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Effects of Vitamin D on Erythrocyte Deformability in STZ-Induced Diabetes Mellitus

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Abstract

Objectives: Diabetes mellitus is a systemic metabolic disorder characterized by chronic hyperglycemia, which contributes to progressive cellular and vascular dysfunction and the development of microvascular and macrovascular complications. Given the role of altered erythrocyte mechanical properties in diabetic microvascular dysfunction, this study aimed to assess temporal changes in erythrocyte deformability and compare the effects of preventive and therapeutic vitamin D administration in streptozotocin-induced diabetic rats on days 7, 14 and 21.

Methods: The study included 64 male rats randomly allocated to four experimental groups: intact control, streptozotocin (STZ)-induced diabetic control, preventive vitamin D and therapeutic vitamin D groups. Experimental diabetes was induced by a single intraperitoneal injection of STZ (30 mg/kg). Vitamin D was administered orally at a dose of 300 IU/day, starting 14 days before STZ administration in the preventive group and on Day 3 after STZ administration in the therapeutic group. Erythrocyte deformability was assessed using a standardized microfiltration technique at three time points (Days 7, 14 and 21). Data were expressed as mean ± standard deviation and statistical significance was set at $p < 0.05$.

Results: Erythrocyte deformability progressively declined in STZ-induced diabetic rats, with a significant reduction in EDI by Day 14 ($p < 0.05$), reaching a 19.5% decrease by Day 21 compared with intact controls. Preventive vitamin D administration resulted in slightly higher EDI values compared with the diabetic control group, although the differences were not statistically significant. In contrast, therapeutic vitamin D administration significantly improved erythrocyte deformability, with EDI increasing by 7.55% on Day 14 and 16.2% on Day 21 compared with the STZ control group ($p < 0.05$).
Conclusions: STZ-induced diabetes progressively reduced erythrocyte deformability with minor changes on Day 7, significant deterioration by Day 14 and the most pronounced alterations on Day 21. Preventive vitamin D administration showed no significant protective effect, whereas therapeutic administration significantly improved deformability by Day 14, with greater improvement observed on Day 21.

KEYWORDS: Vitamin D; STZ-induced diabetes mellitus; Erythrocyte deformability; Preventive and therapeutic approaches.

Introduction

Diabetes mellitus is a systemic metabolic disorder characterized by chronic hyperglycemia, which, over time, contributes to progressive cellular and vascular dysfunction and the development of microvascular and macrovascular complications [1,2]. Alterations in blood rheological properties, together with endothelial dysfunction, are important pathophysiological components of diabetic vascular impairment, collectively compromising vascular homeostasis and microcirculatory blood flow [2-4]. A substantial body of evidence indicates that hyperglycemic conditions induce significant changes in blood rheological properties, with impaired erythrocyte mechanical properties, particularly reduced erythrocyte deformability, being consistently reported [5-7].

One of the principal mechanisms underlying these changes is hyperglycemia-induced oxidative stress, which can damage erythrocyte membrane lipids and proteins, thereby altering membrane structure, fluidity, and mechanical stability [8,9]. Hemoglobin auto-oxidation may further contribute to reactive oxygen species (ROS) generation, while oxidative modifications of erythrocyte membrane lipids and cytoskeletal proteins, including spectrin, may impair membrane integrity and reduce erythrocyte deformability [8,13,22].

It is worth noting that glucose transport into erythrocytes occurs independently of insulin [21]. Consequently, erythrocytes are directly exposed to circulating glucose concentrations, making them particularly susceptible to hyperglycemia-induced biochemical modifications [5,10,11]. One major consequence is the non-enzymatic glycation of hemoglobin as well as membrane-associated and cytoskeletal proteins [9,11,12]. Glycation-induced modification of key structural proteins can disrupt membrane-cytoskeleton interactions and cytoskeletal organization, thereby reducing membrane elasticity [11,12]. In parallel, increased glucose flux through the polyol pathway may contribute to erythrocyte metabolic disturbances under hyperglycemic conditions [7,14]. Aldose reductase utilizes NADPH during the conversion of glucose to sorbitol, potentially reducing NADPH availability for glutathione regeneration [14,23]. Because NADPH-dependent glutathione recycling is essential for maintaining erythrocyte redox homeostasis, alterations in these pathways may increase susceptibility to oxidative membrane damage [8,22]. Collectively, these interconnected hyperglycemia-associated processes promote structural and biophysical alterations in the erythrocyte membrane, driving a shift toward increased erythrocyte rigidity [8,12].

Vitamin D is a secosteroid with a broad range of biological functions beyond its classical role in calcium and bone homeostasis, including antioxidant, anti-inflammatory and immunomodulatory effects [17-19]. Despite growing interest in the pleiotropic effects of vitamin D, its influence on erythrocyte mechanical properties remains insufficiently characterized, particularly with regard to the temporal evolution of erythrocyte deformability and potential differences between preventive and therapeutic administration. Although our previous study reported erythrocyte deformability and the effects of preventive and therapeutic vitamin D administration at the Day 21 endpoint, it did not characterize the temporal development of these effects [20]. The present study extends this work by examining erythrocyte deformability at Days 7, 14 and 21 in an expanded analytical cohort, with particular emphasis on the time-dependent patterns associated with preventive and therapeutic supplementation.

Materials and Methods

1. Experimental Animals and Ethical Approval

Male laboratory rats, 10 weeks of age and weighing approximately 201.70 ± 8.48 g, were maintained under controlled environmental conditions (24 ± 1 °C, relative humidity 55 ± 5%, and a 12-h light/dark cycle) and received a standard diet formulated

for laboratory rats. Male animals were used to minimize potential variability related to sex-dependent hormonal influences and differences in susceptibility to streptozotocin (STZ). All experimental procedures were approved by the Institutional Animal Ethics Committee (Protocol No. 79) and were conducted in accordance with applicable guidelines for the care and use of laboratory animals.

2. Study Design and Experimental Groups

The animals were assigned to four experimental groups: an intact control group, an STZ-induced diabetic group without vitamin D supplementation, a preventive vitamin D group and a therapeutic vitamin D group. In the preventive group, vitamin D administration was initiated 14 days before STZ injection and continued throughout the experimental period. In the therapeutic group, supplementation was started on Day 3 after STZ administration, following confirmation of hyperglycemia and was maintained until the corresponding experimental endpoint.

A total of 64 rats were included in the study: Intact Control Group (n=10): Healthy animals that received no STZ or vitamin D supplementation and were administered sodium citrate buffer as the vehicle control. This group served as a common baseline reference for all observation time points.

STZ-Induced Diabetes Group: Evaluated at three observation time points (6 rats per time point: Days 7, 14 and 21).

Preventive Vitamin D Group: Vitamin D supplementation was initiated before STZ administration (6 rats per time point: Days 7, 14 and 21).

Therapeutic Vitamin D Group: Vitamin D administration was initiated after confirmation of hyperglycemia (6 rats per time point: Days 7, 14, and 21).

3. Induction of Experimental Diabetes

Experimental diabetes was induced in accordance with an established STZ-based protocol with modifications described previously [20]. Before STZ administration, the animals were fasted and subsequently received a single intraperitoneal injection of streptozotocin at a dose of 30 mg/kg. STZ was dissolved immediately before use in freshly prepared 50 mM sodium citrate buffer (pH 4.5) and administered within 10 min of preparation to minimize degradation. Control animals received an equivalent intraperitoneal injection of sodium citrate buffer without STZ. Following STZ administration, animals were provided with a 10% sucrose solution for 24 h. Development of diabetes was confirmed on Day 3 after injection by blood glucose measurement using a glucometer. Successful induction was defined by persistent hyperglycemia exceeding 19 mmol/L, accompanied by polyuria.

4. Vitamin D Administration

Vitamin D was administered orally once daily at a dose of 300 IU/day. In the preventive group, supplementation was initiated 14 days before STZ administration and continued throughout the experimental period. In the therapeutic group, vitamin D treatment was initiated on Day 3 after STZ injection, following confirmation of hyperglycemia and continued until the corresponding experimental endpoint. Vitamin D administration was discontinued 24 h before blood collection.

5. Assessment of Erythrocyte Deformability

Erythrocyte deformability was assessed using a standardized microfiltration technique through microporous membrane filters under controlled suction pressure. The erythrocyte deformability index (EDI,%) was recorded as the primary experimental measure of erythrocyte mechanical behavior. Lower EDI values indicate reduced erythrocyte deformability.

6. Statistical Analysis

Statistical analysis was performed using OriginPro software (version 8.1; OriginLab Corp., Northampton, MA, USA). The erythrocyte deformability index (EDI) is expressed as mean ± standard deviation (SD). Data normality within each group and observation time point was evaluated using the Shapiro-Wilk test and homogeneity of variances across groups was assessed using Levene's test. Statistical comparisons between the intact control group and experimental sub-groups across Days 7, 14 and 21 were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Pairwise comparisons between specific groups at individual time points were verified using Student's two-tailed independent t-test. Differences were considered statistically significant at a threshold of $p<0.05$.

Results

Erythrocyte deformability index (EDI) values varied across experimental groups and observation time points during the 21-day study period (Table 1). In the Intact Control Group , healthy baseline EDI was 2.46±0.17%.

In the untreated STZ Control Group, a progressive impairment of erythrocyte deformability was observed over time: On Day 7, EDI slightly decreased to 2.32±0.08% ($p>0.05$); On Day 14, EDI significantly declined to 2.12±0.09% ($p<0.05$ vs. Control); On Day 21, EDI reached its lowest value of 1.98±0.10% ($p<0.05$ vs. Control), repre-

senting a total reduction in deformability of approximately 19.5% relative to healthy controls.

In the preventive vitamin D group, EDI was higher than in the diabetic control group at all time points, but the differences were not statistically significant.

In the therapeutic vitamin D group, erythrocyte deformability improved significantly compared with the untreated diabetic control group. EDI was 7.55% higher on Day 14 and 16.2% higher on Day 21 than in the corresponding STZ control groups ($p < 0.05$).

TABLE 1. TEMPORAL DYNAMICS OF ERYTHROCYTE DEFORMABILITY INDEX (EDI) ACROSS EXPERIMENTAL GROUPS (MEAN ± SD)

Experimental Group	N	Time (Days)	EDI (%)
Intact Control	10	Baseline	2.46±0.17
STZ Control	6	Day 7	2.32±0.08
	6	Day 14	2.12±0.09*
	6	Day 21	1.98±0.10*
Preventive STZ + Vit D	6	Day 7	2.33±0.07
	6	Day 14	2.15±0.08*
	6	Day 21	2.00±0.08*
Therapeutic STZ + Vit D	6	Day 7	2.38±0.09
	6	Day 14	2.28±0.10**
	6	Day 21	2.30±0.12**

*Note: Data expressed as Mean ± SD. *p<0.05 vs. Intact Control; **p<0.05 vs. STZ Control at the same time point.*

Discussion

The present study evaluated temporal changes in erythrocyte deformability in an STZ-induced diabetic rat model and examined the effects of preventive and therapeutic oral vitamin D supplementation over a 21-day observation period. By assessing the erythrocyte deformability index (EDI) at three experimental time points, this study provides a more detailed characterization of erythrocyte mechanical alterations during the progression of experimental diabetes and following vitamin D administration.

Temporal Dynamics of Erythrocyte Biomechanical Impairment

A central finding of this investigation is the progressive, time-dependent reduction in erythrocyte deformability in untreated STZ-diabetic animals. In healthy intact controls, red blood cells maintain high mechanical compliance (EDI: 2.46±0.17), which is essential for negotiating capillaries whose diameters are smaller than those of relaxed erythrocytes [3, 4]. Following STZ administration, erythrocyte deformability showed a modest, statistically non-significant initial decline on Day 7 (EDI: 2.32 ± 0.08%). However, erythrocyte deformability decreased significantly by Day 14 (EDI: 2.12 ± 0.09%, $p < 0.05$ vs. intact control) and reached its lowest value on Day 21 (EDI: 1.98 ± 0.10%, $p < 0.05$ vs. intact control), representing an approximately 19.5% reduction relative to healthy controls. The reduction in erythrocyte deformability observed at Day 21 is consistent with our previously published findings at the same experimental endpoint [20]. However, the present study extends these observations by including additional animals and evaluating the temporal dynamics of erythrocyte deformability at Days 7, 14 and 21.

The progressive reduction in mean EDI values across the experimental time points suggests sustained impairment of erythrocyte mechanical properties under diabetic conditions [3,6]. Crucially, because mature erythrocytes lack nuclei and organelles, they possess limited synthetic capacity for protein repair or de novo phospholipid synthesis [5,10]. Consequently, molecular damage inflicted upon the erythrocyte membrane and cytoskeletal scaffold during its lifespan accumulates, leading to progressive cell stiffening [8,11].

Biochemical Mechanisms Underlying Impaired Erythrocyte Deformability in Diabetes Mellitus

The biomechanical compliance of the erythrocyte depends on three primary factors: intracellular hemoglobin viscosity, the surface area-to-volume ratio and the viscoelastic properties of the lipid-protein membrane complex [3,6,9]. Hyperglycemia disrupts these physiological parameters through several interacting biochemical pathways. Because glucose transport into erythrocytes occurs independently of insulin [21], intracellular glucose concentrations mirror plasma levels [5,11]. Sustained hyperglycemia promotes

non-enzymatic glycation of hemoglobin and erythrocyte membrane-associated proteins, while the formation of advanced glycation end-products (AGEs) may further alter membrane-cytoskeleton interactions, potentially compromising erythrocyte flexibility and deformability [9,11,12]. In addition to glycation, oxidative stress and lipid peroxidation contribute to erythrocyte membrane dysfunction. Auto-oxidation of elevated intracellular glucose and hemoglobin generates reactive oxygen species (ROS) [8,13], which promote lipid peroxidation of polyunsaturated fatty acids within the erythrocyte membrane bilayer, reducing membrane fluidity [8,9]. Furthermore, oxidative modifications of erythrocyte cytoskeletal proteins, including spectrin, may impair membrane integrity and deformability [8,22]. Concurrently, increased glucose flux through the aldose reductase polyol pathway may increase NADPH consumption, potentially impairing glutathione regeneration and weakening the erythrocyte's antioxidant defenses [14]. This intracellular redox imbalance further increases erythrocyte susceptibility to oxidative stress [8,14].

Differential Impact of Preventive vs. Therapeutic Vitamin D Administration

In the preventive vitamin D group, supplementation initiated 14 days before STZ injection did not significantly attenuate the decline in erythrocyte deformability compared with untreated diabetic controls. On Day 21, the EDI value in the preventive group (2.00 ± 0.08%) was comparable to that in the untreated STZ control group (1.98 ± 0.10%), with no statistically significant difference ($p > 0.05$). These findings suggest that preventive vitamin D supplementation may be insufficient to counteract erythrocyte alterations associated with sustained hyperglycemia under the present experimental conditions. One possible explanation is that persistent hyperglycemia-induced glycation and oxidative stress may contribute to erythrocyte membrane damage despite vitamin D supplementation [8,17]. However, these mechanisms were not directly evaluated in the present study. In contrast, therapeutic vitamin D administration initiated on Day 3 after STZ injection, following confirmation of hyperglycemia, was associated with significantly higher erythrocyte deformability compared with untreated diabetic controls. This effect was evident on Day 14 (EDI: 2.28 ± 0.10%, $p < 0.05$ vs. STZ control) and remained significant on Day 21 (EDI: 2.30 ± 0.12%, $p < 0.05$ vs. STZ control). These findings suggest that the effects of vitamin D supplementation on erythrocyte mechanical properties may depend on the timing of administration, although direct statistical comparisons between the preventive and therapeutic groups are required to confirm this difference.

The observed effects of therapeutic vitamin D administration may involve genomic and non-genomic pathways associated with the pleiotropic actions of vitamin D [17,18]. Vitamin D may exert antioxidant and anti-inflammatory effects through the modulation of cellular signaling pathways and endogenous antioxidant defense mechanisms [17-19]. These properties may contribute to the observed improvement in erythrocyte deformability, although the underlying mechanisms were not directly investigated in the present study. Furthermore, vitamin D may influence systemic inflammatory responses and endothelial function, potentially contributing to changes in the vascular environment [2, 17,19].

Clinical Implications and Methodological Considerations

Reduced erythrocyte deformability compromises microcirculatory hemodynamics by limiting the ability of erythrocytes to traverse narrow capillaries, thereby increasing microvascular flow resistance and contributing to impaired tissue perfusion and microvascular ischemia [3,12,15]. Recent clinical evidence has highlighted reduced red blood cell deformability as a key contributor to diabetic microangiopathy, including retinopathy and nephropathy [12,15,16]. The observed improvement in erythrocyte deformability following therapeutic vitamin D administration suggests a potential role for vitamin D in modulating blood rheological properties under diabetic conditions. However, further experimental and clinical studies are required to determine the translational relevance of these findings.

Study Limitations

Certain limitations should be acknowledged. First, each temporal subgroup utilized n=6 animals; although statistically significant differences were established, expanded cohorts in future studies will further enhance statistical power. Second, biochemical markers of oxidative stress (e.g., malondialdehyde, reduced glutathione), serum 25-hydroxyvitamin D concentrations and advanced glycation end-products were not directly measured in this cohort. Third, microvascular perfusion was not assessed in vivo. Future mechanistic research directly measuring membrane lipid fluidity and cytoskeletal spectrin architecture is recommended to further elucidate these findings.

Conclusions

STZ-induced diabetes was associated with a progressive, time-dependent reduction in erythrocyte deformability, with the most pronounced impairment observed on Day 21. Preventive vitamin D administration did not significantly attenuate this decline, whereas therapeutic administration was associated with significantly improved erythrocyte deformability on Days 14 and 21 compared with untreated diabetic controls. These findings suggest that the effects of vitamin D on erythrocyte mechanical properties may depend on the timing of administration and warrant further mechanistic investigation.

Conflicts of interest. The authors have no conflict of interest to declare.

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