Anemia in Spontaneously Type 2 Diabetic Rats With Renal Impairment

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Abstract

Background: The eSS rats display a mild non obese type 2 diabetic syndrome with an insulin resistant state characterized by hyperglycemia, dyslipidemia and hyperinsulinemia. During the first year, eSS male rats exhibit proteinuria as well as alterations in glomerular filtration.

Methods: In this study, some hematological characteristics were evaluated in 12 month-old eSS males compared to eumetabolic Wistar rats, and the renal histology was also studied.

Results: The diabetic rats were found to have fewer erythrocytes and lower values of hemoglobin. In eSS rats, peripheral blood smears showed immature erythrocytes with polychromatophilia and the presence of erythroblasts was also verified. Thickening of the glomerular basement membrane, areas of tubular dilatation and protein cylinders were observed in kidneys of diabetic animals.

Conclusions: Our results suggest that eSS rats develop anemia because of complex influences of metabolic disturbances and diabetic renal damage and that it might provide new opportunities for studying the pathogenesis of this relevant complication of type 2 diabetics.

Keywords: Anemia; Type 2 diabetes; Renal disease; Rats

Introduction

Anemia is the most common blood disorder in diabetic patients and a significant risk factor for diabetic complications in terms of morbidity, mortality and health care costs [1]. Even though a high incidence of anemia was observed in diabetics without renal impairment especially in those aged and with poor glycemia control [2], anemia is more frequent and more severe in diabetic patients with chronic kidney disease [3-6], being possible to correlate decreased hemoglobin levels with the severity of renal failure [6, 7].

Previous studies carried out in the facilities our laboratory led to demonstrate a mild non obese diabetic syndrome in eSS rats with an insulin resistant state characterized by hyperglycemia, dyslipidemia and hyperinsulinemia with higher expression in males that worsen as they become older [8, 9]. Between 6 to 12 months of age, eSS rats usually develop raising proteinuria and at 12 months of age exhibit lesions of diabetic nephropathy [10, 11].

In consequence, taking advantage of the naturally slow progress of the metabolic derangement in the eSS rats and the consequent long-life-span, we decided to investigate whether 12-month old eSS male rats develop hematological alterations.

Materials and Methods

Animals

Twelve 12-month-old eSS male rats and eleven eumetabolic Wistar (W) rats paired by age were studied. eSS is an inbred rat maintained in the School of Medicine, Rosario University at Rosario, Argentina. Wistar came from the animal breeding facilities in the School of Biochemical Science, Rosario University. Breeding conditions were the same for all the animals, including temperature regulation (24 °C) and light-darkness cycles as well as the artificial air exchange. In all the cases, the individuals had remained housed since they were 21 days old, in hanging collective cages. All animals were fed on a complete commercial diet, special for laboratory rats, and water was ad libitum. These experimental conditions were maintained until the animals were euthanized.

Experimental procedures

After 10-hs overnight fast, all animals were weighed and

Table 1. Body Weight (Bw), Fasting Glycemia (G0), Fructosamine (FH2), Red Blood Cell Count (RBC), Hemoglobin Concentration (Hb), Hematocrit (Ht), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and Mean Cell Hemoglobin Concentration (MCHC) in Diabetic eSS Rats and in Euglycemic Wistar Rats

| Rats	Bw (g)	G0 (mg/dL)	FH2 (µmol/L)	RBC ($\times 10^6/mm^3$)	Hb (g/dL)	Ht (%)	VMC (fl)	MCH (pg)	MCHC (g/dL)
eSS (n = 12)	415 ± 21	147 ± 15	182 ± 17	6.39 ± 0.16	11.09 ± 0.22	34 ± 0.84	49.3 ± 1.1	17.2 ± 0.17	34.9 ± 0.29
Wistar (n = 11)	410 ± 36	103 ± 10	121 ± 14	7.88 ± 0.07	13.9 ± 0.13	39 ± 0.41	48 ± 0.22	16.5 ± 0.06	34.3 ± 0.09
P	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	> 0.05	< 0.01	> 0.05

Values expressed as mean ± standard deviation; fl: femtoliters.
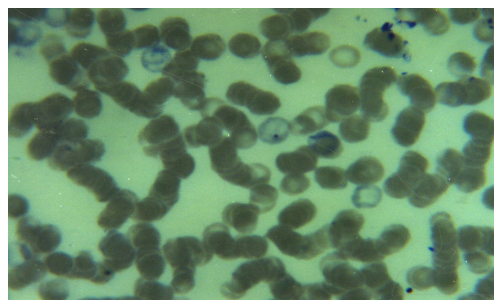

Figure 1. Blood smear of a 12-month old eSS diabetic showing polychromasia, a Howell-Jolly body (center) and Rouleaux formation. May-Grünwald-Giemsa stain. 100 ×.

exsanguinated by cardiac puncture under deep sodium thiopental anesthesia. The animals were sacrificed by this blood sampling. Part of the blood was collected into tubes pretreated EDTA and used as specimen for hematological analyses which required whole blood. Red blood cell count (RBC/mm³), white blood cell count (WBC/mm³) and platelet count (Pt/mm³) as well as the hematocrit (Ht%) and the hemoglobin concentration (Hb; g/dL) were obtained using a multi-parameter hematology analyzer (CELL-DYN 1400 ABBOTT). For the platelet count, blood sample was diluted 1/10. Mean cell volume (MCV; femtoliters), mean cell hemoglobin (MCH; pg) and mean cell hemoglobin concentration (MCHC; g/dL) were also determined. A second aliquot of blood was allowed to clot and centrifuged to obtain hemolysis-free serum which was used for determinations of fasting glycemia (G0; g/dL) and fructosamine levels (FH2; µmol/L). Using a fresh blood drop taken from the tail tip before cardiac puncture, red blood cells on a blood smear were stained with May-Grünwald-Giemsa (Sigma) for morphological analysis.

Immediately after death, both kidneys were removed in six eSS and six Wistar male rats. Sections of renal tissue were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, cut into sections of 4 µm thick, stained with
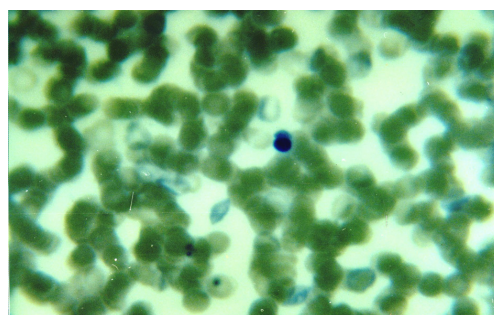
Figure 2. Blood smear of a 12-month eSS diabetic showing an erythroblast (center). May-Grünwald-Giemsa stain. 100 ×.
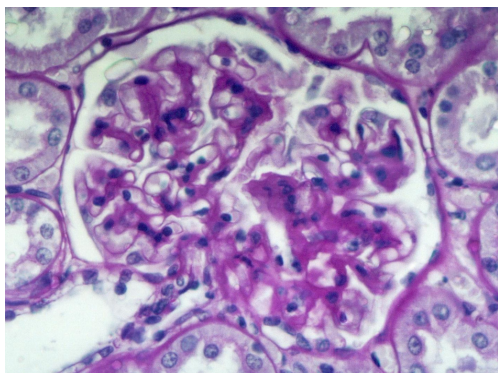
Figure 3. Mild diffuse mesangial thickening of the glomerular basement membrane (12-month old eSS male). HE and Periodic acid-Schiff (PAS) stain. 300 ×.

Figure 4. Moderate tubular dilatation with atrophic epithelium, containing proteinaceous cylinders. HE stain. 100 ×.

hematoxylin-eosin (HE) and Periodic Acid-Schiff (PAS), and examined by light microscopy in a blinded fashion.

Statistical analysis

The results are presented as the mean $\pm$ 1 standard error of mean (SD). Statistical significance was determined by Student's test. P value less than 0.05 (P < 0.05) was accepted as statistically significant.
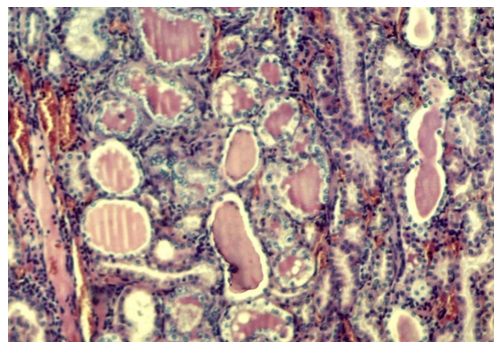
Ethical considerations

All experimental procedures presented in this study were approved by the Bioethics Commission of School of Medicine, which assures adherence to the standards by the Guide for the Care and Use of Laboratory Animals.

Results

As seen in Table 1, eSS rats displayed substantially higher levels of G0 and FH2 and could be considered as being severely hyperglycemic, according to DeFronzo et al (> 140 mg/dL) [12] whereas Wistar rats remained euglycemic. Table 1 also indicates that diabetic rats presented higher levels of RBC, Hb and Ht than those of controls. MCV and MCHC did not differ between groups while MCH was higher in eSS than in W rats.

WBC/mm^3 was lower in eSS than in W (5,175 $\pm$ 528 vs. 7,280 $\pm$ 633; P < 0.05) while Pt/mm^3 was higher (960 $\pm$ 5.6 vs. 870 $\pm$ 3.6; P < 0.001). In the eSS rats leukocyte formula, significant higher neutrophil count (41.3 $\pm$ 2.5% vs. 19.2 $\pm$ 0.3%; P < 0.001) and eosinophil count (3 $\pm$ 0.45 % vs 1.4 $\pm$ 0.7%; P < 0.05) were noted as compared to W. A minor lymphocyte count (54 $\pm$ 2.8% vs. 77.6 $\pm$ 3.3%; P < 0.001) was noticeable in eSS rats.

In eSS rats, the May-Grünwald-Giemsa-stained blood smears showed polychromasia, presence of erythroblasts and Howell-Jolly bodies as well as enhanced Rouleaux formation. The occurrence of erythroblasts in peripheral blood was quantified in eSS (1.17 $\pm$ 0.34) but it was never verified in Wistar eumetabolic controls (Fig. 1, 2).

Histological examination of kidney of eSS rats revealed glomeruli with diffuse thickness increase of mesangial tissue and a certain amount of small glomeruli was also detected; areas of markedly atrophic and dilated tubules containing acidophilic proteinaceous material were also observed (Fig. 3, 4) Neither tubular nor glomerular lesions were detected in any Wistar controls.

Discussion

The anemia of diabetic eSS rats is evidenced by the lower values of the hemoglobin concentration, the hematocrit and the red blood cell when compared to the euglycemic controls that could be related to the chronic metabolic perturbances and the deterioration of renal function of these rats.

Between 6 to 12 months of age, eSS males develop raising albuminuria and impaired glomerular filtration [9]. In diabetics, declining hemoglobin concentration is common among those patients with declining renal function and urinary albumin excretion [13, 14]. The anemia may result from diverse mechanisms, principally associated to both deficiency and hyporesponsiveness to erythropoietin [15].

In eSS rats, the finding of erythroblasts, polychromatophilia and the presence of Howell-Jolly bodies suggest a compensatory attempt that fails to restore the normal values. Since it has been demonstrated that fragility of red blood cells is increased in diabetic patients due to non-enzymatic glycation of proteins [16, 17], attention should be exerted in the elevated FH2 values verified in eSS rats indicating the chronic course of the hyperglycemia. The enhanced Rouleaux formation observed in eSS smears could indicate the

occurrence of hemorheological disorders in the hyperglycemic rats such as the increased erythrocyte adhesiveness and aggregation demonstrated in diabetic patients with poor glycemic control [18].

Because oxidative stress has been identified as the possible cause for producing anemia among diabetics without nephropathy [19], attention should be exerted in the statistical correlation between degree of hyperglycemia, high level of lipidic peroxides and decreased antioxidant defenses verified in eSS rats [20]. An excessive eryptosis related to the oxidative imbalance has been reported in type 2 diabetes patients [21]. Several stressors, including oxidative stress, triggered the red blood cells injury and the resulting eryptosis favors the development of anemia [22, 23]. We suggest that in eSS rats the oxidative stress lead to anemia by impairing the maintenance of erythrocytes. The chronic kidney disease, as it was demonstrated in the eSS rats, would increase the oxidative damage, the red cell fragility and the apoptotic erythrocyte death [24].

The eSS rats had also a significantly lower number of white cells than in controls. Differential count also showed a pattern of lymphopenia and neutrophilia as was reported in critically ill hyperglycemic patients [25, 26]. These white blood cell changes possibly contribute to the increased susceptibility to infection reported for the eSS rats [27].

The high platelet count verified in the eSS animals might indicate an advanced state of their pathology because thrombocytosis has been detected in diabetic and non diabetic end-stage renal patients [28]. This has also been detected in states of diabetic ketoacidosis [29]. Notwithstanding the renal lesions verified in these eSS rats as well as the high mortality already described in this line during second year of life [30], more studies will be necessary to clarify these findings.

Though new experiments could be undertaken to brighten the genesis and evolution of the hematological alterations in spontaneously diabetic eSS rats, we conclude that the anemia occurrence could be useful to study the interactions between chronic hyperglycemia and diabetic nephropathy, involved in the genesis of this relevant cause of morbidity and mortality in the diabetic patients.

Grant Support

Conflict of Interest

The authors disclose no conflict of interest.

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