Long-term Study of Erythropoietin, Losartan, and Combination in Streptozotocin-induced Diabetic Rats

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OBJECTIVES

To evaluate the long-term renal effects of low-dose rHuEPO in diabetic nephropathy (DN) of rats in relation to the novel hypoxia theory[1,2] and endogenous EPO secretion. The effect of rHuEPO was compared to standard drug, losartan (LSR), and the possibility of add-on therapy (EPO and LSR combination) was also investigated.

METHODS

Thirty-four male Sprague-Dawley rats were randomly divided into five groups: control-naïve group, untreated diabetic group, EPO-treated diabetic group (150 U/kg, S.C., TIW)[3], LSR-treated diabetic group (5 mg/kg/day, P.O.), EPO-LSR-treated diabetic group. Drug treatment was started one week after streptozotocin (STZ) injection (60 mg/kg in citrate buffer 10 mmol/L, pH 4.5) and continued for twenty-eight weeks. Albuminuria was evaluated every four weeks. Assessment was done by renal function tests, blood pressure measurement, renal vein oxygen tension, plasma active-renin concentration, endogenous EPO concentration, and complete hematological profile, together with renal histopathological examination using Periodic Acid-Schiff (PAS) and Masson trichrome-stained sections.

Statistics: Differences in continuous variables were analyzed by one-way analysis of variance (ANOVA) followed by posthoc multiple comparisons (Scheffé test). Categorical variables were analyzed by Kruskal-Wallis H, Mann–Whitney's, and chi-square or Fisher exact tests (when appropriate). Albuminuria was analyzed by general linear model for repeated measures. P value < 0.05 was considered statistically significant at confidence interval 95 %.

Table 1: Renal functions, Mean Blood Pressure, Plasma active-renin concentration, Renal Vein Oxygen tension, Hematological parameters, EPO concentration, and Iron status of Control and Diabetic rats Followed for 28 Weeks (Mean ± SD)

	CNT-naïve (n=8)	DM-unt-28W (n=8)	DM-EPO-28W (n=6) (150 U/kg, S.C., TIW)	DM-LSR-28W (n=6) (5 mg/kg/day, P.O.)	DM-EPO-LSR-28W (n=6) (same doses)
Pl Cr (mg/dl)	0.43 ± 0.03	1.04 ± 0.07 *	1.05 ±0.07 *	0.63 ± 0.03 * § ♦	0.96 ± 0.03 * ♪
Cr Cl (ml/min)	1.65 ± 0.04	1.1 ± 0.07 *	1.21 ± 0.13 *	4.39 ± 0.5 * § ♦	1.22 ± 0.09 * ♪
MBP (mmHg)	104.2 ± 1.58	97.5 ± 1.41 *	115.6 ± 1.86 * §	90.8 ± 0.75 * § ♦	95.2 ± 3.65 * ♦
PRₐC (pg/ml)	3.3 ± 0.43	0.9 ± 0.31 *	59.2 ± 14.9 * §	6.5 ± 0.97 * § ♦	21.9 ± 3.2 * § ♦ ♪
RV O₂T (mmHg)	38.5 ± 2.69	14.9 ± 2.62 *	27.2 ± 1.77 * §	25.3 ± 4.01 * §	41.3 ± 4.72 § ♦ ♪
RBCs (M/µL)	8.2 ± 0.49	8.5 ± 0.19	8.2 ± 1.23	7.9 ± 0.21 §	8.7 ± 1.9
Hematocrit (%)	45.2 ± 2.73	48.7 ± 4.03	50.0 ± 6.75	43.8 ± 2.39	53.4 ± 9.63
EPO (pg/ml)	38.7 ± 2.81	53.1 ± 2.66 *	800 ± 163.4 * §	46.3 ± 3.84 ♦	35.9 ± 3.12 § ♦ ♪
TS (%)	42.6 ± 3.00	38.7 ± 1.40	29.1 ± 4.24 * §	41.2 ± 4.36 ♦	37.8 ± 0.89 * ♦
Ferritin (ng/ml)	45.7 ± 4.04	35.8 ± 2.67 *	37.5 ± 2.27 *	41.8 ± 2.36	35.5 ± 3.34 *

Abbreviations: Pl Cr, Plasma Creatinine; Cr Cl, Creatinine Clearance; MBP, Mean Blood Pressure; PRₐC, Plasma active-renin concentration; RV O2T, Renal Vein Oxygen tension; RBCs, Red Blood Cells; EPO, Erythropoietin conc.; TS, Transferrin saturation

Significant difference vs. control-naïve group (*), vs. DM-unt-28W (§), vs. DM-EPO-28W (♦), vs. DM-LSR-28W (♪).


Figure 2: Representative glomerular (A, C) and tubulointerstitial (B) histopathologic findings in EPO-treated diabetic rats (A and B), and LSR-treated diabetic rats (C). PAS stain (X400).

RESULTS

STZ-treated diabetic rats developed progressive albuminuria (figure 1), renal dysfunction (table 1), and significant glomerular changes (table 2) 28 weeks after induction of diabetes. The kidney of untreated diabetic rats showed significant decrease of renal vein oxygen tension associated with significant increase of plasma EPO concentration (table 1).

Chronic administration of rHuEPO led to significant elevation of blood pressure, marked albuminuria progression (figure 1) with profound increase of PRₐC and plasma EPO concentration (table 1), together with significant glomerular changes (table 2, figure 2A) and a picture of acute tubular injury (figure 2B) on renal histopathology examination. LSR sole therapy had the best beneficial effect on DN evolution based on renal function evaluation (table 1), albuminuria (figure 1), and renal histopathology (figure 2C). In addition, LSR improved diabetic renal hypoxia and prevented hypoxia-induced EPO secretion (table 1). Interestingly, administration of rHuEPO in combination with LSR in STZ-induced diabetic rats significantly abolished the renoprotective effect of LSR and worsened renal morphology (table 2), inspite of improving diabetic-renal hypoxia (table 1).
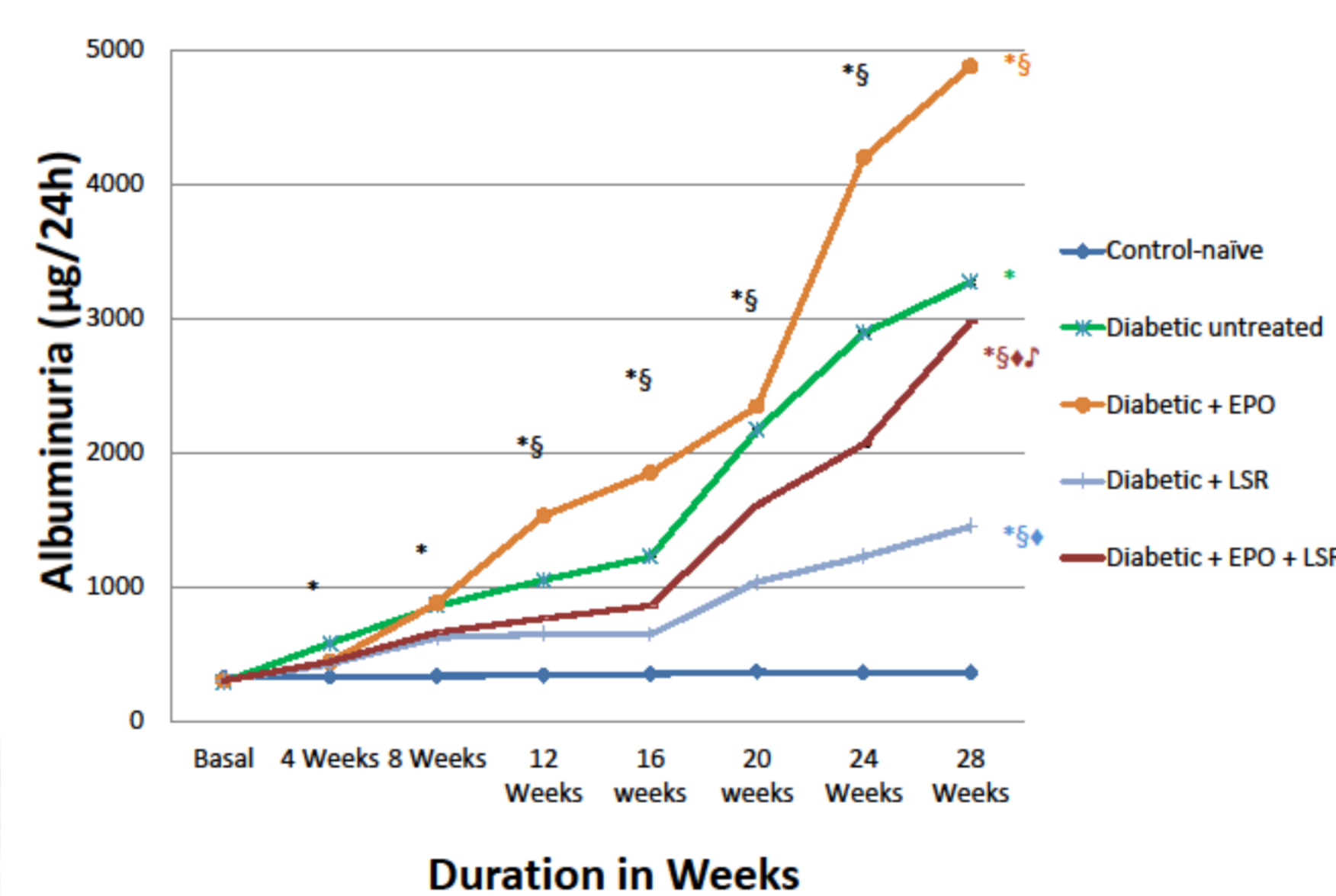
Figure 1: Effect of Erythropoietin (150 U/kg, S.C., TIW), Losartan (5 mg/kg/day, P.O.), or combination on Albuminuria of Control and Diabetic rats (Mean ± SEM)

Table 2: Histopathological scores of Control and Diabetic rats

	CNT-naïve (n=8)	DM-unt-28W (n=8)	DM-EPO-28W (n=6)	DM-LSR-28W (n=6)	DM-EPO-LSR-28W (n=6)
Glomerular score: Median (min-max)	0.00 (0 – 0)	2.00 (1 – 2) *	1.00 (1 – 2) *	1.00 (0 – 1) * §	2.00 (1 – 3) * ♪
IFTA score (%) Present	0.0	37.5	66.7 *	0.0 ♦	100.0 * § ♪
Interstitial Inflammation (%) Present	0.0	37.5	100.0 * §	33.3 ♦	66.7 *
Arteriolar Hyalinosis (%) Present	0.0	25.0	0.0	0.0	83.3 * ♦ ♪

Abbreviations: IFTA; Interstitial Fibrosis Tubular Atrophy.

CONCLUSIONS

The present study confirmed that LSR is a gold standard drug in management of DN where it delayed the progression of albuminuria and stabilized renal function. Contrarily to the expectations, administration of rHuEPO either alone or in combination with LSR for 28 weeks to the STZ-induced diabetic rat did not show beneficial effect on DN evolution, inspite of improving diabetic-renal hypoxia.

Clearly, the combination therapy points to abolishment of the beneficial effect of LSR in experimental DN by the addition of rHuEPO. This may denote that prolonged administration of rHuEPO, even in low dose, has intrinsic harmful renal effect mitigating the effect of a gold standard drug like LSR. This assumption needs confirmation at the molecular level in future experimental studies.

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