Darbepoetin-α treatment enhances glomerular regenerative process in the Thy-1 glomerulonephritis model

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Erythropoietin (EPO) has been described as a hematopoietic cytokine required for the proliferation and differentiation of erythroid progenitor cells regulating the level of circulating red blood cells (4). However, it has been reported that EPO receptors (EPORs) are also expressed in nonerythroid cells (neurons, endothelial cells, cardiomyocytes, renal tubular cells, podocytes, and mesangial cells) which can respond to EPO treatment (17, 18, 22, 24, 27). Therefore, it seems that the therapeutic benefits of EPO treatment on nonhematopoietic tissues are not only the result of the correction of anemia-related tissue hypoxia, suggesting that there are further molecular pathways involved (5, 7, 9, 23, 25, 26).

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Therefore, it seems that the therapeutic benefits of EPO treatment on nonhematopoietic tissues are not only the result of the correction of anemia-related tissue hypoxia, suggesting that there are further molecular pathways involved (5, 7, 9, 23, 25, 26).

It is well established that activation of EPOR by EPO leads to phosphorylation and activation of several signaling pathways that promote cell survival and antiapoptotic effects, including increased activity of antiapoptotic proteins (Bcl-2, Bcl-XL), maintenance of mitochondrial membrane potential, prevention of cytochrome c release, and inactivation of caspases (14, 16).

Anti-Thy-1 glomerulonephritis (Thy-1-GN) is one of the best-studied reversible models of glomerulonephropathy (1, 4, 20, 21). The epitopes of the Thy 1.1 antigen are present predominantly in the mesangial area and to a lesser extent along the glomerular basement membrane of the rat kidney (1). In this model, mesangiolysis leads to severe injury of glomerular structure, characterized by so-called “ballooning lesions.”

The disease has spontaneous resolution in 2 or 3 wk after anti-Thy monoclonal antibody administration. Glomerular recovery occurs by endothelial and mesangial cell proliferation and accumulation of mesangial matrix (1, 21).

EPO has been shown to have a renal-protective effect in ischemic kidney diseases, mainly for its antiapoptotic actions and recruitment of endothelial precursor cells (2, 3, 6, 22). The long-acting recombinant human analog darbepoetin-α ameliorates podocyte injury and decreases proteinuria by a direct effect on podocytes in a model of proteinuria induced by puromycin aminonucleoside (8).

Our aim was to investigate whether darbepoetin-α treatment could prevent or reverse the progression of glomerular lesions in a Thy-1-GN rat model.

MATERIALS AND METHODS

Animals. The study was carried out using 8-wk-old male Wistar rats that weighed 160–180 g. They were fed with standard food and had free access to tap water. All experiments were performed under general anesthesia, and rats were killed by aortic exsanguination. All animals received humane care in compliance with “Principles of Laboratory Animal Care,” formulated by the Spanish National Society for Medical Research and the National Institutes Health Guide for the Care and Use of Laboratory Animals. The experimental protocols were reviewed and approved by the Ethics Committee for Animal Research of Cordoba University.

Induction of Thy-1-GN. To induce glomerulonephritis, anti-Thy-1.1 monoclonal antibodies (ER4 Hibridoma, Antibody Solutions, Palo Alto, CA) were injected into rats intravenously (1 mg/1,000 g body wt). Control rats received a PBS injection (vehicle) instead of the antibody.

Thy-1-GN progression study. To analyze Thy-1-GN progression, animals were divided in five groups and killed at 24 h, 72 h, 7 days, 10 days, or 15 days after anti-Thy antibody administration (n =
10/group). Once rats were killed, their kidneys were perfused with saline and removed. Blood and urine were also collected for evaluating renal function. Because in these experiments (see RESULTS) we found the maximal degree of renal damage and proteinuria, we decided to test the effect of darbepoetin-/H9251 treatment at this time point (see the next subsection below).

Darbepoetin-/H9251 experimental protocol. After anti-Thy antibody or vehicle administration (day 0), rats received two intraperitoneal doses (1 μg/kg) of darbepoetin-/H9251. Two methods of administration were used. In the first group of experiments, darbepoetin-/H9251 was injected at days 0 and 4. Then, a second group of experiments was performed in which darbepoetin-/H9251 was injected at days 4 and 6 (n = 10 for first group, darbepoetin-/H9251 administration at days 0 and 4; n = 6 for the second group, darbepoetin-/H9251 administration at days 4 and 6). Afterward, rats were killed at day 7. In all the experiments, control rats received an intraperitoneal vehicle injection instead of darbepoetin-/H9251.

Blood biochemical analysis. Blood samples were obtained by aortic puncture on the day of death. Hematocrit, total plasma protein (Total Protein Kit, BioSystems), and creatinine (Creatinine Kit, BioSystems) concentrations were measured.

Urinary protein excretion. A spot of urine was obtained by direct cystocentesis once rats were killed. Urinary protein was measured by colorimetric assay with the pyrogallol red method (Protein Urine Kit, BioSystems). Urine protein concentration was evaluated using the total protein-to-creatinine ratio (P/C) in urine spot.

Renal histology. Briefly, renal tissue was fixed in 4% formalin. After dehydration through a graded series of ethanol, the tissue was embedded in paraffin. Three-micrometer paraffin sections were

Fig. 1. Biochemical changes in Thy-1-gglomerulonephritis (GN) progression. Urinary protein excretion (P/C; A) and plasma creatinine concentration (mg/dl) determinations (B) of anti-Thy-1.1 antibody-injected rats [killed 1, 3, 7, 10, or 15 days after injection (n = 10/group)] are shown. The amount of urinary protein was significantly elevated from baseline at days 7 and 10. The highest value for proteinuria was found at day 7. From day 7 to day 15, protein excretion was gradually decreasing. On the other hand, anti-Thy-1.1 antibody injection did not affect plasma creatinine and showed values in the normal range. Values are means ± SE. *P < 0.05 vs. control.

Fig. 2. Histological changes in Thy-1-GN progression. A: renal morphology of control rats. No lesions were observed. B: renal morphology 24 h after anti-Thy monoclonal antibody injection. Early mesangiolysis areas (asterisks), influx of inflammatory cells (white arrow), and some apoptotic bodies (black arrow) can be seen. C: renal morphology 3 days after anti-Thy monoclonal antibody injection. Significant changes in renal morphology can be observed. D: at day 7 after injection of anti-Thy-1.1, there was marked loss of mesangial cells with dissolution of the mesangial matrix and formation of microaneurysms. E: at day 10 after anti-Thy-1.1 injection, glomerular morphology has started to recover. F: at day 15 after anti-Thy-1.1 injection, most glomeruli have a regenerative appearance. Magnification ×40.
stained with hematoxylin-eosin, periodic acid-Schiff (PAS), methenamine silver, and Masson’s trichromic, and examined by normal light microscopy. Semiquantitative morphological studies of glomerular lesions were performed on 25 glomeruli, randomly selected from each specimen, by S. Cañadillas, R. Ortega, and A. Gonzalez-Menchen. The authors were unaware of the origins of the slides. The presence of four types of lesions (mesangiolysis, microaneurysms, crescent and glomerular mesangial cells, or matrix expansion) was studied separately and graded according to the percentage of glomeruli showing each lesion. For this purpose, we used the following criteria: 0 = normal glomeruli with no significant injury; 1 = <25% of glomeruli showing lesions; 2 = 26–50% of glomeruli showing lesions; 3 = 51–75% of glomeruli showing lesions; and 4 = >75% glomeruli showing lesions.

Immunohistochemical analysis. Standard immunohistochemical techniques were used to stain α-smooth muscle actin (SMA), desmin, caspase-3 and Ki67. First, 3-μm sections were deparaffinized and microwave treated in 0.01 mmol/l citrate buffer, pH 7.2, for 15 min. Then, sections were incubated in 0.3% H2O2 in methanol for 30 min. After that, sections were stained with primary antibodies against

![Diagram](image-url)

Fig. 3. Darbepoetin-α administration at days 0 and 4: effect on proteinuria and renal function. A: Thy-1-GN rats treated with darbepoetin-α at days 0 and 4 (Thy-1-GN+Darbe) showed lower protein excretion than untreated rats (Thy-1-GN −Darbe). B and C: neither plasma creatinine concentration nor hematocrit showed significant changes between different groups. Values are means ± SE. *P < 0.05 vs. Thy-1-GN −Darbe; n = 10/group.

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Fig. 4. Darbepoetin-α administration at days 4 and 6: effect on proteinuria and renal function. A: Thy-1-GN rats treated with darbepoetin-α at days 4 and 6 (Thy-1-GN+Darbe) showed lower protein excretion than untreated rats (Thy-1-GN −Darbe). B and C: neither plasma creatinine concentration nor hematocrit showed significant changes between different groups. Values are means ± SE. *P < 0.05 vs. Thy-1-GN −Darbe; n = 6/group.
α-SMA, desmin, and caspase-3 (1:50 dilution) purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Then, sections were incubated for 2 h at room temperature with peroxidase-conjugated anti-rabbit or anti-mouse IgG and treated with 3,3′- diaminobenzidine-tetrachloride (1:100 dilution, En-vision System, DakoCytomation, Glostrup Denmark). Every step was followed by three washes with PBS for 10 min. Sections were counterstained with hematoxylin. To display the degree of immunohistochemical staining, a semiquantitative 0–4 system, with 0 being negative stained and 4 the most positive stained, was used.

Electron microscopy. To study ultrastructure, pieces of kidney tissue obtained from rats were fixed in 2% glutaraldehyde and processed for electron microscopy (EM). The EM level analysis was performed in a qualitative manner in areas of interest selected in the semithin sections. After that, ultrathin sections were cut and studied.

Statistics. All values are expressed as means ± SE. Statistical significance (defined as $P < 0.05$) was evaluated using Student’s t-test or analysis of variance where appropriate.

RESULTS

Thy-1-GN progression. Injection of purified monoclonal antibody against the Thy 1.1 antigen (1 mg/1000 g body wt) induced GN and massive proteinuria in rats. Proteinuria in Thy-1-GN rats gradually increased, reaching its maximum at day 7. Protein excretion decreased toward normal values at day 15 (Fig. 1A). Plasma creatinine concentration did not show significant changes after anti-Thy monoclonal antibody administration (Fig. 1B).

Early mesangiolysis, influx of mononuclear and polymorphonuclear cells, and some scattered apoptotic bodies were noted 24 h after anti-Thy monoclonal antibody administration (Fig. 2B). At day 3, in most glomeruli, mesangial cells disappeared and microaneurysms appeared in the mesangial area in some glomeruli (Fig. 2C). At day 7, most glomeruli showed the full dissolution of the mesangial area, leading to the formation of large microaneurysms in the capillary loops (Fig. 2D). At day 10, the majority of glomeruli showed proliferation areas and matrix expansion (Fig. 2E). After 15 days, mesangial cell proliferation was prominent and an increase in mesangial matrix was observed. Most glomeruli had a regenerative appearance at this time (Fig. 2F).

Darbepoetin-α administration: effect on proteinuria and renal function. To evaluate the effect of darbepoetin-α, Thy-1-GN rats or healthy rats were treated with two doses (1 μg/kg injection of purified monoclonal antibody against the Thy-1.1 antigen (1 mg/1000 g body wt) purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Then, sections were incubated for 2 h at room temperature with peroxidase-conjugated anti-rabbit or anti-mouse IgG and treated with 3,3′- diaminobenzidine-tetrachloride (1:100 dilution, En-vision System, DakoCytomation, Glostrup Denmark). Every step was followed by three washes with PBS for 10 min. Sections were counterstained with hematoxylin. To display the degree of immunohistochemical staining, a semiquantitative 0–4 system, with 0 being negative stained and 4 the most positive stained, was used.

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Darbepoetin-α administration: effect on proteinuria and renal function. To evaluate the effect of darbepoetin-α, Thy-1-GN rats or healthy rats were treated with two doses (1 μg/kg

Fig. 5. Darbepoetin-α administration: effect on glomerular histological lesions. A: renal histology in Wistar rats that received darbepoetin-α treatment or vehicle (PBS) at days 0 and 4 or at days 4 and 6 after anti-Thy monoclonal antibody or vehicle injection. A1–A4: hematoxylin and eosin (HE), periodic acid-Schiff (PAS), methenamine silver (M-silver), and Masson’s trichromic (Masson TR) stain, respectively, of kidneys from control rats that were injected intravenously with vehicle instead of anti-Thy-1.1 antibody. Kidneys did not show any alterations. A5–A8: HE, PAS, M-silver, and Masson TR stain, respectively, of kidneys from control rats that were injected intravenously with vehicle instead of anti-Thy-1.1 antibody. Kidneys did not show any alterations. A5–A8: HE, PAS, M-silver, and Masson TR stain, respectively, of kidneys from control rats that were injected intravenously with vehicle instead of anti-Thy-1.1 antibody. Kidneys did not show any alterations.
of body wt) of darbepoetin-α or vehicle at days 0 and 4 or at days 4 and 6 after Thy-1-GN induction (see METHODS). In both protocols of administration, darbepoetin-α injected into control animals produced no effect on proteinuria at day 7; in contrast, in Thy-1-GN rats, darbepoetin-α significantly reduced the urinary protein content in both experimental groups (Figs. 3A and 4A). However, the early administration of darbepoetin-α had a greater effect on the reduction of proteinuria (Fig. 3A) compared with the group receiving the drug later (at days 4 and 6) (Fig. 4A).

Neither early nor late darbepoetin-α administration modified serum creatinine levels (Figs. 3B and 4B). In both patterns of treatment, hematocrit analysis showed a minimal increase in darbepoetin-α-treated rats, which was not statistically significant (Figs. 3C and 4C).

Darbepoetin-α administration: effect on glomerular histological lesions. At death, kidneys sections were analyzed. In control rats, darbepoetin-α did not alter glomerular morphology (data not shown). In contrast, in Thy-1-GN rats, at day 7 darbepoetin-α-treated rats showed a decrease in glomerular injury: mesangiolysis, microaneurysms, and crescents were significant reduced (Fig. 5A, A9–A16). Interestingly, darbepoetin-α did not affect endocapillary proliferation and matrix expansion (Fig. 5B).

Consistent with proteinuria levels shown above, the improvement observed in glomerular morphology, when darbepoetin-α is administered at days 0 and 4 (Fig. 5A, A9–A12), was greater than that found in rats treated later (at days 4 and 6) (Fig. 5A, A13–A16). However, this difference was not statistically significant between both administration protocols.

Independently of the administration protocol used, we observed that with darbepoetin-α treatment, most of the glomeruli observed at day 7 (see METHODS) were very similar to those observed at day 15 in the Thy-1-GN rats (see METHODS). The comparison of these images is shown in Fig. 6 (data shown correspond to group that received darbepoetin-α at days 0 and 4).

Immunohistochemical studies. We used α-SMA staining for mesangial activation and desmin expression for podocyte damage detection. In control rats, α-SMA staining was negative. In contrast, Thy-1-GN rats had significantly increased mesangial α-SMA expression. Treatment of Thy-1-GN rats with darbepoetin-α significantly reduced α-SMA expression in the mesangial area. Desmin expression in Thy-1-GN rats was also increased. However, treatment with darbepoetin-α did not produce a significant staining reduction (Fig. 7).

To investigate the effect of darbepoetin-α on apoptosis and cell proliferation, we analyzed caspase-3 and Ki67 expression in kidney sections. As shown at Fig. 8, caspase-3 expression was significantly reduced by darbepoetin-α administered at days 0 and 4 compared with Thy-1-GN rats. Similar results were found when darbepoetin-α was administered later (at days 4 and 6).

As expected, in control rats immunohistochemical staining for Ki67 antigen was negative at the glomerulus, being positive at the tubular compartment. Control rats that received darbepoetin-α did not show any proliferative changes compared with control. In contrast, Thy-1-GN rats treated with darbepoetin-α did show significant cellular proliferation at the glomerular level. This increase in cell proliferation was attributed to the regenerative process induced by darbepoetin-α. Interestingly, an increment in Ki67 staining was found in Thy-1-GN rats not treated with darbepoetin-α. The number of proliferating cells in this group was significantly higher compared with control rats. This fact could be due to natural glomerular recovery, which occurs after injury (Fig. 9).

Nevertheless, our results demonstrated that Thy-1-GN rats treated with darbepoetin-α showed the highest level of proliferating cells, and these data correlated with a reduction in proteinuria as well as an amelioration of glomerular lesions (Ki67 data shown correspond to group which received darbepoetin-α at days 0 and 4). We did not observe significant differences when darbepoetin-α was administered at days 4 and 6.

EM studies. Glomerular ultrastructure in control rats treated with darbepoetin-α showed a conserved structure without any morphological changes (Fig. 10B). On the other hand, glomerular characteristics in Thy-1-GN rats were quite different; rats injected with the anti-Thy monoclonal antibody presented an altered renal morphology. At the ultrastructural level, glomerular architecture could be observed as totally disorganized. Mesangiolysis and microaneurysms were the most observed lesions (Fig. 10C). Ultrastructural analysis of Thy-1-GN rats

Fig. 6. darbepoetin-α-treated rats (7 days after disease induction) vs. Thy-1-GN rats (15 days after disease induction). HE-stained kidney of Thy-1-GN rats 7 days after disease induction (A), Thy-1-GN rats 15 days after disease induction (B), and Thy-1-GN rats 7 days after disease induction and treated with 2 doses (day 0 and 4) of darbepoetin-α (C). Glomeruli from darbepoetin-α-treated rats at day 7 after nephritis induction were very similar to glomeruli from Thy-1-GN rats at day 15 after disease induction. Magnification ×40.
treated with darbepoetin-α showed a glomerular lesion pattern quite similar to that found in light microscopic analysis (Fig. 10D). At the ultrastructural level, the predominant events were cell proliferation and matrix expansion (Fig. 10; data shown correspond to group that received darbepoetin-α at days 0 and 4).

DISCUSSION

In the present study, we analyzed the effect of treatment with darbepoetin-α in the rat Thy-1-GN model using two different protocols of administration. We showed that independently of the administration pattern, early administration (at days 0 and 4) or later (at days 4 and 6), of darbepoetin-α reduced proteinuria, mitigated glomerular damage, and accelerated glomerular recovery.

In the rat Thy-1-GN model, mesangiolysis leads to severe destruction of glomerular structure, characterized by balloononing lesions. Glomerular recovery begins with migration of mesangial cells from vascular poles and angiogenesis by immature endothelial cells (10–12, 19).

Following intravenous injection, glomerular binding of the anti-Thy-1.1 antibody is classically observed during the first 3 days in the mesangium and along the glomerular basement membrane (1). The nephrotoxic effect is mediated by direct binding of anti-Thy-1.1 and not by circulating immune complexes (1). Single injection of a Thy-1 monoclonal antibody initiates an acute phase of mesangiolysis and matrix dissolution, leading to microaneurysm formation and distortion of the capillary network. This acute phase is followed by an intense proliferation of resting mesangial and endothelial cells and an increase in mesangial matrix. The majority of the destroyed glomeruli are gradually reconstituted, returning to an almost normal structure 2 or 3 wk after Thy-1 monoclonal antibody administration (1, 20).

In the last decade, growing evidence has accrued to indicate that EPO and its derivates have effects outside the hematopoietic system, enhancing angiogenesis and tissue repair.

**Fig. 7.** α-Smooth muscle actin (SMA) and desmin analysis expression. A, A1–A4: α-SMA immunostaining of kidney from control rats (A1), Thy-1-GN rats (A2), Thy-1-GN rats treated with darbepoetin-α (at days 0 and 4; A3), and Thy-1-GN rats treated with darbepoetin-α (at days 4 and 6; A4). Darbepoetin-α treatment significantly reduced α-SMA expression. A5–A8: desmin immunostaining of kidney from control rats (A5), Thy-1-GN rats (A6), Thy-1-GN rats treated with darbepoetin-α (at days 0 and 4; A7), and Thy-1-GN rats treated with darbepoetin-α (at days 4 and 6; A8). Desmin expression was not significant modified by darbepoetin-α treatment. B: semiquantitative analysis of α-SMA and desmin expression (0–4). Magnification ×40. Values are means ± SE. *P < 0.05 vs. Thy-1-GN group; n = 10/group that received darbepoetin-α at days 0 and 4 and n = 6/group that received darbepoetin-α at days 4 and 6.
etic system and serve as a novel cytoprotective agent in organs expressing EPOR (5, 7, 9, 23, 25, 26). EPOR expression occurs in several tissues, including renal tissue, at tubular and glomerular cells, including mesangial cells, endothelial cells, and more recently demonstrated in podocytes (15, 22).

Here, we employed darbepoetin-α, which is long-acting recombinant human EPO analog with increased glycosylation. Darbepoetin-α stimulates erythropoiesis via the same mechanism as the native hormone and has greater in vivo metabolic stability.

The major finding of the current study was that darbepoetin-α significantly reduced protein excretion at day 7 after nephritis induction in treated rats compared with nontreated rats. Consistently, glomerular histology in Thy-1-GN rats treated with darbepoetin-α revealed a significant decrease in glomerular lesions. Mesangiolysis, microaneurysms, and crescents were significantly reduced in rats treated with darbepoetin-α. In contrast, darbepoetin-α did not reduce endocapillary proliferation and matrix expansion. EM analysis revealed an altered structure in Thy-1-GN rats showing mesangiolysis areas and microaneurysm formation. These lesions were mitigated in darbepoetin-α-treated Thy-1-GN rats, but high cell proliferation and matrix expansion were observed in most glomeruli.

We tested two patterns of administration and found that regardless of the form of administration (days 0 and 4 or days 4 and 6), in both groups of experiments darbepoetin-α improved renal morphology and decrease proteinuria. Nevertheless, when darbepoetin-α was administered at days 0 and 4, both the improvement in glomerular morphology as well as the decrease in proteinuria levels were higher compared with rats that received darbepoetin-α later (at days 4 and 6).

Therefore, we could argue that early administration of the drug could have more effect on glomerulonephritis progression simply because the beneficial effects of darbepoetin-α are acting in the animal longer and since the beginning of the disease.

In summary, our data have demonstrated that protein excretion and glomerular lesions can be partially prevented with early administration of darbepoetin-α (days 0 and 4) and ameliorated if darbepoetin-α is administered later (days 4 and 6), once GN is established.

Hence these results suggest that darbepoetin-α possesses both a protective effect as well as the capacity to accelerate the intrinsic resolution process in a Thy-1-GN rat model.

Interestingly, in both protocols of darbepoetin-α administration, the improvement in renal function and renal morphology occurring at day 7 in darbepoetin-α-treated Thy-1-GN rats, could not be observed in Thy-1-GN rats (without darbepoetin-α treatment) until day 15. These data indicated that darbepoetin-α treatment enhanced the endogenous mechanisms which lead to disease resolution.

Glomerular cellular protection by darbepoetin-α has been addressed by other authors in renal and nonrenal tissues (13). Recently, it has been reported that darbepoetin-α treatment protects podocytes and prevents them from undergoing apoptosis in a puromycin aminonucleoside-induced model of nephritic syndrome (17).

After binding of darbepoetin-α to EPOR, studies in nonrenal cells have demonstrated that there is activation of a variety of pathways involved in cell survival, proliferation, and differentiation.
Fig. 9. Ki67 analysis expression. A: Ki67 immunostaining of kidney from control rats (A1), control rats treated with darbepoetin-α at days 0 and 4 (A2), Thy-1-GN rats (A3), and Thy-1-GN rats treated with darbepoetin-α (at days 0 and 4; A4). Ki67 expression increased significantly in Thy-1-GN rats treated with darbepoetin-α as well as in Thy-1-GN rats compared with control group. Values are means ± SE. *P < 0.05 vs. Thy-1-GN without Darbe; n = 6/group.

Fig. 10. Electron microscopy. Shown are glomerulus electron micrographs from control rats (A), control rats treated with darbepoetin-α (B), Thy-1-GN rats (C), and Thy-1-GN rats that received 2 doses of darbepoetin-α (at days 0 and 4 after nephritis induction; D). Glomerular ultrastructure from Thy-1-GN rats treated with darbepoetin-α (D) showed a lower degree of lesion and more conserved structure than untreated rats (C).
intracellular signaling pathways that results in a prosurvival effect. Caspases are proteins implicated in apoptotic signaling. Activation of EPOR by EPO promotes cell survival and antiapoptotic effects, including inactivation of caspases. The current study revealed that caspase-3 expression was upregulated in Thy-1-GN rats while darbepoetin-α-treated rats showed lower caspase-3 expression. The decrease in caspase-3 staining was statistically significant compared with nontreated rats. Our results indicate that renoprotection mediated by darbepoetin-α might be due, in part, to its antiapoptotic effect on glomerular cells.

Seven days after Thy-1-GN induction, extensive expression of α-SMA and desmin was observed in the mesangial area. Darbepoetin-α administration significantly decreased α-SMA expression, whereas it did not lead to any significant reduction of desmin expression, at variance with other reports in which darbepoetin-α treatment significantly reduced desmin expression in a model of proteinuria induced by puromycinaminonucleoside (8).

Neither mesangial cell proliferation nor matrix expansion was reduced by darbepoetin-α treatment. This phenomenon could be explained by arguing that during repair of glomerular structures after damage, mesangial cell proliferation and extracellular matrix expansion are factor-determining in the evolution of glomerular injury. In this sense, the determination of Ki67 antigen expression revealed that darbepoetin-α treatment increases cell proliferation at the same time that it improves proteinuria and ameliorates glomerular lesions in the Thy-1-GN rats compared with nontreated rats. In our opinion, the increment of proliferating cells found at the glomerular area is the result of a natural recovery process, which is enhanced when rats are treated with darbepoetin-α. Mesangial cells in Thy-1-GN not only proliferate but also undergo a transient phenotypic transformation to myofibroblasts that express α-SMA in their cytoplasm (28). The transient increase in mesangial cell proliferation presumably reflects a physiological response required for successful glomerular reconstitution and renal tissue repair. This glomerular repair process involves regeneration of capillary architecture by angiogenesis. Also, it is known that myofibroblasts can produce extracellular matrix components, such as type I and type III collagens, which have an important role during the angiogenic process.

These observations suggest that excess and abnormal mesangial matrix may provide a suitable microenvironment for migration and proliferation of endothelial cells and may constitute a benefit in capillary remodeling. However, our results indicate that darbepoetin-α, through an unknown mechanism, helps mesangial cells regain their original phenotype, showing less expression of SMA at the mesangium. Consistent with these findings, Zhang et al. (28) have recently published a study demonstrating that, in accordance with the increase of the capillary, mesangial cells and matrix decreased gradually, and most glomeruli almost recovered their normal structure.

Based on our results and those of others, we may hypothesize that darbepoetin-α might modify the progression of glomerular diseases, accelerating endogenous repair processes through cytprotective cellular responses, including mitogenesis, angiogenesis, inhibition of apoptosis, proliferation of glomerular cells, and mobilization of progenitor cells from the bone marrow (13).

Therefore, our data suggest that darbepoetin-α treatment improves Thy-1-GN disease in two ways: 1) preventing disease progression, if treatment is performed early; and 2) accelerating glomerular recovery/repair, if treatment is performed later, once many lesions have already appeared.

Nevertheless, the beneficial effects of darbepoetin-α treatment in glomerular disease must be balanced against potential adverse effects related to hematocrit increase. In the current study, although the darbepoetin-α dose employed was higher than the dose used in humans, this did not alter hematocrit significantly. Furthermore, a novel molecule of EPO has been recently developed that exerts a cytostatic effect without promoting erythropoiesis (carbamyalted EPO). Carbamyolated EPO may be of clinical utility in patients with kidney disease (13).

In conclusion, this study has demonstrated that darbepoetin-α treatment improves renal function and stimulates glomerular cell repair. These effects could be mediated, in part, by two mechanisms: the activation of antiapoptotic pathways and the stimulation of glomerular cell proliferation.

Nonetheless, although our study suggests that darbepoetin-α might limit the irreversible degeneration of glomerular cells, further studies are necessary to elucidate the applicability of these findings to glomerular diseases in humans.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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