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Lipid Peroxidation in Erythrocytes of Rats Fed a High Copper Diet

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Summary To study the effect of increased copper levels on erythrocyte lipid peroxidation, we fed a diet containing 2 g Cu/kg diet fed to normal and bile-duct ligated rats. No significant change was observed either in endogenous lipid peroxide content or in stimulated lipid peroxidation in the erythrocytes of rats with increased copper levels compared with the levels in control groups.

Key Words: lipid peroxidation, copper, erythrocyte

The high copper levels seen either in Wilson's disease or in copper toxicity have been reported to cause hemolytic anemia [1–3]. Although several studies have been done to detect the relation between copper and accelerated destruction of erythrocytes [4, 5], the mechanism of copper-induced hemolytic anemia is not well understood yet. The formation of fluorescent chromolipids, the end products of lipid peroxidation, has been reported in human erythrocytes incubated with copper ions [5]. In most of these studies, isolated systems and very high copper concentrations have been used.

In this study, we wanted to investigate the effect of moderately increased erythrocyte copper levels of endogenous and stimulated lipid peroxidation and to show if an oxidative mechanism is involved in this copper toxicity.

MATERIALS AND METHODS

Four groups of male Wistar rats weighing 200–250 g were used. Bile-duct ligated rats fed a normal diet for 15 days were used as controls (Groups I and III, respectively). Other normal and bile-duct ligated rats were fed a copper sup-

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plemented diet (2 g copper as copper sulphate added per kg diet) for 15 days (Groups II and IV, respectively).

Bile-duct ligation was done in ether-anesthetized rats according to Alemdaroğlu *et al.* [6] and was used to elevate copper levels in erythrocytes. Heparinized blood was taken by heart puncture from the rats following their decapitation. Erythrocytes were separated from the plasma by centrifugation and washed three times with 0.9% NaCl.

Plasma copper concentration was determined with "Böhringer Mannheim" kits. Erythrocyte copper levels were measured by the method of Gubler *et al.* [7]. Erythrocyte susceptibility to lipid peroxidation was determined according to Stocks and Dormandy's procedure [8]. Lipid peroxidation during a 1-h incubation period at 37°C was expressed in terms of malondialdehyde. The final composition of the incubation mixture was 6.5 mM H_2O_2, 1.6 mM sodium azide, and erythrocyte suspension in phosphate-buffered saline (14 mg Hb/incubation mixture). Values were expressed as nanomoles of malondialdehyde per gram of hemoglobin. Hemoglobin concentration of erythrocyte suspensions was measured with Drabkin's reagent. Erythrocyte glutathione (GSH) content was measured by the method of Beutler *et al.* [9].

Statistical significance between groups was determined using Student's *t*-test.

Table 1. Plasma and erythrocyte copper levels in normal and bile-duct ligated rats fed a normal diet or copper supplemented diet for 15 days.

Group	Plasma copper (μg Cu/100 ml plasma)	Erythrocyte copper (μg Cu/100 ml erythrocyte)
I	128.2±12.3	58.9±5.9
II	214.1±23.3*	86.6±9.5*
III	170.0±17.5	74.9±7.6
IV	386.1±51.3**	132.2±31.3**

Means±SD ($n=8$ each). *$p<0.001$ as compared with Group I; **$p<0.01$ as compared with Group III.

Table 2. Erythrocyte endogenous lipid peroxide, stimulated lipid peroxide and glutathione levels in normal and bile-duct ligated rats fed a normal diet or copper-supplemented diet for 15 days.

Group	Endogenous lipid peroxide	Stimulated lipid peroxide	Glutathione (mg/dl)
I	73.0±9.3	1,076.2±99.3	94.3±8.7
II	69.9±8.1	998.3±97.2	92.4±11.2
III	77.2±8.7	1,117.8±101.7	93.2±8.9
IV	78.6±9.7	1,110.7±87.5	96.1±9.9

Means±SD ($n=8$ each). Lipid peroxide levels are expressed in terms of nmol malondialdehyde/g Hb/h.

RESULTS AND DISCUSSION

Plasma and erythrocyte copper levels were increased significantly in Group II compared with Group I and in Group IV compared with Group III (Table 1). No significant change was observed either in endogenous lipid peroxide content or in stimulated lipid peroxidation of Groups II and IV compared with Groups I and III, respectively (Table 2). Erythrocyte GSH content was also found unaltered (Table 2).

Copper-induced lipid peroxidation as a possible cause of the destruction of erythrocytes has been reported in several studies [4, 5]. It has been proposed that copper ions catalyze a hydroperoxide-dependent free radical chain reaction which leads to the decomposition of unsaturated fatty acids of membrane phospholipids and thus to cause oxidative damage to the red cell membrane [4]. On the other hand, Piriou *et al.* [10] studied the lipid peroxidation in blood containing different concentrations of copper (0, 4×10^{-4} M, 12×10^{-4} M or 20×10^{-4} M) and they did not observe any induction of lipid peroxidation in erythrocytes incubated for 24 h even with the highest copper concentrations where they have observed hemolysis.

In our study, the erythrocyte copper concentration was elevated only 2–3 fold, as seen in Wilson's disease. Our model may be proposed to be a more natural system for the detection of the relation between copper and lipid peroxidation in erythrocytes, since we used "*in vivo*" conditions. We could not observe any induction either in erythrocyte susceptibility to lipid peroxidation or in endogenous lipid peroxide content in erythrocytes of rats with increased copper concentration.

As a result, we suggest that in the case of a 2–3 fold elevation in the erythrocyte copper level, copper-induced oxidative mechanism is not the cause of erythrocyte destruction. The antioxidant defense system of erythrocytes may play a protective role against the toxic effects of copper ions which have prooxidant activity.

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