Effect of Prolonged Nitric Oxide Synthesis Inhibition on Plasma Fibrinogen Concentration in Rats

Koh-ichi Sugimoto*, Shuichi Tsuruoka and Akio Fujimura
Department of Clinical Pharmacology, Jichi Medical School, 3311-1 Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan

Received August 21, 2000 Accepted November 20, 2000

ABSTRACT—We examined whether nitro-L-arginine-methyl ester (L-NAME) causes a sustained elevation in plasma fibrinogen concentration in rats. Oral dosing of L-NAME (100 mg/kg per day) for 7 days significantly raised plasma fibrinogen concentration in rats. The increase in plasma fibrinogen, however, returned to control levels by the treatment for more than 7 days, in spite of progressive hypertension. Candesartan failed to reverse the transient hyperfibrinogenemia, indicating that the rise in plasma fibrinogen may occur through the mechanisms other than angiotensin II receptor activation. These data suggest that a prolonged L-NAME treatment does not cause chronic hyperfibrinogenemia in rats.

Keywords: Nitro-L-arginine-methyl ester (L-NAME), L-NAME-induced hypertension, Fibrinogen

A number of investigations revealed that elevated fibrinogen is one of the independent risk factors for cardiovascular events (1, 2). Plasma fibrinogen concentrations are shown to be elevated in hypertensive patients (1, 2), often associated with other cardiovascular risk factors such as smoking, diabetes mellitus, obesity and hyperlipidemia. The combination of these risk factors enhances the chance of hypertensive patients for developing cardiovascular diseases. Therefore, to obtain a maximal preventive effect against cardiovascular events in patients with hypertension, a reduction in additional risk factors is also needed during antihypertensive therapy. Although the precise mechanism(s) of elevated plasma fibrinogen in hypertensive patients has not been fully understood, an antihypertensive agent with a fibrinogen-lowering effect would have great merit for the treatment of hypertension.

To examine the effects of various antihypertensive agents on plasma fibrinogen, an animal hypertensive model with a sustained elevation in plasma fibrinogen is useful. Previous studies showed that the inhibition of nitric oxide (NO) synthesis by nitro-L-arginine-methyl ester (L-NAME) raises blood pressure (BP) in animals (3, 4). In addition, a short-term (7 days) treatment with L-NAME elevates plasma fibrinogen concentration in rats (5). However, a change of plasma fibrinogen during a prolonged treatment with L-NAME was not examined in rats. In this study, we administered L-NAME to rats for 4 weeks and investigated the potential usefulness of L-NAME-treated rats as an animal hypertension model with hyperfibrinogenemia. We also examined the effect of candesartan cilexetil, an angiotensin II type 1 (AT1) receptor blocker, on plasma fibrinogen concentration in these animals, because the renin-angiotensin system is activated in L-NAME-induced hypertension (6, 7).

Experiments were performed in accordance with the Jichi Medical School Guide for Use of Laboratory Animals. Male Wistar rats were purchased from Japan SLC (Tokyo) and used for experiments at 8 weeks of age. All rats were kept under a specific pathogen-free environment with controlled temperature and humidity conditions. They were housed two per cage with free access to standard rat chow and water before and during the experiment. After the basal BP measurement, vehicle (distilled water) alone or L-NAME (Sigma Chemical Co., St. Louis, MO, USA) was given orally by gastric gavage twice daily to rats. To examine the dose-dependency of L-NAME, 30 and 100 mg/kg of the agent was given to animals for 7 days. The effect of an AT1-receptor blocker, candesartan cilexetil (Takeda Pharmaceutical Industries, Osaka), on the elevated plasma fibrinogen concentration induced by L-NAME was also determined. Candesartan cilexetil dissolved in a small amount of dimethyl sulfoxide (Wako Pure Chemicals, Osaka) was diluted in distilled water to adjust the dose. Candesartan or its vehicle was co-administered by gavage once daily. At the end of the treatment period, a blood sample for plasma fibrinogen was obtained from the abdominal aorta under pentobarbital anesthesia (50 mg/kg, i.p.). Citrated blood

*Corresponding author. FAX: +81-285-44-7562
E-mail: ksugi@jichi.ac.jp
To examine the effect of a prolonged treatment with L-NAME on plasma fibrinogen concentration, L-NAME (100 mg/kg, daily) was administered to rats for 4 weeks. At 2 weeks after the initiation of the treatment, a blood sample for plasma fibrinogen was obtained from the tail vein. Maximal care was taken to avoid blood coagulation during citrated blood collection. At the end of the treatment period, citrated blood was collected from the abdominal aorta under pentobarbital anesthesia. Systolic BP was measured by the standard tail-cuff sphygmomanometer (KN-210-1, Natsume Seisakusho, Tokyo) in awake, prewarmed rats. Plasma fibrinogen concentration was measured by the thrombin time method.

Data are expressed as the means ± S.E.M. Statistical differences were analyzed by analysis of variance followed by Scheffe’s test. Data between two groups were analyzed by the unpaired t-test. A P less than 0.05 was considered to be significant.

After the 7 day-treatment with L-NAME, systolic BP increased in a dose-dependent manner. Daily dose of 100 mg/kg, but not 30 mg/kg of L-NAME significantly caused the elevation in plasma fibrinogen concentration at the end of the treatment period (Table 1). To examine the effect of AT1-receptor blockade, candesartan was co-administered with L-NAME to rats. Co-administration with candesartan mildly reduced the L-NAME-induced BP elevation, but it was not significant. The increase in plasma fibrinogen concentrations induced by 7 day-treatment of L-NAME was not diminished by co-administration with candesartan (Table 1). Treatment with candesartan alone did not significantly influence BP or plasma fibrinogen concentration.

To determine BP and plasma fibrinogen concentration during a prolonged L-NAME treatment, the agent was administered for 4 weeks in the second experiment. Repeated administration of L-NAME for 4 weeks raised BP further in rats (Table 2). However, the elevation in plasma fibrinogen concentration was not detected at 2 and 4 weeks during treatment with L-NAME (Table 2).

Table 1. Changes in blood pressure and plasma fibrinogen concentration induced by L-NAME for 7 days and effects of co-administration of candesartan in male Wistar rats

| Treatment          | n  | Blood pressure (mmHg) | Plasma fibrinogen concentration (mg/dl) |
|--------------------|----|-----------------------|----------------------------------------|
|                    |    | basal 2 weeks 4 weeks | 2 weeks 4 weeks                        |
| Vehicle            | 8  | 141.8 ± 2.7 149.4 ± 2.2 | 151.8 ± 2.6 168.2 ± 3.6*               |
| L-NAME, 30 mg/kg   | 8  | 149.4 ± 2.2 145.7 ± 2.5 | 171.5 ± 4.3* 209.4 ± 10.4*           |
| L-NAME, 100 mg/kg  | 8  | 145.7 ± 2.5 147.2 ± 2.6 | 169.3 ± 2.2* 206.1 ± 9.2*           |
| Vehicle + Candesartan, 1.0 mg/kg | 8  | 144.4 ± 2.2 144.4 ± 2.2 | 169.3 ± 2.2* 206.1 ± 9.2*           |
| Vehicle + Vehicle  | 8  | 143.2 ± 1.9 143.2 ± 1.9 | 153.3 ± 2.1 183.6 ± 5.4              |
| Vehicle + L-NAME, 100 mg/kg | 8  | 144.4 ± 2.2 | 169.3 ± 2.2* 206.1 ± 9.2*           |
| L-NAME, 100 mg/kg + Candesartan, 0.3 mg/kg | 8  | 144.2 ± 1.3 144.2 ± 1.3 | 163.5 ± 2.5 217.6 ± 4.8**           |
| L-NAME, 100 mg/kg + Candesartan, 1.0 mg/kg | 8  | 142.5 ± 1.8 142.5 ± 1.8 | 165.2 ± 4.4 201.1 ± 5.8            |

*P<0.05, **P<0.01 vs the vehicle-treated rats.

Table 2. Changes in blood pressure and plasma fibrinogen concentration induced by a prolonged treatment with L-NAME in male Wistar rats

| Treatment          | n  | Blood pressure (mmHg) | Plasma fibrinogen concentration (mg/dl) |
|--------------------|----|-----------------------|----------------------------------------|
|                    |    | basal 2 weeks 4 weeks | 2 weeks 4 weeks                        |
| Vehicle            | 16 | 129.3 ± 4.0 133.8 ± 3.1 | 140.0 ± 2.5 166.0 ± 4.8*               |
| L-NAME, 100 mg/kg  | 16 | 133.8 ± 3.1 133.8 ± 3.1 | 141.4 ± 2.3 201.3 ± 3.8**             |

*P<0.05, **P<0.01 vs the vehicle-treated rats.
velopment and onset of cardiovascular diseases. A number of epidemiological studies have demonstrated that various inflammatory factors including fibrinogen contribute to the development of cardiovascular events (2). Although the mechanism of elevated plasma fibrinogen is unclear, it remains possible that inflammatory cytokines produced in some tissues enhance fibrinogen synthesis in the liver.

It has been shown that chronic NO synthesis inhibition by L-NAME induces marked monocyte infiltration into the coronary vessels associated with induction of monocyte chemoattractant protein-1 (MCP-1) during the first week of the treatment (8). Locally developed inflammation caused by NO synthesis inhibition could stimulate fibrinogen synthesis in the liver. In this study, a prolonged treatment with L-NAME failed to cause persistent hyperfibrinogenemia. At present, we have no explanation for the transient increase in plasma fibrinogen concentrations. Plasma fibrinogen concentration would progressively elevate in L-NAME-treated rats, if inflammatory changes last during chronic inhibition of NO synthesis with L-NAME. We think that some compensative mechanisms are involved in the lack of persistent hyperfibrinogenemia by chronic L-NAME treatment. For example, inhibition of NO synthesis with L-NAME induces the expression of transforming growth factor-β1 (TGF-β1) (9). TGF-β1 exhibits both proinflammatory and anti-inflammatory activities (10, 11). During a chronic inhibition of NO synthesis, TGF-β1 might act as an anti-inflammatory rather than a proinflammatory cytokine, resulting in a suppression of fibrinogen synthesis in the liver.

Candesartan cilexetil, an AT₁-receptor antagonist, did not influence plasma fibrinogen concentration in intact rats or L-NAME-treated rats. These data suggest that the increase in plasma fibrinogen induced by L-NAME may not be mediated by AT₁-receptor-related stimuli.

In summary, this study confirmed earlier findings that the short-term inhibition of NO synthesis increases plasma fibrinogen concentration associated with the BP elevation in rats. The increase in plasma fibrinogen concentration, however, returned to control levels along with the repeated L-NAME administration, indicating that prolonged inhibition of NO synthesis does not result in chronic hyperfibrinogenemia in rats. Short-term inhibition of NO synthesis raises plasma fibrinogen concentration probably through mechanisms other than AT₁-receptor activation. To examine the pathophysiology of hyperfibrinogenemia and the potential fibrinogen-lowering profile of antihypertensive agents, a hypertensive animal model with a persistent elevation of plasma fibrinogen concentration needs to be developed.

Acknowledgment
We wish to thank Mariko Hojo for excellent technical assistance.

REFERENCES
1. Kannel WB: Influence of fibrinogen on cardiovascular disease. Drugs 54, Suppl 3, 32 – 40 (1997)
2. Danesh J, Collins R, Applegey P and Petto R: Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease. JAMA 279, 1477 – 1482 (1998)
3. Lehera V, Salom MG, Miranda-Guardiola F, Moncada S and Romero JC: Effects of N³-nitro-L-arginine methyl ester on renal function and blood pressure. Am J Physiol 261, F1033 – F1037 (1991)
4. Johnson RA and Freeman RH: Sustained hypertension in the rat induced by chronic blockade of nitric oxide production. Am J Hypertens 5, 919 – 922 (1992)
5. Kawabata A: Evidence that endogenous nitric oxide modulates plasma fibrinogen levels in the rat. Br J Pharmacol 117, 236 – 237 (1996)
6. Pollock DM, Polakowski JS, Divish BJ and Opgenorth TJ: Angiotensin blockade reverses hypertension during long-term nitric oxide synthase inhibition. Hypertension 21, 660 – 666 (1993)
7. Melaragno MG and Fink GD: Role of ANG II in hypertension produced by chronic inhibition of nitric oxide synthase in conscious rats. Am J Physiol 271, H806 – H811 (1996)
8. Tomita H, Egashira K, Kubo-Inoue M, Usui M, Koyanagi M, Katoh M, Shimokawa H, Takeya M, Yoshimura T and Takeshita A: Inhibition of nitric oxide synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat heart and vessels. Arterioscler Thromb Vase Biol 18, 1456 – 1464 (1998)
9. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Takami K, Shimokawa H and Takeshita A: Early induction of transforming growth factor-β via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthase in rats. Hypertension 32, 273 – 279 (1998)
10. Border WA and Ruoslahti E: Transforming growth factor-β in disease: The dark side of tissue repair. J Clin Invest 90, 1 – 7 (1992)
11. Wahl SM: Transforming growth factor-β: The good, the bad, and ugly. J Exp Med 180, 1587 – 1590 (1994)