Tetradecylthioacetic acid prevents the inflammatory response in two-kidney, one-clip hypertension

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Bivol LM, Berge RK, Iversen BM. Tetradecylthioacetic acid prevents the inflammatory response in two-kidney, one-clip hypertension. Am J Physiol Regul Integr Comp Physiol 294: R438–R447, 2008. First published November 21, 2007; doi:10.1152/ajpregu.00590.2007.—ANG II promotes inflammation through nuclear factor-κB (NF-κB)-mediated induction of cytokines and reactive oxygen species (ROS). The aim of the present study was to examine the effect of tetradecylthioacetic acid (TTA), a modified fatty acid, on NF-κB, proinflammatory markers, ROS, and nitric oxide (NO) production in two-kidney, one-clip (2K1C) hypertension. The 2K1C TTA-treated group had lower blood pressure (128 ± 3 mmHg) compared with 2K1C nontreated (178 ± 5 mmHg, P < 0.001). The p50 and p65 subunits of NF-κB were higher in the clipped kidney (0.44 ± 0.01 and 0.22 ± 0.01, respectively) compared with controls (0.25 ± 0.03 and 0.12 ± 0.02, respectively, P < 0.001). In the 2K1C TTA-treated group, these values were similar to control levels. The same pattern of response was seen in the nonclipped kidney. In 2K1C hypertension, cytokines plasma were higher than in control: TNF-α was 13.5 ± 2 pg/ml (P < 0.03), IL-1β was 58.8 ± 10 pg/ml (P = 0.003), IL-6 was 210 ± 33 pg/ml (P < 0.001), and monocyte chemoattractant protein-1 was 429 ± 21 pg/ml (P = 0.04). In the 2K1C TTA-treated group, these values were similar to controls, and the same pattern was seen in the clipped kidney. Clipping increased 8-iso-PGF-2α (P < 0.01) and decreased NO production (P < 0.01 vs. control) in the urine. TTA treatment normalized these values. NO production was also lower in clipped and nonclipped kidney (P < 0.001). After TTA treatment, these values were similar to controls. The results indicate that TTA has a potent anti-inflammatory effect in 2K1C by inhibition of p50/p65 NF-κB subunit activation, reduction of cytokines production and ROS, and enhanced NO production. The present study was designed to assess the impact of TTA treatment on renal proinflammatory and radical oxidant marked.

Hypertension plays an important role in development of chronic renal disease. Inhibition of the renin-angiotensin system (RAS) is known to reduce progression of renal damage, indicating that RAS activation is an important mediator in the development of chronic renal disease (22). Angiotensin II (ANG II) is involved in the development of hypertension, as is seen in the two-kidney, one-clip (2K1C) hypertension (25, 31, 32), but ANG II is also involved in vascular and tubulointerstitial inflammation (39), which are both important mechanisms in the progression of kidney diseases. Due to activation of these systems, 2K1C hypertension is a suitable model to study drugs that may have an effect on both hypertension and inflammation.

Inflammatory cytokines and chemokines (38), imbalance between nitric oxide (NO) and reactive oxygen species (ROS) production (2, 27), have been shown to contribute to blood pressure elevation and to the progression of renal disease. Recruitment of inflammatory cells in the renal tissue is seen in various renal diseases, either immunemediated or hypertension induced (36). Despite the link between the inflammatory response and hypertension, the molecular mechanisms of this relationship are poorly understood. ANG II-induced expression of cytokines and chemokines is mediated by nuclear factor-κB (NF-κB) (14, 42), a major initiator of intracellular inflammation pathways. However, other triggers of NF-κB activation may also be involved (4, 28, 43). The active state of NF-κB induces further the activation of numerous proinflammatory genes, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) (41). Several studies have shown that NF-κB inhibition suppresses the tissue injury in the kidney. On the other hand, NF-κB is known to be a strong activator of ROS (10). An increased level of ROS may cause tissue injury, as well as decreased bioavailability of NO (34), which plays an important role in functional alterations of endothelial vascular cells and may contribute to smooth muscle cell hypertrophy and remodeling of the vascular wall in hypertension.

Tetradecylthioacetic acid (TTA) is an analog fatty acid, with a carbon atom substituted with sulphur one in the third position of the carbon chain, counted from the carboxyl end of the normal saturated fatty acid (1). Due to this substitution, TTA does not undergo mitochondrial β-oxidation (6, 7). TTA is known to be a stimulator of all three types of peroxisome proliferator activated receptors (PPARs) (7), but also acts via PPAR-independent pathways (5). It had been shown that TTA attenuates the development of renal hypertension (8, 19), but does not affect established genetic high blood pressure, as seen in the spontaneously hypertensive rat (SHR) (8).

TTA reduces the inflammation in circumstances not connected with hypertension (17, 18). It is generally accepted that drugs that interfere with RAS, such as angiotensin-converting enzyme inhibitors and AT1-receptor (AT1R) blockers, reduce vascular and tubular inflammation in chronic kidney disease (11, 13, 37), but, in contrast to TTA, these drugs also lower the blood pressure in SHR. An important issue that needs to be explored is whether TTA has any effect on inflammation in hypertension.

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ers. Our working hypothesis was that TTA inhibits activation of NF-κB, reduces production of cytokines and ROS, and increases NO production.

MATERIALS AND METHODS

Animals. The study was performed in 24 Wistar male rats, Han.Tac: WH M from Møllegaard Breeding Colony (Skensved, Denmark), with a body weight of 180–200 g at the start of the study. The experiments were performed in accordance with, and under the approval of, the Norwegian State Board for Biological Experiments, the Guide for the Care and Use of Laboratory Animals, and the Guidelines of the Animal Welfare Act.

The rats were kept in cages with constant temperature (25°C) and humidity. They were exposed to a 12:12-h light-dark cycle (light from 0600 to 1800) and had unrestricted access to tap water and food.

Induction of 2K1C hypertension. The right kidney was exposed through a lumbar incision, and the right renal artery was dissected free and clipped by a rigid U-shaped silver clip with an internal opening of 0.25 mm. The animals were anesthetized by isoflurane anesthesia 1 l/min (Forene-Abbott) mixed with 2 l/min O₂ using an Omehda Isotec 3 anesthesia utility (BOC-Health Care). The rats received Temgesic 1 ml/kg body wt subcutaneously, immediately after surgery and for the following 2 days. The rats were followed for the next 6 wk when plasma, urine, and tissue collections were made.

Measurements of blood pressure. Systolic blood pressure (SBP) was measured in unanesthetized animals before clipping and weekly during the development of hypertension by means of the tail-cuff method (UGO BASILE). Before each measurement, the rats were warmed to 35°C for 10 min in a cupboard.

The study groups. The animals were randomized in four groups: two control groups (one TTA treated, one nontreated) and two groups with 2K1C hypertension (one TTA treated, one nontreated). Six animals were used in each group. One plasma and two urine samples per animal were collected, in addition to one kidney sample per animal in the control groups (collected from the left kidney) and two kidney samples per animal from the 2K1C groups (one collected from the clipped and other from the nonclipped kidney). Urine was collected both before clipping or start of TTA treatment and 6 wk later, when plasma and renal cortex tissue were also collected. Plasma and urine were used for measurement of cytokines, NO production, and 8-iso-PGF-2α. The renal cortex was used for measurement of NF-κB, cytokines, and NO production. The controls and 2K1C hypertensive nontreated groups were fed with standard pellet rat food (0.25 g/Na, 14.7% proteins), whereas the TTA-treated controls and 2K1C hypertensive groups were given food pellets mixed with TTA dissolved in acetone (2.7 kg pellet to 9 g TTA in 1 liter acetone). Acetone was allowed to evaporate for 3 days before administration. To ensure that TTA and acetone did not change the food intake, the amount of food was monitored for 2 wk in all four groups and was found similar.

Collection of urine. Urine was collected on ice for 24 h using metabolic cages. The urine volume was recorded, and 2 ml of each sample were filtered and frozen at −80°C until analyzed.

Collection of plasma. The rats were anesthetized with pentobarbital sodium (65 mg/kg body wt) 6 wk after the start of experiments, and blood samples were collected on ice in EDTA-coated collection tubes. Samples were gentle rocked for 30 s and then centrifuged at 1,800 g for 15 min at 4°C. The supernatant was kept at −80°C until analyzed.

Measurements of the p50/65 heterodimer subunits of NF-κB (p50/65 NF-κB) from renal cortex. An assay kit from Chemicon International was used (CA 92590). In short, during the assay, the capture probe, a double-stranded biotinylated oligonucleotide containing the flanked DNA consensus sequence for NF-κB (5’-GGGACTT-TCC-3’), was mixed with nuclear extract from the samples in transcription factor assay buffer containing salmon sperm DNA to block nonspecific DNA binding activity. The mixture of the samples of nuclear extraction, probe, and buffer were transferred to the streptavidin-coated plate. The active NF-κB protein was immobilized on the biotinylated double-stranded oligonucleotide capture probe bound to the streptavidin plate well, and inactive unbound material was washed away.

The NF-κB transcription factor subunits, p50 and p65, were detected with specific rabbit primary antibodies anti-NF-κB p50 and anti-NF-κB p65. A highly sensitive horseradish peroxidase-conjugated secondary antibody was used for the colorimetric detection read in a plate reader at dual 450/650 nm. The results were expressed by optical density at 450/650 nm.

Measurements of cytokines and chemokines from renal cortex and plasma. A multiplex assay kit (Linco Research, RCYTO-80K-PMX) was used for simultaneous quantification of the following cytokines and chemokine: TNF-α, IL-1β, IL-6, and MCP-1. The cytokine/chemokine multiplex assay uses microbeads based on the luminex technology. In the quantitative assay, surfaces of fluorescence-coated microbeads were conjugated to specific capture antibody able to identify the specific target analyte. All of the samples were centri-

Table 1. Raw food, Na⁺ intake, and body weight of the studied groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control TTA</th>
<th>2K1C</th>
<th>2K1C TTA</th>
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<td>Raw food intake, mg/day</td>
<td>25.1 ± 1.2</td>
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<tr>
<td>Na⁺ intake, mmol/day</td>
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<td>Body weight before, g</td>
<td>212 ± 4</td>
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<td>Body weight after 3 wk, g</td>
<td>286 ± 4</td>
<td>283 ± 3</td>
<td>280 ± 5</td>
<td>267 ± 3</td>
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Values are means ± SE. TTA, tetradecylthioacetic acid; 2K1C, two-kidney, one-clip hypertension. *P = 0.04, lower body weight of 2K1C TTA-treated animals compared with 2K1C group.
fuged again at 3,000 g for 5 min before assay setup and were assayed in duplicate. In short, the plasma and urine samples were diluted 1:5 in 5 ml serum containing 0.08% sodium azide, reconstituted with 1 ml deionized water, and 4-ml PBS with 0.08% sodium azide and 1% bovine serum albumin. The tissue samples were diluted 1:7 with lysis buffer used for homogenization, followed by incubation with specific cytokine primary antibodies at 4°C for 20 h. The secondary antibody was added the next day and was incubated at room temperature for 2 h. The streptavidin-phycoerythrin was used as a detection dye, and the fluorescence was measured with Luminex. The results are determined based on a five-parameter standard curve generated automatically.

Measurements of NO by nitrate/nitrite concentration in plasma, urine, and renal cortex. The nitrate/nitrite (NO$_3$/NO$_2$) concentrations were measured by lactate dehydrogenase colorimetric assay (Cayman Chemical, no. 760871) based on Griess reaction (26). Each sample of plasma, urine, and renal cortex from both clipped and nonclipped kidney was assayed in duplicate. In short, the NADPH, NO$_2$ reductase, and coenzyme factors were added, and the samples were incubated for 3 h at room temperature. Thereafter, the Griess reagents [sulphanilamide and N-(1-naphyl) ethylenediamine] were added, and the absorbance was read at 540 nm after 10-min incubation at room temperature. The results were expressed in micromoles per milliliter for plasma and urine and micromoles per gram for tissue.

Measurements of 8-iso-PGF-2α concentration in urine. The 8-iso-PGF-2α from urine was analyzed by an enzymatic immunoassay (Cayman Chemical, no. 51653), as a marker of oxygen radical production (15). The assay was based on the competition between 8-iso-PGF-2α and an 8-iso-PGF-2α-acytcholylcholsterase (AChE) conjugate (tracer) for a limited number of 8-iso-PGF-2α-specific rabbit antiserum binding sites. A molecule of the analyte covalently attached to a molecule of AChE (serves as the tracer). Quantification of the tracer was achieved by measuring its AChE activity with Ellman’s reagent. The urine samples, taken at the beginning and at the end of the study, were purified by using a purification kit (Cayman Chemical, no. 416359) and diluted 1:10 with assay buffer. Each sample was assayed in duplicate. The tracer and antiserum were added, and the samples were incubated at room temperature for 18 h. The Ellman’s reagent was added, and the absorbance was read at 410 nm.

Statistical methods. Data are presented as means ± SE. Statistical analyses were done using SPSS software for Windows 2003. Differences between groups were assessed by ANOVA, with post hoc tests using Scheffe adjustment for multiple comparisons. P values <0.05 were considered statistically significant.

RESULTS

Food, Na$^+$ intake, and body weight before and after the measurements. The raw intake of food, as well as Na$^+$ intake, was similar in all studied groups, independent of TTA treatment (Table 1). The initial body weight of the animals was also similar (Table 1), but 2 wk later, the body weight in the 2K1C TTA-treated group was significantly lower than in the 2K1C nontreated group or controls (Table 1).

SBP. SBP was measured before clipping and weekly for the following 6 wk in all groups. In the 2K1C hypertensive group, the SBP increased from 116 ± 2 to 178 ± 5 mmHg (P < 0.001) 6 wk after clipping. In 2K1C TTA group, the treatment attenuated the development of high blood pressure. SBP was 128 ± 3 mmHg 6 wk after clipping (P < 0.001, compared with 2K1C untreated), but higher than in controls (112 ± 3 mmHg,
p50/65 NF-κB concentration in kidney cortex. The p50/65 subunits of NF-κB were similar in controls, independent of treatment (Fig. 2, left). The p50 subunit was 0.25 ± 0.03 in the nontreated group and 0.27 ± 0.03 in the TTA-treated group, while the p65 subunit was 0.12 ± 0.02 in the nontreated and 0.13 ± 0.01 in the TTA-treated group. Both NF-κB subunits were higher in the clipped kidney of the 2K1C group compared with both controls (0.44 ± 0.01 for p50 and 0.22 ± 0.01 for p65, P < 0.001 for both subunits). After TTA treatment in the 2K1C group, p50 was 0.20 ± 0.01 and p65 was 0.10 ± 0.01, both not different from controls (Fig. 2, middle). The same pattern was observed also in the nonclipped kidney of the 2K1C group, where the subunits of NF-κB were higher compared with the control group (0.36 ± 0.01 for p50 and 0.17 ± 0.005 for p65, P = 0.01 for both subunits). NF-κB subunits in the nonclipped kidney of the 2K1C TTA group were 0.26 ± 0.03 for p50 and 0.12 ± 0.01 for p65 (both values were different from controls) (Fig. 2, right).

**Plasma and tissue TNF-α.** TNF-α concentration in plasma was similar in the control groups. It was 7.4 ± 0.5 pg/ml in the nontreated control and 7.3 ± 0.3 pg/ml in the TTA-treated control group. In the nontreated 2K1C hypertensive animals, the TNF-α concentration was 13.5 ± 2 pg/ml (P = 0.03, compared with control). In the 2K1C TTA-treated group, TNF-α concentration was 6.6 ± 0.8 pg/ml, not different from control plasma values (Fig. 3A).

TTA treatment did not affect TNF-α concentration in the renal cortex from the control rats (Fig. 4A). It was 77.5 ± 2 pg/g in nontreated control and 80.6 ± 3 pg/g in the TTA-treated control group. In the clipped kidney, TNF-α was 129.7 ± 4 pg/g (P < 0.001, compared with control), and, after TTA treatment, the TNF-α in the 2K1C TTA-treated group was 99.3 ± 1 pg/g, lower than in untreated 2K1C (P = 0.01), but higher than in control (P = 0.02) (Fig. 4B). In the nonclipped kidney, the TNF-α concentration was lower than control (64.5 ± 4 pg/g, P = 0.02, Fig. 4C), but, in the 2K1C TTA-treated group, TNF-α concentration was similar to control value (Fig. 2C).

**Plasma and tissue IL-1β.** IL-1β concentration in plasma was similar in the control groups. It was 20.6 ± 2 pg/ml in the nontreated control group and 19.1 ± 2 pg/ml in the TTA-treated control group. In the nontreated 2K1C hypertensive animals, IL-1β concentration was higher than controls (58.8 ± 10 pg/ml, P = 0.003), whereas, in the TTA-treated 2K1C animals, IL-1β concentration was below control values (12.9 ± 1 pg/ml, P = 0.01, Fig. 3B).

IL-1β concentration in renal cortex of the control animals did not change with TTA treatment (Fig. 4D). It was 221 ± 8 pg/g in nontreated control and 225 ± 21 pg/g in TTA-treated control group. In the clipped kidney, IL-1β concentration was higher than in controls (414 ± 26 pg/g, P < 0.001). In the 2K1C TTA-treated group, IL-1β was similar to control values.

![Graphs](http://ajpregu.physiology.org/)
**in MCP-1 in the clipped kidney compared with control (Fig. 4G).** In the renal cortex from the clipped kidney of the nontreated group, IL-6 was similar to that in the control rats (33±6 pg/ml) (Fig. 3C).

The IL-6 concentration in the renal cortex from the controls was similar, independent of treatment. It was 833±63 pg/g in control nontreated and 945±66 pg/g in TTA-treated control group (Fig. 4G). In the renal cortex from the clipped kidney of the nonclipped group, IL-6 was 1,184±111 pg/g (P = 0.01, compared with controls). The TTA-treated group had IL-6 concentration similar to the controls (737±80 pg/g, Fig. 4H).

In the renal cortex from the nonclipped kidney of nonclipped animals, IL-6 was lower than control values (460±106 pg/g; P = 0.02). In the 2K1C TTA-treated group, IL-6 was similar to control values in the nonclipped kidney (802±94 pg/g, Fig. 4f).

**Plasma and tissue MCP-1.** MCP-1 concentrations in plasma were similar in the controls, independent of TTA treatment. It was 362±23 pg/ml in nontreated control and 329±30 pg/ml in TTA-treated control group. In 2K1C, MCP-1 in plasma was 429±21 pg/ml (P = 0.04, compared with control), while in the 2K1C TTA-treated group, MCP-1 concentration was not different from control values (303±24 pg/ml, Fig. 3C).

MCP-1 concentration in the renal cortex from the control groups was similar, independent of treatment. It was 935±95 pg/g in control nontreated and 1,169±358 pg/g in TTA-treated control group (Fig. 2J). In the renal cortex from the clipped kidney, MCP-1 was 3,889±341 pg/g (P < 0.001, compared with control). In the TTA-treated 2K1C group, MCP-1 was lower than in 2K1C-untreated group (2,071±189 pg/g, P = 0.01), but still higher than in the control group (P = 0.02, Fig. 2K).

In the renal cortex from the nonclipped kidney of the 2K1C group, MCP-1 was 3,554±214 pg/g (P = 0.02, compared with control), and in the 2K1C TTA-treated group, MCP-1 was lower than in 2K1C untreated (2,028±276 pg/g, P = 0.01, Fig. 2L), but still higher than in the control group (P = 0.02).

**Plasma, tissue, and urine NO2/NO3 concentration.** The NO3/NO2 concentrations in plasma from controls were similar, independent of TTA treatment (90±15 μmol/ml in control nontreated, 101±22 μmol/ml in control TTA treated). In the 2K1C group the NO3/NO2 was lower than in controls (35±5 μmol/ml, P = 0.006), while, in the TTA-treated group, NO3/NO2 was higher than in the untreated 2K1C group (53±3 μmol/ml, P = 0.03). NO2/NO3 in this group was lower than in untreated control (P = 0.03, Fig. 5a).

NO2/NO3 in renal cortex was similar, independent of TTA treatment in control groups (Fig. 5a). It was 4.2±0.3 μmol/g in control nontreated and 6.1±0.1 μmol/g in control TTA treated. In the clipped kidney of 2K1C, renal NO2/NO3 was lower compared with control (1.8±0.3 μmol/g, P < 0.001).

In the 2K1C TTA-treated group, NO2/NO3 was similar to control value (5.1±0.3 μmol/g, Fig. 5B).

**Radical oxidant marker (8-iso-PGF-2α in urine).** 8-iso-PGF-2α concentration in urine was measured in all groups before and 6 wk after clipping and start of TTA treatment. Urine NO3/NO2 was not different between groups before clipping or start of TTA treatment (Fig. 6A). After 6 wk, the urine NO3/NO2 remained unchanged in control groups, independent of treatment (Fig. 6B). Clipping reduced NO3/NO2 to 35±9 μmol/ml at the end, compared with 71±12 μmol/ml before the experiments (P = 0.04). These values were lower than control (78±16 μmol/ml, P = 0.02). TTA treatment increased urine NO3/NO2 in the 2K1C group, from 74±16 μmol/ml before to 180±6 μmol/ml after 6 wk (P < 0.001, Fig. 6B).

**DISCUSSION**

The main finding in the present study is that TTA has a strong effect on inflammatory markers and oxidative stress in renal hypertension. We have shown that TTA treatment 1) reduces activation of NF-κB p50/p65 subunits in the renal cortex of the clipped and nonclipped kidney; 2) inhibits the activation of TNF-α, IL-1β, IL-6, and MCP-1 in plasma and the clipped kidney of 2K1C; 3) increases NO production in plasma, urine, and renal tissue from the clipped kidney; and 4) inhibits the production of 8-iso-PGF-2α synthesis. Overall, these results indicate that TTA prevents activation of inflammation and increased oxidative stress in renovascular hypertension. The present study adds new information regarding the different inflammatory responses in the clipped and nonclipped kidney in 2K1C hypertension.

2K1C is a classic model of renovascular hypertension, where ANG II plays a pivotal role in development and maintenance of the high blood pressure (25, 31, 33). ANG II production is enhanced due to the reduced perfusion pressure.
behind the clip (20). ANG II mediates vasoconstriction through the AT1R, increases salt reabsorption through the same receptor, and enhances aldosterone synthesis (3, 29). Differences in RAS activation in the clipped and nonclipped kidney have been described before (9, 21), findings that may explain the results from the present study. The concentrations of renin and ANG II are high in the clipped kidney. On the other hand, in the nonclipped kidney, the renin concentration is very low (32), while ANG II is increased, probably due to an active plasma uptake mechanism in this kidney (46).

TTA is a modified fatty acid known to attenuate the blood pressure development and prevent kidney damage in 2K1C hypertension (8, 19). Mechanisms shown to be involved in the blood pressure-lowering effect of TTA are reduction of renin mRNA levels in the clipped kidney (19), followed by decreased plasma renin activity and ANG II levels (8). It has also been shown that TTA has only a minor effect on the ANG II levels in the nonclipped kidney (8). As the level of ANG II in this kidney seems to be dependent on ANG II plasma uptake, it is reason to suggest that TTA has no effect on this mechanism. In the present study, we have confirmed previous reports that TTA did not completely normalize blood pressure, a finding that may be explained by the lack of full normalization of plasma ANG II levels (8, 19). The renal vascular response to exogenous ANG II is, however, increased in the nonclipped kidney, probably due to reduced circulating levels of ANG II after TTA treatment (8).

Inflammatory markers are increased in hypertension (40) and may contribute to vascular and tubulointerstitial inflammation followed by fibrosis (39). Cytokines are increased in models with high ANG II levels in both plasma and renal tissue (38), and ANG II is known to influence the synthesis of NF-κB, TNF-α, IL-1β, IL-6, and MCP-1 in hypertension (14, 41, 42). In the present study, we have shown that clipping enhanced levels of the p50/65 subunits of NF-κB in both kidneys, but with a stronger effect in the clipped than in the nonclipped kidney (8). The cytokine production was enhanced in the clipped kidney, while in the nonclipped kidney the pattern of response was different. MCP-1 was increased, IL-1β was unchanged, while TNF-α and IL-6 were reduced. That may be due to the different content of ANG II in the clipped and nonclipped kidney of 2K1C hypertensive animals, causing different ability to trigger the proinflammatory markers. All inflammatory markers were enhanced in plasma from 2K1C hypertensive rats. After the TTA treatment, the plasma cytokine concentrations were reduced to control levels. In the clipped kidney, the TTA treatment reduced the NF-κB synthesis and the levels of most cytokines, but TNF-α and MCP-1 did
not reach the control levels. In the nonclipped kidney, MCP-1 was increased after induction of 2K1C hypertension, and TTA treatment reduced MCP-1, but the concentration did not reach control levels. TTA had no effect on IL-1β in this kidney, and, surprisingly, TNF-α and IL-6 were enhanced. The explanation for these different patterns is not clear. However, it has been shown that IL-6 provides renal protection, independent of blood pressure through epoxygenase cytochrome P-450-dependent production, and this may be the case in the nonclipped kidney (24). TNF-α has been shown to protect the kidney by inhibition of renin gene expression via blocking of p65 subunit of NF-κB (43). The present study shows that TTA treatment enhanced TNF-α and reduced p65 subunit of NF-κB in the nonclipped kidney. Whether this finding may contribute to the low concentration of renin in the nonclipped kidney remains to be proven.

The present study investigated further the impact of TTA on the balance between NO and ROS production. Increased oxidative stress is known to contribute to the development of hypertension and renal damage (2, 27). Antioxidant treatment attenuates activation of NF-κB by reducing both oxidative stress and blood pressure (28). AT1R blockers attenuate blood pressure and suppress renal NF-κB activity and immune cell number in the kidney. It is well established that ANG II induced hypertension in the rat and humans (32) and is accompanied by increased oxidative stress in blood vessels (27). ROS production is known to quench the release of NO, and this may induce vasoconstriction due to loss of vasodilators (35). In the present study, clipping reduced NO2/NO3 level in both kidneys, and TTA treatment restored the NO2/NO3 levels. A similar pattern was also seen in plasma and urine. Furthermore, 8-iso-PGF-2α, as a marker of ROS production, was substantially increased in the hypertensive animals, as it has been shown in different types of hypertension, such as ANG II-infused rats (12), 2K1C hypertension (44), and SHR (45). 8-iso-PGF-2α is produced from free radical peroxidation of everted arachidonic acid and is an extremely potent renal vasoconstrictor (30). Our results show that TTA treatment reduced the 8-iso-PGF-2α to control values.

The reduced blood pressure, inflammation, and ROS production shown after TTA treatment in this study are in accordance with the effect of other PPAR stimulators on inflammation and oxidative stress (16) and may be explained by reduced ANG II production reported by previous studies (8, 19). We have gathered more information showing that TTA increases NO and reduces ROS production, mechanisms that both may have a blood pressure-lowering effect. The reduction of inflammatory markers induced by TTA may be mediated through reduced ANG II production, and there is reason to suggest that most of our results can be explained by this mechanism. However, we cannot exclude that TTA affects cytokine production by ANG II-independent pathways, and further studies are needed to explore how TTA may reduce high blood pressure and the inflammatory response. TTA is also known to reduce body weight due to its interference with lipid metabolism (23). We have earlier shown that TTA reduces body weight and downregulates angiotensinogen in the white adipose tissue (8, 19), and the results from the present study confirm these findings.

**Perspectives and significance.** This study adds new information regarding the inflammatory response in the two kidneys in 2K1C hypertension, a model with high levels of ANG II. The results indicate strong activation of inflammatory markers 6 wk after induction of hypertension when minor organ damage occurs. The reduced production of cytokine and ROS indicate that the drug reduces inflammatory markers of main importance in chronic renal disease. Further studies are needed to explore whether TTA could be a promising drug for treat-
ment of patients with renovascular hypertension, one major challenge in clinical practice.

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