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Possible mechanism for the anemia induced by candesartan cilexetil (TCV-116), an angiotensin II receptor antagonist, in rats

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Abstract

Candesartan cilexetil (TCV-116), an angiotensin II receptor antagonist, was administered orally to male F344/Jcl and Crj:CD (SD) rats at 1000 mg kg^{-1} day^{-1} for 1–28 days, and the possible mechanism for the anemia induced by TCV-116 was investigated. In the TCV-116 group, the erythrocyte count, hematocrit value and hemoglobin concentration were decreased by 7–8% as compared with the values in the control group after dosing for 28 days. The plasma and renal erythropoietin levels, the reticulocyte count in the peripheral blood and the erythroid cell count upon bone marrow examination were decreased on day 7, but there were no accompanying histopathological renal lesions. Renal blood flow was increased, and mean blood pressure was decreased after TCV-116. These results suggest that the primary cause of the anemia induced by TCV-116 treatment is the increase in renal blood flow followed by a decrease in erythropoietin production.

Keywords: Anemia; (Rat); TCV-116; Erythropoietin; Renal blood flow; Angiotensin II

1. Introduction

Candesartan cilexetil, (±)-1-(cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate (TCV-116), has been found in animal experiments to exhibit angiotensin II antagonistic activity (Shibouta et al., 1993; Inada et al., 1993). Dose-dependent decreases in the erythrocyte count, hematocrit value and hemoglobin concentration were observed in F344 rats after treatment with TCV-116 at 300 mg kg^{-1} day^{-1} or more (Table 1) (Sato et al., 1996; Naeshiro et al., 1997a).

Captopril and losartan potassium, an angiotensin converting enzyme inhibitor and an angiotensin II receptor antagonist, respectively, are known to cause anemic changes in Sprague–Dawley rats (Imai et al., 1985; Wong et al., 1991), and repeated administration of ramipril has been reported to reduce the plasma erythropoietin level in Wistar rats (Gould and Goodman, 1990). Erythropoietin is the principal physiological regulator of erythrocyte production. Colony-forming unit erythroid cells are late-stage erythroid precursor cells and targets of erythrocytes. An oxygen sensor in the kidney controls erythrocyte production by detecting oxygen supply and consumption (Bauer and Kurtz, 1989). A level of erythrocyte production inadequate to sustain erythropoiesis is probably the primary cause of renal anemia (McGonigle et al., 1984). The cause for a decrease in erythrocyte count after angiotensin converting enzyme inhibitor treatment is considered to be suppression of erythrocyte production as a result of increased renal blood flow (Hirakata et al., 1986; Bailey and Sizeland, 1989; Gould and Goodman, 1990).

The purpose of the present study was to investigate the possible mechanism for the anemia induced by TCV-116 in F344 and Sprague–Dawley rats. Firstly, general characteristics of TCV-116-induced anemia were assessed hematologically, biochemically and histopathologically using F344 rats (Experiment 1). Secondly, hemodynamic effects of TCV-116 were examined using Sprague–Dawley and F344 rats (Experiment 2). Thirdly, the effects of saline supplementation on the anemia-related changes were assessed to determine whether the pharmacological effect of

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Table 1
Erythrocyte count, hematocrit value and hemoglobin concentration in male F344 rats treated with TCV-116 for 4 weeks

Report	Dose (mg kg^{-1})	Erythrocyte count ($\times 10^4$ μl^{-1})	Hematocrit value (%)	Hemoglobin concentration (g%)
I	0	978 ± 13	47.1 ± 0.5	16.0 ± 0.2
	30	932 ± 20[a]	45.4 ± 0.8[a]	15.5 ± 0.3[a]
	100	918 ± 23[a]	44.6 ± 1.0[a]	15.4 ± 0.3[a]
	300	901 ± 28[a]	44.3 ± 1.1[a]	15.2 ± 0.4[a]
II	0	868 ± 20	47.2 ± 1.0	15.5 ± 0.3
	1000	765 ± 14[a]	41.9 ± 0.9[a]	14.0 ± 0.4[a]
	3000	743 ± 60[a]	40.2 ± 2.7[a]	13.6 ± 0.9[a]

[a] Significantly different from the respective control, $P < 0.01$.
I: Sato et al. (1996).
II: Naeshiro et al. (1997a).

TCV-116 is involved in the anemic changes (Experiment 3).

2. Materials and methods

2.1. Materials and chemicals

TCV-116 was synthesized at Takeda Chemical Industries, TCV-116 was suspended in a 5% gum arabic solution and administered orally to rats in each experiment. Ketamine hydrochloride and droperidol were purchased from Sankyo (Japan). The other chemicals used were of reagent grade.

2.2. Animals

The animals used were male F344/Jcl rats aged 6 weeks (weight; 74–118 g, Clea Japan, Japan) in Experiments 1, 2 and 3 and male Crj:CD(SD) rats aged 7 weeks (weight; 193–237 g, Charles River Japan, Japan) in Experiment 2. The animals were allowed free access to tap water and a powdered laboratory animal diet (CE-2, Clea Japan) and were housed in a temperature- and humidity-controlled room (20–26°C, 40–70%) with a 12-h light/dark cycle.

2.3. Experimental protocol

2.3.1. Experiment 1: assessment of anemia

TCV-116 was administered to F344/Jcl rats once daily at a dose of 1000 mg kg^{-1} day^{-1} for 7, 14 or 28 days (day 0 = the first dosing day). The dosage level was selected on the basis of the results of previously conducted toxicity studies of TCV-116 in F344 rats (Table 1): anemia without severe toxic changes was observed at 1000 mg kg^{-1} (Sato et al., 1996; Naeshiro et al., 1997a). The control animals received a 5% gum arabic solution in the same manner. About 24 h after dosing on day 6, 13 and 27, 10 animals in each control and treated group were killed and autopsied to assess anemia-related changes. Erythrocyte morphology and osmotic fragility were assessed on day 28. Bone marrow was examined on days 7 and 28.

2.3.2. Experiment 2: renal blood flow and blood pressure measurement

Crj:CD(SD) rats were used for measurement of renal blood flow. The age of 7 weeks was selected because a decrease in the plasma erythropoietin level was observed on day 7 in Experiment I (age of 7 weeks). It was found to

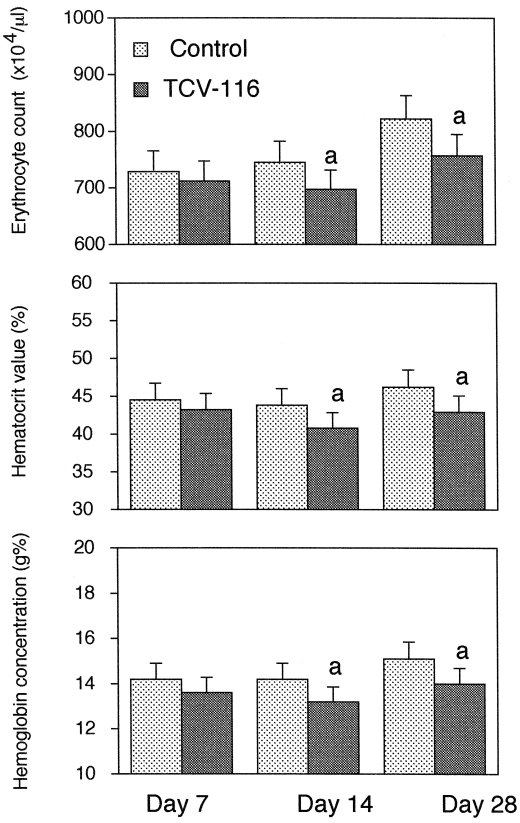


Fig. 1. Erythrocyte count, hematocrit value and hemoglobin concentration in F344 rats after administration of TCV-116 for 7, 14 and 28 days without saline supplementation. Results are expressed as means ± S.D. for 10 animals. (a) Significantly different from the time-matched control, $P < 0.01$.

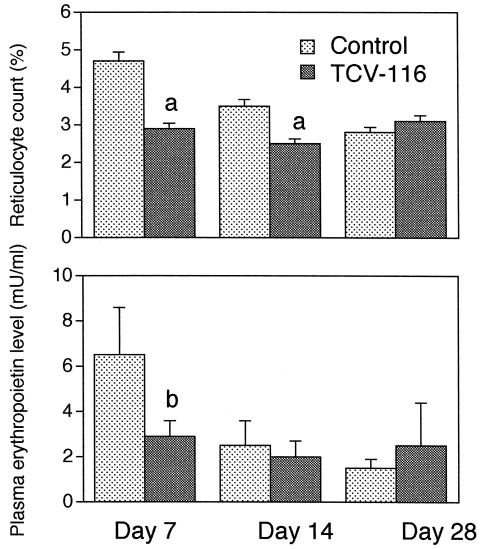


Fig. 2. Reticulocyte count and plasma erythropoietin level in F344 rats after TCV-116 for 7, 14 and 28 days without saline supplementation. Results are expressed as means ± S.D. for 10 animals. (a) Significantly different from the time-matched control, $P < 0.01$.

be difficult to operate on F344 rats at 7 weeks of age for measurement of renal blood flow because the animals were small and could not tolerate the surgical procedure (catheterization of the left ventricle). Furthermore, we confirmed that anemia and a decrease in the plasma erythropoietin level (13.3 ± 3.7 vs. 7.4 ± 3.3 mU ml^{-1}, $P < 0.01$) could be seen in Crj:CD(SD) rats treated orally with TCV-116 for 28 days. The animals were anesthetized with a mixture of ketamine hydrochloride (100 mg kg^{-1}, i.m.) and droperidol (2 mg kg^{-1}, i.m.). A catheter for measurement of blood pressure and sampling reference blood was positioned in the abdominal aorta via the right femoral artery. A second catheter for infusion of microspheres was inserted into the left ventricle via the right carotid artery. The day following surgery, mean blood pressure was measured before dosing using a pressure transducer (DX-360, Nihon Kohden, Japan), an amplifier for measurement of blood pressure (AP-641G, Nihon Kohden) and a polygraph (RM-6000, Nihon Kohden). TCV-116 was administered orally once at a dose of 1000 mg kg^{-1}. The dosage level was selected on the basis of the results of a hemodynamic study and Experiment I: TCV-116 reduced the mean blood pressure at 100 mg kg^{-1} in normotensive rats (Sato et al., 1997) and anemia without severe toxic changes was observed at 1000 mg kg^{-1}. Five h after the dose, mean blood pressure was measured in the same manner, and renal blood flow was then determined by the colored dye extraction microsphere method (Kowallik et al., 1991; Kanagawa et al., 1997). To investigate the effects of saline supplementation, the animals were allowed free access to saline in place of tap water beginning 5–7 days before the single dose of TCV-116.

F344 rats were used for measurement of blood pressure. The animals were anesthetized with a mixture of ketamine hydrochloride and droperidol. A catheter for measurement of blood pressure was positioned in the abdominal aorta via the right femoral artery. TCV-116 was administered at a dose of 1000 mg kg^{-1} orally for 7 days. The day following surgery, mean blood pressure was measured before the 7th dose of TCV-116 using a pressure transducer (DX-360), an amplifier for measurement of blood pressure (AP-641G) and a polygraph (RM-6000). Eight hours after the 7th dose, mean blood pressure was measured in the same manner.

2.3.3. Experiment 3: saline supplementation

The protocol was essentially the same as that for Experiment 1 except for the drinking fluid. Erythrocyte morphology and osmotic fragility were not examined. Bone marrow was examined on day 28. Physiological saline (a 0.9% NaCl solution) was given as the drinking fluid in place of tap water beginning 1 day before the start of treatment.

2.4. Hematological and biochemical examinations

Blood samples were withdrawn from the abdominal aorta with heparinized syringes under ether anesthesia. Part of the heparinized blood was used for hematology and to determine the osmotic fragility and morphology of the erythrocytes, and the remainder was centrifuged to obtain

Table 2
Erythrocyte parameters in F344 rats after administration of TCV-116 for 28 days with saline supplementation

Days on drug	Group	Erythrocyte count ($\times 10^4$ ml^{-1})	Hematocrit value (%)	Hemoglobin concentration (g%)	Reticulocyte count (%)	Plasma erythropoietin level (mU ml^{-1})
7	Control	744 ± 25	44.9 ± 1.7	14.3 ± 0.3	4.3 ± 0.4	5.6 ± 2.2
	TCV-116	744 ± 32	45.1 ± 2.3	14.3 ± 0.4	4.0 ± 0.8	4.9 ± 2.2
14	Control	752 ± 10	44.0 ± 0.5	14.3 ± 0.2	3.1 ± 0.3	3.4 ± 1.1
	TCV-116	740 ± 17	43.4 ± 0.9	14.0 ± 0.3	3.5 ± 0.4	4.3 ± 1.1
28	Control	822 ± 23	46.3 ± 1.0	15.1 ± 0.3	2.7 ± 0.5	1.5 ± 0.7
	TCV-116	823 ± 15	46.2 ± 0.8	15.2 ± 0.3	2.4 ± 0.6	1.5 ± 0.7

Results are expressed as means ± S.D. for 10 animals.
Not statistically significant.
Control animals received 5% gum arabic solution with saline supplementation.

Table 3
Bone marrow examination in F344 rats after administration of TCV-116 for 7 days without saline supplementation

	Control	TCV-116
Erythrocyte series (total)	90.2 ± 7.8 (100.0)	65.7 ± 7.6[a] (100.0)
Proerythroblasts	1.6 ± 0.3 (1.8)	1.0 ± 0.7[b] (1.5)
Basophilic erythroblasts	5.4 ± 1.5 (6.0)	3.6 ± 1.4[b] (5.7)
Polychromatic erythroblasts	49.4 ± 7.7 (54.8)	35.9 ± 6.0[a] (54.5)
Orthochromatic erythroblasts	31.8 ± 6.0 (35.3)	24.2 ± 4.1[a] (36.9)
Mitotic erythroid cells	1.8 ± 0.7 (2.1)	0.9 ± 0.4[a] (1.4)
Myeloid series (total)	41.4 ± 5.8	44.4 ± 6.8
Neutrophilic series (total)	37.1 ± 5.9	40.2 ± 6.1
Myeloblasts	0.3 ± 0.2	0.3 ± 0.2
Promyelocytes	0.7 ± 0.3	0.7 ± 0.3
Myelocytes	5.6 ± 1.6	4.8 ± 0.7
Metamyelocytes	9.3 ± 1.8	10.4 ± 2.8
Staff form nuclear cells	8.3 ± 2.0	9.4 ± 1.4
Polymorphonuclear cells	12.4 ± 2.8	14.1 ± 3.2
Mitotic neutrophilic cells	0.4 ± 0.3	0.5 ± 0.2
Eosinophils (total)	4.2 ± 1.2	4.2 ± 1.1
Basophils (total)	0.0 ± 0.1	0.0 ± 0.0
Others (total)	90.0 ± 12.8	92.9 ± 7.2
Monocytes	1.4 ± 0.9	1.5 ± 0.7
Lymphocytes	81.3 ± 12.1	83.9 ± 7.7
Plasma cells	0.1 ± 0.1	0.1 ± 0.1
Reticulum cells	1.4 ± 0.4	1.7 ± 0.6
Megakaryocytes	1.9 ± 0.9	1.5 ± 0.5
Mast cells	3.9 ± 0.9	4.2 ± 0.7
Total of nucleated cells	221.6 ± 16.5	203.0 ± 14.4[a]
Myeloid/erythroid	0.4 ± 0.1	0.6 ± 0.1[a]

Results are expressed as means ± S.D. for 10 animals.
Number of nucleated cells in bone marrow (10^4 mm^{-3}).
Numbers in parentheses denote percentage of the total erythroid cell count.
Significantly different from the control, [a] $P < 0.01$, [b] $P < 0.05$.
Control animals received 5% gum arabic solution without saline supplementation.

plasma for blood chemistry and determination of the erythropoietin level. The erythrocyte count, hematocrit value, hemoglobin concentration, leukocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were determined or calculated with an automated hematology analyzer (E-5000, Toa Medical Electronics, Japan). The reticulocyte count was done with an automated reticulocyte analyzer (R-1000, Toa Medical Electronics). Erythrocyte osmotic fragility was examined with a Coil planet centrifuge (Sanki Engineering, Japan) (Kitajima and Shibata, 1975). Whole blood was fixed with 1% glutaraldehyde and osmium tetroxide in 0.1 M phosphate buffer (pH 7.4), and then erythrocyte morphology was examined by light microscopy. The plasma urea nitrogen, creatinine, total bilirubin and lactate dehydrogenase activity were determined with an automated blood chemistry analyzer (Hitachi 7150, Hitachi, Japan) and standard reagents (Wako Pure Chemical Industries, Japan). The kidneys were removed and homogenized in 3 volumes of 10 mM phosphate-buffered saline (pH 7.4). The homogenates were centrifuged at $3000 \times g$ for 30 min, and the erythropoietin concentration in the supernatant was assayed (Fried et al., 1982).

2.5. Determination of erythropoietin

The plasma and renal erythropoietin levels were determined by an enzyme immunoassay using a commercially available kit (Immunoelitte EPO, Toyobo, Japan). Rat erythropoietin purified from the serum of anemic rats was a gift from Professor Ryuzo Sasaki (Kyoto University, Japan). The rat erythropoietin gave a dose–response line parallel to that obtained with the isolated human erythropoietin used as a standard in the kit.

2.6. Histopathological examination

The liver, kidneys, spleen and a femoral bone marrow sample were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin–eosin and examined by light microscopy.

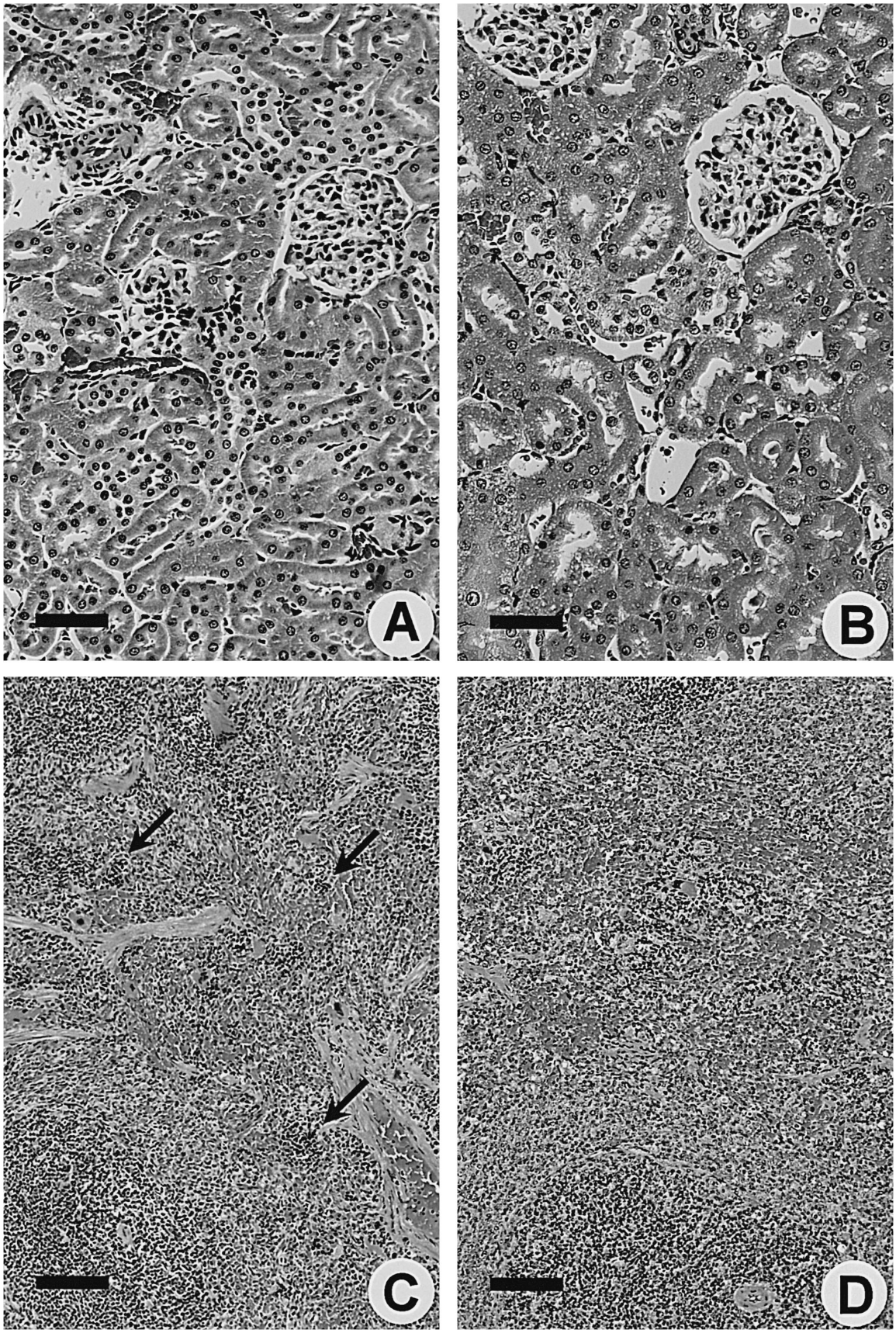
2.7. Bone marrow examination

Samples of the femoral bone marrow were collected and suspended in fetal bovine serum. The number of nucleated cells in the suspension was counted with an automated hematology analyzer (E-5000). Smears of bone marrow cells were made and stained with May–Giemsa's solution (Ramsell and Yoffey, 1961), and the differential count for 1000 nucleated cells was determined (Wintrobe, 1974).

2.8. Statistical analysis

Data are expressed as the means ± S.D. for 5 to 10 animals. Statistical analyses were performed between the TCV-116 group and time-matched control group using the F test for homogeneity of variance followed by Student's *t*-test or the Aspin and Welch *t*-test. All statistical tests

Fig. 3. Photomicrographs of the kidney and spleen. Kidney in a control (A) and TCV-116-treated F344 rat (B) without saline supplementation. (A, B) No abnormalities were observed on day 7 (×110, bar = 50 μm). Spleen in a control (C) and TCV-116-treated F344 rat (D) without saline supplementation. (C) Extramedullary hematopoiesis (arrows) was observed on day 7. (D) Decreased extramedullary hematopoiesis was observed on day 7 (×230, bar = 100 μm).


A
B
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D

were conducted at the 5 and 1% two-tailed probability levels.

3. Results

In the TCV-116 group without saline supplementation, decreases in the erythrocyte count, hematocrit value and hemoglobin concentration were observed on day 14 and to a lesser extent (7–8% reductions) on day 28 (Fig. 1). In the control group without saline supplementation, an increase in the erythrocyte count with time was observed from day 7 to day 28 (Fig. 1). In the TCV-116 group, there were no changes in mean corpuscular indices (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration), platelet count or leukocyte count on day 7, 14 or 28 (data not shown). There were also no abnormalities in morphology or osmotic fragility of the erythrocytes on day 28. The reticulocyte count in peripheral blood was decreased on days 7 and 14 (Fig. 2). The plasma erythropoietin level was decreased on day 7 but comparable to the value in the control group on days 14 and 28 (Fig. 2). In the control group, decreases in the reticulocyte count and plasma erythropoietin level with time were observed from day 7 to day 28 (Fig. 2). The renal erythropoietin level in the TCV-116 group was also decreased on day 7 (3.2 ± 0.7 vs. 1.8 ± 0.6 mU g^{-1}, $P < 0.05$). An increase in plasma urea nitrogen was seen on each examination day, but no changes were observed in plasma creatinine, total bilirubin or lactate dehydrogenase activity.

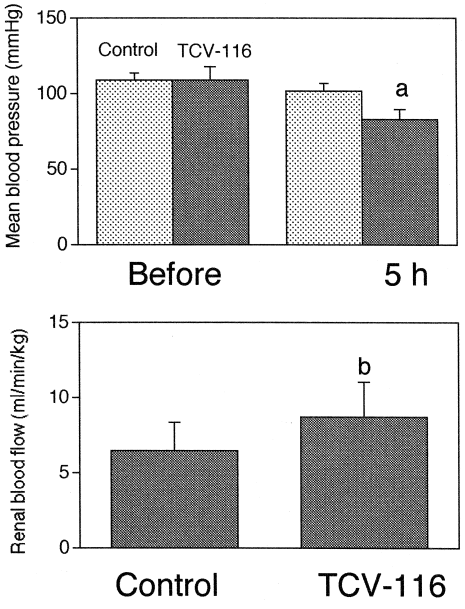

Fig. 4. Mean blood pressure and renal blood flow in CD(SD) rats 5 h after TCV-116 without saline supplementation. Results are expressed as means ± S.D. for 7–10 animals. (a) Significantly different from the control, $P < 0.01$. (b) Significantly different from the control, $P < 0.05$.

Table 4
Mean blood pressure in F344 rats after the 7th dose of TCV-116 without saline supplementation

Group	Mean blood pressure (mmHg)	
	Before dosing	8 h after dosing
Control	119 ± 9	116 ± 8
TCV-116	92 ± 7[a]	84 ± 7[a]

Results are expressed as means ± S.D. for 5–8 animals.
[a]Significantly different from the respective control, $P < 0.01$.
Control animals received 5% gum arabic solution.

The erythrocyte count, hematocrit value, hemoglobin concentration, reticulocyte count, plasma erythropoietin level and plasma urea nitrogen in the control animals and the TCV-116-treated animals receiving saline were comparable at all measurement times, indicating that the development of anemia was suppressed by saline supplementation (Table 2).

Upon bone marrow examination on day 7, the erythroid cell count was decreased, and the myeloid/erythroid ratio was increased in the TCV-116-treated animals not receiving saline (Table 3). There were no changes in the myeloid cell count or the ratio of any type of erythroid cell to the total erythroid cell count on day 7. Upon histopathological examination of the liver, kidneys, spleen and bone marrow, blood chemical and gross pathologic analyses and organ weight measurement on days 7, 14 and 28, no evidence of hemorrhage, hemolysis or compensatory erythropoiesis was observed in the TCV-116-treated animals not receiving saline. Mild basophilic changes in the renal tubules in the cortex and hypertrophy of the juxtaglomerular cells were observed on day 28; however, no lesions such as necrosis or degeneration were observed in the renal tubules or peritubular cells on any of the days when they were examined (Fig. 3B). Decreased extramedullary hematopoiesis was observed in the spleen on day 7 (Fig. 3D).

Renal blood flow was increased and mean blood pressure was decreased significantly 5 h after the single dose of TCV-116 as compared with the control value (Fig. 4). Also, mean blood pressure was decreased before and 8 h after the 7th dose of TCV-116 (Table 4). Saline supple-

Table 5
Mean blood pressure and renal blood flow in CD(SD) rats before and 5 h after a single dose of TCV-116 with saline supplementation

Time after dosing	Group	Mean blood pressure (mmHg)	Renal blood flow (ml min^{-1} kg^{-1})
Before	Control	108 ± 7	Not examined
	TCV-116	109 ± 7	Not examined
5 h	Control	106 ± 3	6.15 ± 2.16
	TCV-116	100 ± 8	6.59 ± 1.72

Results are expressed as means ± S.D. for 7–10 animals.
No statistical significance.
Control animals received 5% gum arabic solution with saline supplementation.

mentation suppressed the increase in renal blood flow and decrease in mean blood pressure caused by TCV-116 treatment (Table 5).

4. Discussion

Anemic changes were observed in rats and beagle dogs after repeated administration of TCV-116 (Sato et al., 1996; Ishimura et al., 1996; Sakura et al., 1996; Nishida et al., 1996; Naeshiro et al., 1997a). A slight anemia was also seen in rats after repeated administration of angiotensin converting enzyme inhibitors and an angiotensin II receptor antagonist (Imai et al., 1985; Iida et al., 1985; Kobayashi et al., 1989; Wong et al., 1991; Inui et al., 1992; Narama et al., 1993), and it was thought that the anemia and erythrocytic changes could be due to the suppression of erythropoietin production (LaRocca et al., 1986; Gould and Goodman, 1990). In the present study, TCV-116 induced a slight but significant reduction in the erythrocyte count, hematocrit value and hemoglobin concentration in rats receiving 1000 mg kg^{-1} day^{-1} daily for up to 28 days. Decreases in the reticulocyte count on days 7 and 14, and plasma erythropoietin level on day 7 were also observed. These findings suggest that the decrease in the plasma erythropoietin level and the subsequent reduction in the reticulocyte count are involved in the anemia.

Decreases in the plasma erythropoietin level and reticulocyte count and an increase in the erythrocyte count with age were observed in F344 rats from 6 to 10 weeks of age (Naeshiro et al., 1998), and similar changes were observed in the control animals from day 7 and day 28 (Figs. 1 and 2). Erythrocyte formation increased rapidly during the period of accelerated growth and erythrocyte formation and body weight gain were linearly correlated. When the erythrocyte count and body weight almost reached a plateau, the plasma erythropoietin level and reticulocyte count decreased and became almost constant. Therefore, it was considered that the plasma erythropoietin level and reticulocyte count were evidence of erythrocyte formation during the growth period. Decreases in plasma erythropoietin level and reticulocyte count with age have been reported previously (Bozzini et al., 1989; Kurtz et al., 1990).

Other hematological parameters such as mean corpuscular indices and morphology and osmotic fragility of the erythrocytes in the various groups were comparable at the end of the 4-week treatment period, indicating that there were no abnormalities in the erythrocytes. No evidence of hemorrhage, hemolysis or compensatory erythropoiesis was found on hematology, blood chemistry, bone marrow examination, gross pathology or histopathology analyses. The above findings suggested that the anemia induced by TCV-116 is not hemorrhagic or hemolytic. The decrease in the erythroid cell count observed on bone marrow examination on day 7 was not considered to be a direct effect of TCV-116 on the bone marrow because there were no changes in the myeloid cell count or the erythroid cell stages nor in the leukocyte count or platelet count on days 7, 14 and 28.

Erythropoietin production is regulated by the oxygen level. An oxygen sensor located in the kidney is thought to monitor oxygen pressure (Bauer and Kurtz, 1989). Hypoxia stimulates the production of erythropoietin and enhances erythrocyte formation, whereas hyperoxia lowers the erythropoietin level and decreases erythrocyte formation. When erythrocyte count and function are normal, the renal oxygen pressure or oxygen supply depends on renal blood flow. Under normal circumstances, there is a constant relationship between global renal blood flow and global renal oxygen consumption (Bauer and Kurtz, 1989). TCV-116 suppresses the renin–angiotensin system and reduces blood pressure by decreasing angiotensin II-induced peripheral vessel resistance as do angiotensin converting enzyme inhibitors (Harrap et al., 1990; Giudicelli et al., 1991; Oizumi et al., 1992; Wang et al., 1992) and other angiotensin II receptor antagonists (Wong et al., 1991). Also, a single dose and repeated administration of TCV-116 increased renal blood flow by decreasing renal vascular resistance in conscious spontaneously hypertensive rats (Kanagawa et al., 1997). Repeated administration of TCV-116 could cause a sustained increase in renal blood flow in rats because a sustained decrease in mean blood pressure was observed in normotensive rats after a single dose of TCV-116 at 100 mg kg^{-1} (Sato et al., 1997) and Experiment 2. In the present study, an increase in renal blood flow and a decrease in the plasma erythropoietin level were observed in rats after TCV-116 treatment. Therefore, suppression of erythropoietin production could be caused by the increased renal blood flow, due to the pharmacological effects of TCV-116, which gives a false signal to the oxygen sensor.

Gentamicin, an aminoglycoside, antibiotic and a nephrotoxic agent, causes extensive tubular necrosis and regeneration in the renal cortex (Rougemont et al., 1981; Heller, 1984). Anemia and a decrease in the plasma erythropoietin level have been observed in gentamicin-treated rats (Nagano et al., 1990: Naeshiro et al., 1997b). A major cause of gentamicin-induced anemia is considered to be a deficiency in erythropoietin during renal failure because the site of erythropoietin production is damaged (Fisher, 1980). Erythropoietin production was decreased in Experiment 1; however, the change was not thought to be due to impairment of the erythropoietin producing site because histopathological examination of the kidney revealed no lesions such as necrosis or degeneration of the tubular or peritubular tissues, the erythropoietin producing sites (Koury, 1988: Lacombe et al., 1988). Therefore, the renal basophilic tubules could not be considered to indicate regeneration of damaged tubules.

Concomitant saline supplementation prevented the anemia-related changes and suppressed the decrease in mean

blood pressure and increase in renal blood flow induced by TCV-116 (Tables 1, 2 and 4). The anemia observed after TCV-116 treatment was considered to be due to the pharmacological effects of this compound. With saline supplementation, the pharmacological action of the compounds, i.e., the effect of the renin–angiotensin system, is reduced because the physiological significance of the renin–angiotensin system in the maintenance of body fluid homeostasis is weakened, and the overall activity of the renin–angiotensin system is decreased. Also, it has been reported that the increased blood urea nitrogen level and decrease in erythrocyte count and heart weight after treatment with enalapril or imidapril are decreased or abolished by saline supplementation, and that these changes are caused by angiotensin converting enzyme inhibition (Bagdon et al., 1985; Kawai et al., 1992).

From these results, a possible mechanism for the anemia after TCV-116 treatment in rats could be as follows: (1) TCV-116 increases the renal blood flow due to its pharmacological effect resulting in an increase in oxygen supply to the kidneys, (2) erythropoietin producing cells in the kidneys do not produce a sufficient amount of erythropoietin and (3) the slightly decreased plasma erythropoietin level does not stimulate the bone marrow to produce a normal number of new erythrocytes.

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