganese, zinc and copper have a role in the antioxidant defense system (Zidenberger-Cherr and Keen 1991; Ebadi et al. 1996) and magnesium is present in every cell membrane in membrane ATP-ase and magnesium activates several enzymes which catalyse polyphosphate hydrolysis (Fox et al. 2001).

In the diseases where metal ion supplementation is neccessery, it is important to know the complex form (ligand) of the metal. The absorption of metal and other physicochemical properties can change, depending on the ligand (Hallberg et al. 1970; Kaltwasser et al. 1987; Rauch et al. 1990; Szentmihályi et al. 1998) and the physiological effect of the metal compound varies depending on the ligand as well.

Therefore, the hydroxyl radical generating ability and scavenging activity of some metal (Mg, Mn, Zn) compounds were studied by chemiluminescence method in the $H_2O_2$-luminol system with or without microperoxidase.

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## Materials and Methods

The weight ratio of vitamin E to vitamin C was 1:2, and that of vitamins to metals was 2:1 in the experimental system. Concentrations of the metal compounds in the basic solutions were 1 mg/ml. The compounds examined were of pharmacopoeal quality. Since the result is matrix dependent, the measurements were always made under same conditions (temperature, pH, concentration ratios).

Chemiluminescence techniques were applied for the studying of metal catalysis or scavenger activity. Light emission of luminol was measured by a method of Blázovics et al. (1999) using a Berthold Lumat LB-9501 luminometer. The intensity of chemiluminescence light is given as the relative light unit (RLU).

The reaction mixture for measuring free radical generating ability was as follows: hydrogen peroxide ($10^4$ dilution) 300 µl, luminol ($7x10^{-7}$ M) 100 µl, the sample (1 mg metal compound/ml solution) 50 µl and bidistilled water 250 µl.

The reaction mixture for measuring the hydroxyl scavenging activity was: hydrogen peroxide ($10^4$ dilution) 300 µl, microperoxidase ($3x10^{-7}$ M) 300 µl, luminol ($7x10^{-7}$ M) 50 µl and the sample (1mg/ml ) 100 µl diluted with bidestilled water to 1 ml. The intensity of chemiluminescence light is given as the relative light unit (RLU) reduced by free radical scavenging substances.

## Results and Discussion

Since the decomposition of luminol in the presence of hydrogen peroxide is catalysed by metal ions, some metal compounds were investigated for the catalytic activity of the reaction.

**Table 1.** Free radical generating or scavenging abilities of metal compounds (RLU %) and standard deviation (SD %) in the $H_2O_2$/•OH- luminol system.

| Samples | RLU % | SD % |
|---|---|---|
| Background int | 100 | |
| Magnesium oxi | 125 | 1.2 |
| Magnesium chl | 71.0 | 0.8 |
| Magnesium sul | 20.6 | 0.4 |
| Magnesium cit | 5.1 | 1.0 |
| Magnesium glu | 125 | 1.1 |
| Manganese dic | 26.8 | 1.3 |
| Manganese sul | 12.5 | 0.3 |
| Manganese glu | 102 | 1.5 |
| Zinc chloride | 4.9 | 0.6 |
| Zinc sulfate | 6.6 | 0.2 |
| Zinc citrate | 13.5 | 1.7 |
| Zinc gluconate | 6.0 | 1.4 |
| Vitamin E and C | 15.6 | 0.5 |

**Table 2.** Free radical generating or scavenging abilities of metal compounds (RLU %) and standard deviation (SD %) in the $H_2O_2$/•OH- microperoxidase-luminol system.

| Samples | RLU % | SD % |
|---|---|---|
| Background int | 100 | |
| Magnesium oxi | 177.4 | 0.9 |
| Magnesium chl | 104.7 | 0.9 |
| Magnesium sul | 106 | 0.3 |
| Magnesium cit | 2.3 | 2.5 |
| Magnesium glu | 388 | 4.9 |
| Manganese dic | 364 | 2.5 |
| Manganese sul | 366 | 0.4 |
| Manganese glu | 203 | 3.0 |
| Zinc chloride | 201.6 | 1.1 |
| Zinc sulfate | 82.6 | 0.1 |
| Zinc citrate | 74.3 | 3.6 |
| Zinc gluconate | 82.1 | 6.6 |
| Vitamin E and | 91.6 | 0.4 |

**Table 3**. Free radical generating or scavenging abilities of metal compounds in the presence of Vitamin E and C (RLU %) and standard deviation (SD %) in the $H_2O_2$/•OH-microperoxidase-luminol system

| Samples | RLU % | SD % |
|---|---|---|
| Background intensity | 100 | |
| Magnesium sulfate | 191.5 | 1.2 |
| Magnesium gluconate | 182.9 | 0.8 |
| Manganese sulfate | 240 | 0.3 |
| Manganese gluconate | 160.5 | 0.4 |
| Zinc sulfate | 95.9 | 0.1 |
| Zinc gluconate | 94.4 | 0.4 |

In the applied system and concentrations neither vitamins nor metal compounds (except for magnesium oxide, magnesium gluconate and manganese gluconate) generated the decomposition of luminol to aminophtalate in $H_2O_2$/•OH-luminol system (Table 1). When we applied the microperoxidase in the system ($H_2O_2$/•OH-microperoxidase-luminol) different metal compounds have different radical scavenging activity (Table 2). The metal ions, depending on the quality of the metal, ligand and concentration, may induce free radicals. Among magnesium compounds, only magnesium citrate has significant scavenging activity whereas other magnesium compounds enhance the free radical level formation. None of the manganese compounds have hydroxyl radical scavenging activity, while zinc compounds (except for zinc chloride) show scavenging activity. The mixture of vitamin E and C in the applied concentration has only a weak scavenging activity in this system.

The presence of vitamin E and C in the metal ion-containing solution changes the free radical scavenging activity. The vitamins increased the relative light unit (RLU %) of the system ($H_2O_2$/•OH-microperoxidase-luminol) together with magnesium sulfate, zinc sulfate and zinc gluconate solutions, while decreased free radical formation together with magnesium gluconate, manganese sulfate and manganese gluconate solutions (Table 3).

According to the results it has been stated that chemiluminescence measurement is an efficient method for the determination of free radical generating or scavenging activity of metal compounds as well.

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