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Brief communication

In vitro effects of zinc gluconate and acetate on red cell aggregability

S. Khaled [a], J.F. Brun [a,*], G. Cassanas [b], L. Bardet [b], J. Mercier [a] and C. Préfaut [a]
[a] *Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie 34295 Montpellier-cédex 5, France*
[b] *Laboratoire de Physique Industrielle Pharmaceutique, Faculté de Pharmacie, 34060 Montpellier, France*

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1. Introduction

Erythrocyte aggregation is a reversible physiological process which plays an important role in blood rheological properties at low shear conditions [1,2]. Although emphasis is now given on red cell intrinsic factors [2], it is clear that the suspending medium has a key role in promoting interactions among adjacent cells [3], due to specific plasma proteins (fibrinogen and globulins) which have been reported to bind to the cell surface [4], although characteristics of this binding are poorly known [2]. While the bridging theory was generally believed to explain most of this aggregation process, there is an alternative hypothesis assuming that aggregation may rather result from electrostatic interactions explained by macromolecular depletion near the cell surface [5,6]. Red cells thus bind to form rouleaux, which may explain part of the Fåhraeus–Lindqvist effect and thus improve blood flow in terminal arterioles [2] while it is clear that they increase whole blood viscosity in veins and venules. Dissociation of rouleaux occurs when a shear stress is applied to the red cell suspension [7,8].

Studies have demonstrated that some bivalent cations can influence blood rheology by inducing alterations in red cell properties. In this respect, Ca^{2+} (at high concentrations) decreases red cell deformability [9], and increases red cell aggregation [10]; on the opposite, Zn^{2+} improves red cell deformability [11–18] but its effect on aggregation remains unknown.

However, there are preliminary results suggesting that zinc may modify red cell aggregability, since *in vivo* zinc supplementation has been shown to significantly decrease erythrocyte aggregation in healthy adult volunteers [19].

In order to determine whether red cell aggregation is directly influenced by zinc, we *in vitro* investigated the effect of increasing concentrations of two zinc salts versus a control buffer.

*Corresponding author: Dr. J.F. Brun, MD, PhD, Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme Musculaire (CERAMM), CHU Lapeyronie 34295 Montpellier-cédex 5, France. Tel.: +334 67 33 82 84; Fax: +334 67 33 89 66; Telex: CHR MONTP 480 766 F; E-mail: drjfbrun@aol.com.

2. Subjects and methods

Ten volunteer women (age 34.4 ± 2.75 yrs, weight 91.54 ± 5.37 kg, height 160.93 ± 2.62 cm, body mass index (BMI) 35.28 ± 1.78 kg/m^2) underwent a venepuncture for drawing 9 ml of blood after an overnight fast. Blood samples were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer) from a catheter set in the antecubital vein. Blood was divided into 9 aliquots (volume: 1 ml) in which we added (100 μl) increasing concentrations of zinc gluconate (0.25, 0.5, 1 and 2 mg/l) or zinc acetate (0.25, 0.5, 1 and 2 mg/l) or isotonic NaCl (9 g/l) as control medium. Incubation lasts 30 minutes and measurements were performed within three hours after venepuncture.

Erythrocyte aggregation is determined by the "Myrenne aggregometer" (manufactured by Myrenne Gmbh, Roetgen, W. Germany) at room temperature, at two differents shear rates. The aggregometer records the transmitted light among a sline red blood cell thickness. First the sample is submitted to a high shear rate (600 s^{-1}) which is the disaggregation phase. After ten seconds the shear rate abrupt stops, so the red blood cell aggregates in the stasis phase: this is the "M" measurement. The "M1" measurement is realized on the low shear phase which means that, first of all the sample is submitted to the high shear rate of 600 s^{-1} during ten seconds, and then shearing acutely decreases, resulting in aggregation of the red blood cells under conditions of low shear rate at 3 s^{-1} ("M1" > "M") [20].

2.1. Statistics

Statistical analysis was performed by repeated measures analysis of variance (ANOVA) and Wilcoxon signed-rank test for pairs. Results are presented as mean ± the SE of the mean. A value of $p < 0.05$ was considered as significant.

3. Results

Figure 1 shows the relationship between zinc concentration and the index of aggregation "M". Zinc salts (either with gluconate or acetate), have no effect at 0.25 mg/l (ANOVA, $p = 0.67$). However,

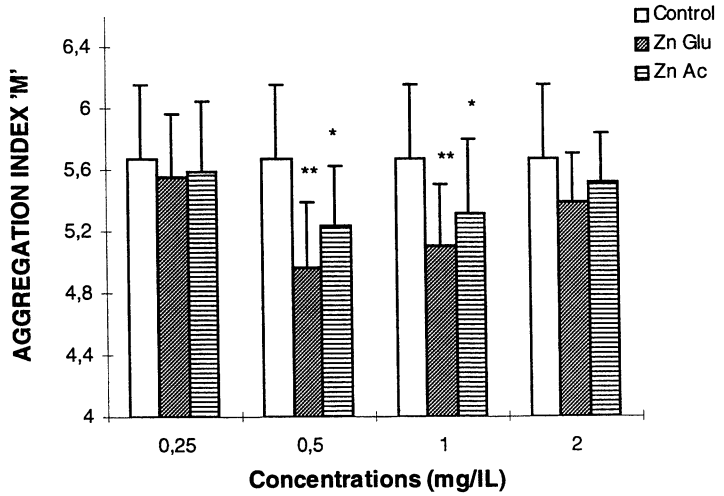

Fig. 1. *In vitro* effects of zinc gluconate and zinc acetate (compared to control) on aggregation index "M" ($^*p < 0.05$, $^{**}p < 0.01$).

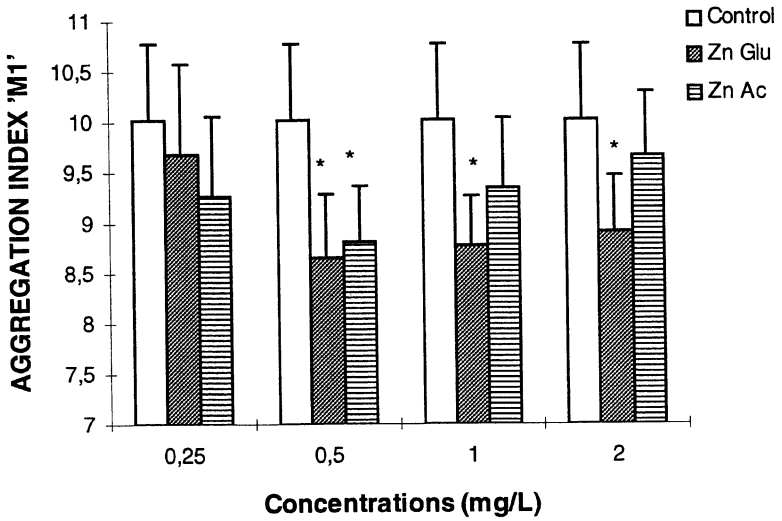

Fig. 2. *In vitro* effects of zinc gluconate and zinc acetate (compared to control) on aggregation index "M1" (*$p < 0.05$).

at 0.5 mg/l, both zinc salts compared to the control buffer, significantly decrease red cell aggregation (ANOVA, $p = 0.0015$). This decrease is more significant with zinc gluconate compared to zinc acetate (12.15%, $p < 0.01$ vs 7.64%, $p < 0.05$). At 1 mg/l, both zinc salts significantly decrease red cell aggregation (ANOVA, $p = 0.008$). For this concentration also, the decrease in aggregation is more significant with zinc gluconate compared to zinc acetate (10.2%, $p = 0.007$ vs 6.36%, $p = 0.032$). When the concentration of zinc reaches the supraphysiological level of 2 mg/l, the red cell aggregation index is no longer decreased by zinc addition, either with gluconate or acetate (ANOVA, $p = 0.54$).

As shown in Fig. 2 the index of aggregation "M1" is influenced by the concentration of zinc salts compared to the control buffer. At 0.25 mg/l, no effect of zinc (gluconate or acetate) can be detected (ANOVA, $p = 0.21$). When the concentration reaches 0.5 mg/l, both zinc salts significantly decrease the red cell aggregation index "M1" (ANOVA, $p = 0.0046$). At the concentration of 1 mg/l this effect is still observed (ANOVA, $p = 0.04$) but is only significant for gluconate which significantly decreases "M1" by 12.28% ($p < 0.05$). At 2 mg/l (ANOVA, $p = 0.097$), only zinc gluconate significantly decreases "M1" (9.8%, $p < 0.05$) while no effect of acetate can be detected.

4. Discussion

This study shows that red cell aggregation in conditions of stasis, as evaluated with the index "M", is decreased *in vitro* by zinc salts, at the concentrations of 0.5 and 1 mg/l while concentrations of 0.25 and 2 mg/l does not significantly modify aggregation. When red cell aggregability is stimulated by a low shear flow (index "M1") zinc gluconate decreases aggregation at concentrations lower than 0.25 mg/l, while the inhibitory effect of zinc acetate on aggregation becomes significant only at concentrations higher than 0.5 mg/l. Therefore, there appears to be a minimal threshold value below which zinc does not significantly influence aggregation, while at supraphysiological levels these effects of zinc become less apparent.

Our ten 10 blood samples were drawn from healthy women with some degree of overweight, keeping in mind that obesity increases blood viscosity and red cell aggregation [21,22]. Thus, we planned in this experiment to study the effects of zinc on moderately hyperaggregable erythrocytes.

Interestingly, zinc gluconate appears to exhibit a more significant effect (compared to acetate) on erythrocyte aggregation, consistent with our previous findings on zinc action on red cell deformability [16].

The red cell aggregation process is regulated by several factors, including shear rate, hematocrit, physico-chemical properties of the suspending medium, intrinsic properties of the red cell [23] and possibly, at least *in vivo*, interactions with the vessel wall [24]. In our study, two possible impacts of alterations in zinc concentrations may be involved in the effect on aggregation: first, the physico-chemical properties of the suspending fluid, and, on the other hand, properties of red cell membrane or cytoplasm.

A membrane effect can be hypothesized on the basis of previous studies showing that zinc may act on membranes by several ways. First, this cation influences enzymatic function of superoxide dismutase and glutathion peroxidase, which are involved in protector mechanisms against oxidative damage of cell structures [25]. This effect explains some of its protective effect in maintaining cell and tissue integrity.

Some investigations [11,15] have shown that zinc increases red cell filterability in sickle cell anemia, a situation where red cells are submitted to an overload of Ca^{2+}, by inhibiting the binding of Ca^{2+} to ATPase. This is consistent with the therapeutic use of orally administered zinc salts in sickle cell disease which is commonly used in some countries [12,13]. In addition, calcium at low concentrations exhibits important regulatory effects on membrane functions, while at high concentrations it induces marked disturbances of membrane functions [26] which play are associated with a decrease in red cell deformability [9]. It is also known that calcium and zinc exert antagonistic effects in most biological systems [26]. For instance, concerning more specifically blood rheology, we previously reported that zinc, *in vitro* and at physiological concentrations, improves the deformability of red cells artificially hardened with a hypercalcic buffer [16,17].

Results of this study could suggest that, like calcium, zinc, which circulates in plasma at concentrations close from those employed in our experiment, plays a role in the regulation of membrane functions, while at supraphysiological concentrations it may induce abnormal function of this membrane.

Alternatively, our results may reflect a calcium-antagonistic effect which reverses to some extent the adverse effects of calcium of erythrocyte rheological properties. An increase in red cell Ca^{2+} concentration induces hemoglobin binding to the cell membrane, which becomes stiffer [27]. Moreover, this binding modifies surface properties, resulting in increased aggregability of the cells [10]. At this level, zinc may thus compete with calcium, and thus reduce hemoglobin binding to the cell membrane. Such alterations in cell surface properties may modify the properties of binding sites involved in the process of aggregability.

Another explanation may be that divalent cations such as Ca^{2+} and Zn^{2+} modify physico-chemical properties of the suspending fluid. Ca^{2+} increases the interaction between plasma proteins (especially fibrinogen) and red cells, increasing aggregation [28]. Thus Zn^{2+} could decrease Ca^{2+} related interactions between plasma proteins and erythrocytes, resulting in a lower aggregability.

On the whole, this study shows that, consistent with *in vivo* findings, Zn^{2+} addition (which had previously been shown to increase erythrocyte deformability) decreases the aggregability of moderately hyperaggregable erythrocytes. The cellular mechanism of this effect of zinc remains to be investigated, and might be related to either a direct membrane effect, an alteration in hemoglobin–membrane interactions, or a modification of interactions between plasma proteins and the red cell membrane. To what extent this process is explained by an antagonism with calcium remains also to be investigated.

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