Urol Res (1999) 27:312–314

ORIGINAL PAPER

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A preventive effect of a selective endothelin-A receptor antagonist, S-0139, on the erythropoietin-induced reduction of the renal cortical blood flow

Received: 28 January 1998 / Accepted: 9 April 1998

Abstract We have confirmed that renal cortical blood flow (RCBF) is significantly decreased by recombinant human erythropoietin (EPO). Endothelin-1 (ET_1) is thought to be a mediator because its level increased significantly when EPO was administered. The present study was performed to clarify the effect of a selective ET-A receptor antagonist, S-0139, on EPO-induced RCBF reduction. Ten-week-old male Wistar rats, weighing about 250 g, were divided into five groups. Group 1 ($n = 5$), a control group, received normal saline solution (NSS). Group 2 ($n = 5$) received 200 U/kg body weight (BW) per hour of EPO. Group 3 ($n = 5$) received 400 U/kg BW per hour of EPO. Group 4 ($n = 5$) received both 200 U/kg BW per hour of EPO and 4 mg/kg BW per hour of S-0139. Group 5 ($n = 5$) received both 200 U/kg BW per hour of EPO and 40 mg/kg BW per hour of S-0139. Drugs were administered intravenously via the right femoral vein using a microinfusion pump for 4 h under urethane anesthesia. The RCBF was measured every 30 min by the hydrogen gas clearance method. When the 4 h had elapsed, the concentrations of plasma creatinine (Cr), ET1 and renin activity (RA) were measured. Compared with group 1, groups 2 and 3 showed significant ($P < 0.001$) decreases of RCBF, while the ET1 levels of these two groups increased significantly ($P < 0.03$). The ET1 of groups 4 and 5 also increased significantly ($P < 0.03$), however, the RCBF of these two groups did not decrease. No significant differences were observed in either Cr or RA between the five groups. EPO induces ET_1 secretion. The reduction of RCBF is due to ET_1-derived vasoconstriction. S-0139 has potential for preventing EPO-induced and ET_1-mediated RCBF reduction.

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Key words Recombinant human erythropoietin · Renal cortical blood flow · Endothelin · Selective endothelin-A receptor antagonist · Hypertension

Introduction

An animal study that we performed revealed that recombinant human erythropoietin (EPO) has potential for decreasing renal cortical blood flow (RCBF). In that study we also noticed that plasma endothelin-1 (ET_1) had significantly increased. We thought therefore that perhaps ET_1 was a mediator for EPO-induced RCBF reduction. The present study was performed to clarify the effect of selective ET-A receptor antagonist, S-0139 on EPO-induced and ET_1-mediated RCBF reduction.

Materials and methods

Ten-week-old male Wistar rats, weighing about 250 g, were used in this study. The animals were divided into five groups and each group consisted of five rats. Group 1, a control group, received normal saline solution (NSS). Group 2 received 200 U/kg body weight (BW) per hour of EPO. Group 3 received 400 U/kg BW per hour of EPO. Group 4 received both 200 U/kg BW per hour of EPO and 4 mg/kg BW per hour of S-0139. Group 5 received both 200 U/kg BW per hour of EPO and 40 mg/kg BW per hour of S-0139. EPO was purchased from the Chugai Pharmaceutical Co. (Tokyo, Japan); S-0139, (27-O-3-[2-(3-carboxy-acryloylamino)-5-hydroxyphenyl]-acryloyloxy myricerone sodium salt) was a generous gift from the Shionogi & Co. (Osaka, Japan).

The drugs were dissolved in NSS and administered intravenously via the right femoral vein using a microinfusion pump for 4 h under urethane anesthesia. To compensate for fluid loss during the RCBF measurement, all solutions were prepared prior to infusion in order to maintain a constant speed of 0.5 ml/h.

On the day of experiment, animals were anesthetized with an intraperitoneal injection of urethane at a dose of 1000 mg/kg BW. The right femoral vein was exposed with a minimal (i.e., about 1 cm in length) incision and a 24-gauge intravenous cannula was inserted into the femoral vein and fixed with 4–0 silk suture and then connected to the microinfusion pump line. The wound was then closed with 4–0 silk suture. Next, the abdomen was opened

with a minimal (i.e., about 4 cm in length) median incision and the left kidney was gently exposed. RCBF was measured every 30 min by the hydrogen gas clearance method [10] using an electrolytic flow meter (Model RBF 2, Biomedical Science Co., Ishikawa, Japan). Blood pressure was not monitored.

A monopolar needle-shaped electrode was introduced into the renal cortex to a depth of 1 mm at the lower pole of the left kidney, and a disk-shaped counter-electrode was set just behind the left kidney. The abdominal cavity was then filled with an adequate volume of NSS. The wound was covered with a 4-cm square-shaped cotton cloth during RCBF measurement to lessen insensible fluid loss.

After 4-h of RCBF measurement, about 8 ml of whole blood was taken from the left femoral artery to estimate the concentrations of plasma creatinine (Cr), ET_1 and renin activity (RA). Cr was measured by using the Creatinine Analyzer 2 (Beckman Instruments, Galway, Ireland). ET_1 and RA were measured by the radio immunoassay method.

Both kidneys were removed and prepared for light microscopic examination.

The data was analyzed by one-way analysis of variance followed by Student's *t*-test for differences between means.

Results

All the rats were well anesthetized by urethane during the measurement of RCBF. Spontaneous respiration was regular and stable, and bleeding from the wound was almost 0 ml. Therefore reliable data were obtained.

All the data are summarized in Figs. 1 and 2, and Table 1. The values are described as the mean ± standard deviation of five rats of each group.

Compared with group 1, groups 2 and 3 showed significant ($P < 0.001$) decreases of RCBF, while the ET_1 levels of these two groups increased significantly ($P < 0.03$). The ET_1 of groups 4 and 5 also increased significantly ($P < 0.03$); however, the RCBF of these two groups did not decrease. No significant differences were observed in either Cr or RA between the five groups; or were any histological changes detected.

Discussion

EPO is a potent erythrocyte-producing agent mainly used for the treatment of anemia in hemodialysis patients [2, 13]. However, it has the side effect of hypertension [1, 3, 9, 11]. There are many reports on the mechanism of EPO-induced hypertension [6].

ET is a strong vasoconstrictive peptide [8, 14, 15]. Recently attention has been paid to the role of ET in EPO-induced hypertension. We have tried, therefore, to apply our established methodology of cyclosporine-induced nephrotoxicity [4] to the present study to evaluate the relation between EPO and ET.

Tojo et al. [12] reported that EPO-induced ET_1 secretion was observed in spontaneous hypertensive rats but not in Wistar rats; however, we confirmed ET_1 elevation in Wistar rats. Although Ongil et al. [7] reported that there was no relation between EPO and ET, EPO

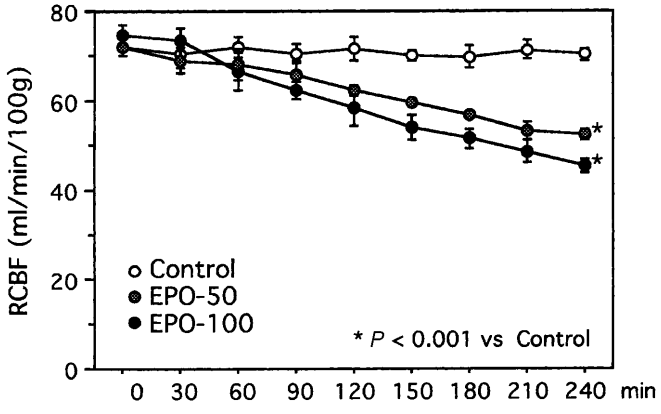

Fig. 1 Renal cortical blood flow (RCBF) of groups 1, 2, and 3

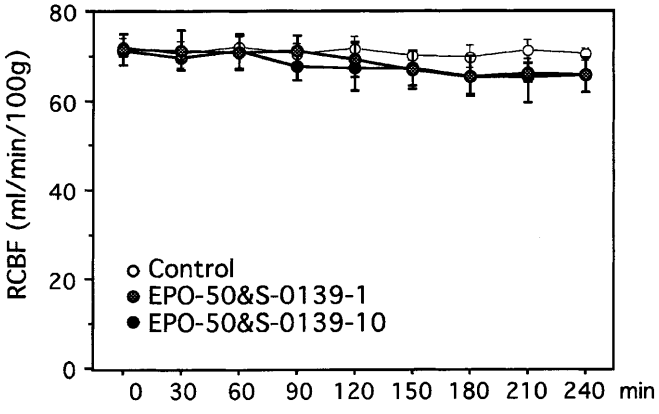

Fig. 2 NCBF of groups 1, 4, and 5

Table 1 Results of experiments 1 and 2. *Cr* createnine, *ET-1* endothelin-1, *RA* renin activity

	Cr (mg/dl)	ET-1 (pg/ml)	RA (ng/ml per hour)
Group 1 (Control; $n = 5$)	0.5 ± 0.1	2.7 ± 1.4	16.8 ± 6.1
Group 2 (EPO-50; $n = 5$)	0.5 ± 0.1	4.2 ± 1.8*	16.3 ± 5.6
Group 3 (EPO-100; $n = 5$)	0.6 ± 0.1	4.4 ± 1.6*	17.2 ± 5.9
Group 4 (EPO-50 + S-0139-1; $n = 5$)	0.5 ± 0.1	3.8 ± 1.2*	15.9 ± 7.2
Group 5 (EPO-50 + S-0139-10; $n = 5$)	0.5 ± 0.2	4.1 ± 1.5*	16.5 ± 6.4

* $P < 0.03$ vs. Control

certainly stimulated ET_1 secretion. We think that a decrease of RCBF must be due to ET-induced vasoconstriction.

S-0139 is a nonpeptide selective ET-A receptor antagonist [5]. EPO-induced RCBF reduction was completely blocked by S-0139 administration at a dose of 4 mg/kg per hour. This is strong evidence that RCBF reduction is mediated by ET_1. Since ET_1 increased in S-0139-administered groups, the vasoconstrictive effect of ET_1 was thought to be blocked at the receptor site. Tojo et al. [12] has also reported that BQ-123, another type of selective ET-A receptor antagonist, has potential for preventing EPO-induced reduction of glomerular filtration rate. From our data, S-0139 also has this beneficial effect.

The detailed mechanism of ET_1 elevation is unknown. But a direct effect of EPO on endothelial cells is suspected because ET_1 elevation and RCBF reduction were observed within only 4 h.

In conclusion, EPO induces ET_1 secretion. The reduction of RCBF is due to ET_1-derived vasoconstriction. S-0139 has potential for preventing EPO-induced and ET_1-mediated RCBF reduction.

Acknowledgements This study was partly supported by a grant from the Renal Anemia Society Foundation.

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