EFFECT OF ZINC ON INCREASING OXYGEN AFFINITY OF SICKLE AND NORMAL RED BLOOD CELLS

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Summary--We have hypothesized a state of zinc deficiency in sickle cell disease (SCD). This could at least partially explain the growth problems, hypogonadism, and slow healing leg ulcers associated with SCD. Preliminary findings revealed abnormally low red blood cell zinc levels in 10 of 16 patients studied. Before suggesting zinc supplementation in SCD we thought it important to look at the effect of zinc on red cell metabolism and function. It was found that zinc chloride added to normal and SCD blood to a final concentration of 1.5×10^{-3} M caused a left-shift of the blood oxygen affinity curve (increased oxygen affinity) varying from 1.5 to 3.5 mm Hg change in half saturation (p50). This curve shifting property has important implications for SCD since recent work with cyanate suggests that such shifts are very beneficial in treatment of SCD. Thus zinc supplementation in SCD, in addition to its potential role in correcting wound healing and growth problems, may have a beneficial effect on the basic pathological process. Data are given which suggest that zinc and 2,3-diphosphoglycerate may not be competing for the same site on the hemoglobin molecule.

Zinc deficiency has been described in humans (1, 2). Several of the clinical symptoms associated with zinc deficiency such as growth retardation and hypogonadism are also associated with sickle cell disease (SCD) (3). Persons with SCD also have a tendency to develop leg ulcers which are refractory to healing and treatment. There are indications that zinc treatment is effective in accelerating wound healing (4). These observations led to the hypothesis that persons with SCD may be zinc-deficient as a result of hyperzincuria (5). Since the red blood cell (RBC) is a relatively rich source of zinc, the mechanism for the hyperzincuria may be the release of RBC zinc into the plasma via the hemolytic process associated with SCD. We have gathered preliminary

data indicating that RBC zinc levels in 10 of 16 SCD persons are low (5, and Prasad et al., unpublished). Because of this and because of our interest in RBC metabolism and function we initiated a study seeking possible effects of zinc on the RBC. In particular, since zinc is known to have a high binding affinity for histidine and since histidine residues have major roles in the biophysics of the hemoglobin molecule, we looked for possible effects of zinc on the oxygen affinity of blood from SCD patients. Herein we wish to report observations demonstrating that zinc produces a left-shift (i.e., increase in affinity) of the oxygen affinity curve for blood from both normal and SCD patients. The clinical implications of these observations will also be discussed. Further, studies demonstrating the lack of competition between zinc and 2,3-diphosphoglycerate (DPG) for binding sites on hemoglobin will be reported.

MATERIALS AND METHODS

Blood from persons with SCD or from normal subjects was drawn from antecubital veins either into heparinized "Vacutainer" tubes or into heparinized syringes. The SCD blood was obtained in Detroit and transported to Ann Arbor. Blood was chilled (4°C) until use and was never used when more than one day old.

For study of the effect of zinc on blood oxygen affinity, 1.0 ml of a zinc chloride-physiological saline solution was mixed with 4.0 ml of blood so that the final solution would be 1.5×10^{-3} M in zinc chloride. The hematocrit of the blood used was adjusted to about 37 volumes per cent (either by removal of plasma or by removal of RBC's) so that the hematocrit after zinc addition would be about 30 volumes per cent. This was done in order to make the blood samples comparable. The time of incubation before oxygen affinity measurement varied from 1 to 3 hours but averaged about 2 hours. Control samples were treated similarly with the exception that zinc chloride was not added to the physiological saline.

Blood oxygen affinity curves were obtained by tonometry of blood at two different oxygen tensions in the presence of 5.5% CO_2. The pH and partial pressure of oxygen (pO_2) of these blood samples were measured at 37°C with a Radiometer Blood Gas System. Hemoglobin saturation was determined by a Co-oximeter (Instrumentation Laboratories, Lexington, Mass.). The plot of % oxyhemoglobin against pO_2 was used to determine the p50 (i.e., the pressure of O_2 at which the hemoglobin is 50% oxygenated). The p50 was corrected to pH 7.4 (6).

For the study of possible competition between zinc and DPG we added 13.0 ml of 0.15 M bis Tris, pH 7.3 (at 37°C), to 26 ml of washed packed RBC's This solution was hemolyzed by freezing and thawing twice in a dry ice-acetone bath. After centrifugation at 48,200 g for 40 minutes the hemolysate was then divided into two 6 ml aliquots and 0.3 ml of a DPG solution added to each aliquot to give final DPG concentrations as reported in the Results section. Zinc chloride (0.2 ml, 4.87×10^{-2} M) was added to one aliquot and physiological saline (0.2 ml) added to the other. These aliquots were then refrozen and then thawed immediately before use so that the DPG levels would remain high. The hemoglobin levels of these final solutions were from 23 to 26 g/100 ml. The p50's were determined as with the whole blood samples with the modification that each sample was divided and one part was tonometered with O_2 and the other part with N_2. Proportions of the O_2 and N_2 tonometered samples were mixed to obtain points for p50 determinations (uncorrected for pH) much as before. This allowed us to straddle the p50 values.

DPG was determined by a method similar to that described by Keitt (7). In experiments employing zinc, weighed amounts of commercial zinc chloride (Baker Analyzed Reagent), assuming 100% purity, were used.

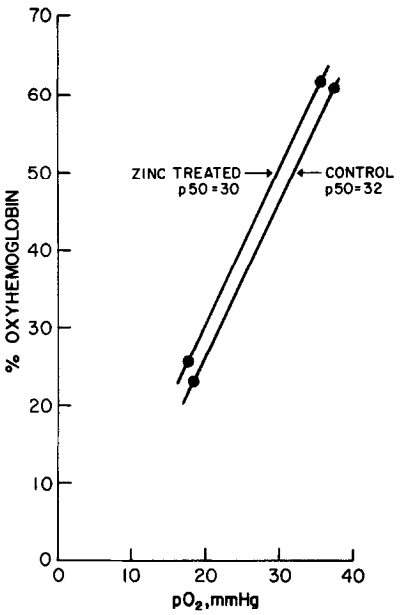

Figure 1. Effect of Zinc Chloride (1.5×10^{-3} M) on Oxygen Affinity Curve of Sickle Cell Blood (Hematocrit Adjusted to 30 Volumes Per Cent).

RESULTS

During our preliminary trials we found that very high levels of zinc chloride can lead to precipitation of plasma proteins, most likely the γ-globulin fraction. Further, high levels of zinc chloride can cause clumping of washed red cells. We settled on 1.5×10^{-3} M zinc chloride as a concentration which did not cause obvious abnormalities and which produced substantial effects.

Figure 1 demonstrates the left shift of the blood oxygen affinity curve observed when a solution of zinc chloride (1.5×10^{-3} M final concentration) was incubated with blood from a SCD patient. In this case a left shift in p50 of 2 mm was noted. There were differences in the degree of blood oxygen affinity changes noted in different SCD blood samples. With the eight SCD blood samples and three normal samples studied we noted decreases in p50 as given in Table I. The mean p50 decrease in SCD blood was 2.9 and in normal blood it was 2.5 mm Hg. The variability in the SCD samples may result partially

Table I. Oxygen Affinity Increasing Effects of Zinc on SCD Blood and Normal Blood

Blood Sample	p50 without zinc	Decrease in p50 with 1.5×10^{-3} M zinc chloride
SCD Patient Number:		
0022	32.0 mm Hg	2.0 mm Hg
0005	36.2	2.5
0027	33.0	1.5
0033	34.0	2.5
0004	28.7	1.9
0018	33.6	3.3
0014	32.5	6.0
0025	35.7	3.5
mean	33.2	2.9
Normal Blood Samples:		
TL	26.8	2.3
FO	26.0	2.7
NN	26.5	2.5
mean	26.4	2.5

from differences in pre-experiment levels of zinc and from other biological differences.

Zinc-DPG competition experiments were performed on hemolysates with and without zinc and with varying amounts of DPG. If zinc competes with DPG then the magnitude of the curve shift effected by zinc should lessen with increasing DPG levels. If zinc does not effectively compete with DPG then the magnitude of the curve shift will not be directly dependent upon DPG levels. The results of such studies as indicated in Table II are somewhat variable, but overall there is no apparent competitive effect of DPG with zinc.

DISCUSSION

We have shown that 1) some patients with SCD are zinc deficient in terms of RBC zinc (5), and 2) zinc has a left-shifting effect on the oxygen affinity curve of blood samples from both normal and SCD patients.

The clinical implication of these observations is that zinc supplementa-

Table II. Effect of DPG Levels on Curve Shifting Properties of Zinc (See text for details)

Experiment number	DPG levels, μmole/g hemoglobin	Δp50 due to zinc chloride, mm Hg
1	16.3	5.2
	26.4	5.1
	29.9	2.7
2	8.9	4.4
	22.8	5.0
	31.9	7.5
3	8.0	3.5
	23.1	5.0
	31.9	6.5
4	9.6	3.2
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	37.3	3.5
5	13.3	4.6
	27.3	5.8
	30.5	4.6
6	12.9	3.6
	26.2	3.9
	29.3	4.7

tion may be useful in the treatment of SCD. We have previously discussed the possible benefit of zinc supplementation in the amelioration of growth problems, hypogonadism and leg ulcers seen in some cases of SCD. Perhaps more importantly, the curve shifting property of zinc suggests that zinc may also beneficially effect the primary pathogenic process (i.e., the sickling phenomenon). Cyanate, which is presently believed to have great potential in the treatment of SCD (8) is thought to act by a mechanism of left-shifting the oxygen affinity curve. This, of course, allows for more oxyhemoglobin and presumably less sickling at any given blood pO_2. Present clinical trials of cyanate on patients with SCD suggest that small *in vivo* left-shifts of 2 mm or less can cause noticeable hematological improvement (8). Thus zinc with its left-shifting properties may have similar beneficial effects on the hematological course of SCD. Zinc in moderate oral doses is apparently non-toxic and can be taken for long periods without any apparent detrimental effects (4).

However, it remains to be seen whether zinc levels in SCD RBC's can be obtained in vivo high enough to cause significant curve shifts and if such levels have an effect on the hematological course of SCD.

The molecular basis of the curve shifting property of zinc is not known. There are at least two obvious types of sites where zinc binding to amino acid residues could cause a shift in the oxygen affinity curve. The first type of site is the DPG binding site which includes histidine-143, valine-1, and lysine-82 of the β chain of the hemoglobin molecule (9). If zinc binds here, its curve shifting effect should be less with increasing levels of DPG. Our competition experiments seem to exclude this binding mechanism. The second possible mechanism for a curve shifting effect would be the binding of zinc to an amino acid residue at one of the sites concerned with the Bohr effect, i.e., histidine-146 of the β chain, and histidine-122 and valine-1 of the α chain (9). We are presently carrying out experiments to test for this mechanism.

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