

Beneficial effects of long-term administration of ONO-3144, a free radical scavenger, on stroke-prone SHR¹

HIGASHINO Hideaki^{2,4}, MAEDA Kana², KAWAMOTO Masazumi^{2,3}, ENDO Hiroshi^{2,3}, LEE Wen-Hsiung²
¹Department of Pharmacology, Kinki University School of Medicine, Osaka-Sayama, Osaka 589-8511; ³Department of Acupuncture, Kansai College of Oriental Medicine, Kumatori, Osaka 590-0482, Japan)

KEY WORDS ONO-3144; anti-inflammatory agents; free radical scavengers; antioxidant; inbred SHR rats; longevity

ABSTRACT

AIM: To clarify the preventive effects of ONO-3144, a free radical scavenger, on stroke-prone spontaneously hypertensive rats (SHRSP) and free radicals related to the hypertensive disorders. **METHODS:** Drugs with powdered chow were administered orally to the rats from 2- to 14-month-old. Body weight, blood pressure, rectal temperature, oxygen consumption rate, thyroid hormones, lipids, platelet number, ocular fundus, autopsy, and life span were investigated. **RESULTS:** ONO-3144 did not affect the growth, blood pressure, concentrations of thyroid hormones and lipids in the blood, but decreased the rectal temperature and oxygen consumption rate. ONO-3144 also prevented the platelet number decrease, sclerotic change of retinal artery, edematous and hypertrophic changes in parenchymal and circulatory organs. Both the average life-span and the longest life time of SHRSP given 40 mg/kg ONO-3144 were longer than those of normotensive rats and no administration hypertensive rats. **CONCLUSION:** The oxidative free radicals closely relate to hypertension-induced pathophysiological changes in SHRSP, and ONO-3144 prevents the changes of those disorders, and then brings longevity.

INTRODUCTION

A free radical scavenger, 2-aminomethyl-4-tert-butyl-6-propionylphenol (ONO-3144, Fig 1), was developed as a basic nonsteroidal anti-inflammatory drug (NSAID) which had no effect on cyclooxygenase activity during the process of prostaglandin synthesis¹⁻³. Some reports have shown that this compound has several preferable effects such as inhibitions of TXA₂ synthesis, peroxide lipid production together with radical scavenging action, and no ulcerative effect on the stomach besides analgesic and antipyretic effects in comparison with usual antiinflammatory drugs⁴⁻⁷. Meanwhile, undesirable effects of radical groups such as peroxide lipids and peroxyradicals produced in the course of life activity have been clarified and come up to a topical subject for maintaining a further human healthy life⁸⁻¹⁰.

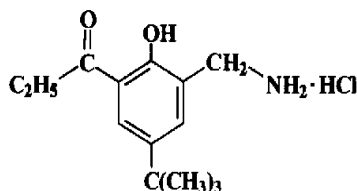


Fig 1. Chemical structure of 2-aminomethyl-4-tert-butyl-6-propionylphenol hydrochloride (ONO-3144).

On the development of hypertension and related disorders such as atherosclerosis, brain ischemia, and heart attack, it is estimated that several types of free radicals deeply relate to the onset of these disorders. On the present study, we designed to investigate the preventive effects of ONO-3144 on the hypertensive disorders of SHRSP and to confirm the elongation of life span in ONO-3144 treated SHRSP.

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² Correspondence to Prof HIGASHINO Hideaki.

Phn 81-72-366-0221, ext 3166. Fax 81-72-367-1820.

E-mail higasino@med.kindai.ac.jp

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MATERIALS AND METHODS

Animals and medication Four groups of SHRSP^[11] and one group of normotensive Wistar Kyoto rats (WKY) aged 2 months, each group consisting of 8 male rats which were bred in our animal center, were used. Rats were divided into the following 5 groups; SHRSP-0; none of drug (control group), SHRSP-10; ONO-3144 10 mg·kg⁻¹·d⁻¹, SHRSP-20; ONO-3144 20 mg·kg⁻¹·d⁻¹, SHRSP-40; ONO-3144 40 mg·kg⁻¹·d⁻¹, and WKY-0; none of drug. ONO-3144 was provided by courtesy of Ono Pharmaceutical Co, Ltd (Osaka, Japan). Drugs were administered orally to the rats after mixed with powdered commercial chow (SP, Funahashi, Shizuoka, Japan) every day until the end of the experiment at 14-month-old. Since food intake decreases with age owing to a decrease in demand, quantities of given diet were regulated as follows; 80 g·kg⁻¹·d⁻¹ at 2–3-month-old, 70 g·kg⁻¹·d⁻¹ at 4–5-month-old, 60 g·kg⁻¹·d⁻¹ after 6-month-old.

Observation methods The measurements of body weight and blood pressure taken by a tail cuff oscillometric method^[12] were done in the afternoon every week, and rectal temperature taken by a rectal thermometer for rats, oxygen consumption rate taken by a hand-made consumption meter, thyroid hormones, thyroid stimulating hormone (TSH), total cholesterol and triglyceride levels in the serum, and platelet number were measured in the peripheral blood taken from the tail vein at 6-month-old. Photographs of ocular fundus were taken with a fundus camera (RC-2, Kowa, Osaka, Japan) at ages of 5, 6, 9, 11, and 12 months, and analyzed under modified Scheie's classifications^[13] [hypertensive changes (H); 0–4 scores, and sclerotic changes (S); 0–4 scores]. On the rats with a spontaneous death after 7-month-old and the rats which were sacrificed at the end of experiment (14-month-old), an autopsy was carried, and each macroscopic tissue change was investigated.

Statistical analysis All values were presented as $\bar{x} \pm s$, and compared control and administered groups by a paired or unpaired Student's *t* test after confirming the same distribution of the data between two groups followed by the *F*-test, or *Chi*-square test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Growth rates of rats At the beginning of the

experiment, the rates of body weight increase of SHRSP-20 and -40 were somewhat temporally declined compared with that of the control group (SHRSP-0; 240 g ± 3 g, SHRSP-10; 242 g ± 4 g, SHRSP-20; 233 g ± 4 g, SHRSP-40; 230 g ± 5 g, WKY-0; 238 g ± 3 g at 12 weeks of age). However, each body weight's loss and gain recovered to the same level until 6-month-old, and there was no difference between the values in the 4 groups of SHRSP (data not shown).

Systolic blood pressure changes Although the systolic blood pressure (SBP) of WKY-0 changed within 100–120 mmHg, each group of SHRSP developed from 150 to 264 mmHg according to the aging. However, there was no significant difference in the SBP among 4 groups of SHRSP.

Comparison of rectal temperatures Rectal temperatures of 6-month-old SHRSP and WKY during oral administration of ONO-3144 were determined. The value of SHRSP-0 group was significantly higher than that of WKY-0 group [(37.9 ± 0.1) °C vs (37.1 ± 0.1) °C, Fig 2]. However, administration of ONO-3144 decreased the temperatures at the doses of 10, 20, and 40 mg/kg near to the value of WKY-0 ($P < 0.05$ vs SHRSP-0 group, Fig 2).

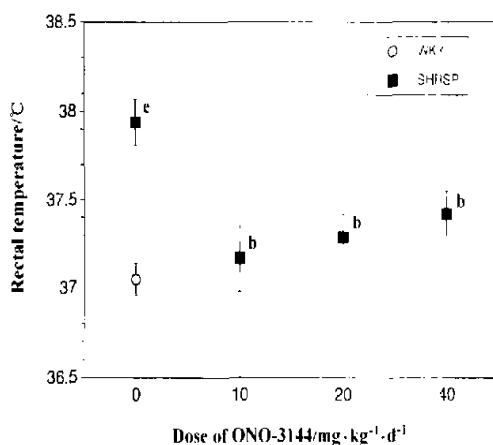


Fig 2. Rectal temperatures during oral administration of ONO-3144 in SHRSP and WKY. $n = 8$. $\bar{x} \pm s$. ^b $P < 0.05$ vs SHRSP control group. ^a $P < 0.05$ vs WKY control group.

TSH and thyroid hormone levels At 6-month-old, TSH and thyroid hormones levels in the serum taken from the tail vein were measured. Although triiodothyronine (T3) level of WKY-0 was higher than that of SHRSP-0 [(0.68 ± 0.05) μg/L vs (0.44 ± 0.02) μg/L], there was no difference in the values of TSH and

thyroxine (T4) between both control groups of SHRSP and WKY. The values of T3 and TSH were elevated by treatment with ONO-3144 $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in SHRSP. T4 levels of SHRSP ($40 - 47 \text{ } \mu\text{g/L}$) did not change at any doses (data not shown).

Oxygen consumption rate of each group

Oxygen consumption rate of SHRSP-0 measured at 6-month-old during the administration of ONO-3144 was significantly higher than that of WKY-0 [$(1.41 \pm 0.08) \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ vs $(1.15 \pm 0.07) \text{ L} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Fig 3]. ONO-3144 decreased the oxygen consumption of SHRSP in a dose-dependent manner, and ONO-3144 $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ significantly decreased the oxygen consumption to the same level of WKY-0.

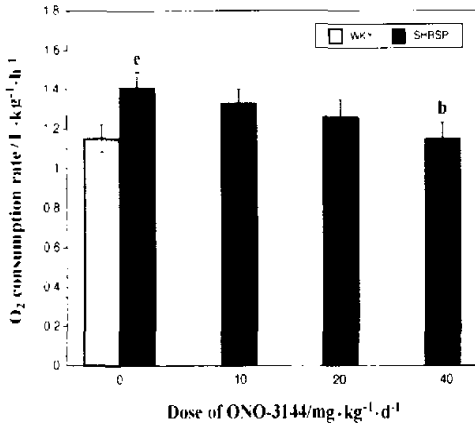


Fig 3. Oxygen consumption rates during oral administration of ONO-3144 in SHRSP and WKY. $n = 8$. $\bar{x} \pm s$. $^a P < 0.05$ vs SHRSP control group. $^b P < 0.05$ vs WKY control group.

Levels of serum total cholesterol and triglyceride

There was no significant difference in the levels of serum total cholesterol [(890 ± 40) , (990 ± 50) , (810 ± 20) , (950 ± 40) , and $(920 \pm 30) \text{ mg/L}$ for SHRSP-0, -10, -20, -40, and WKY-0, respectively], and in the levels of triglyceride among 5 groups (data not shown).

Platelet numbers in the tail venous blood

The platelet numbers in the tail venous blood of SHRSP group was decreased [$(51 \pm 3) \times 10^4$ per μL] in comparison with that of WKY group [$(59 \pm 3) \times 10^4$ per μL]. On the other hand, ONO-3144 20 and $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ increased the platelet numbers to [$(65 \pm 2) \times 10^4$ per μL] and [$(61 \pm 2) \times 10^4$ per μL], respectively.

Changes in retinal arteries of ocular fundus

In each SHRSP group, H scores which showed the

hypertensive change of the retinal artery were elevated with age, while the elevation was unaffected by ONO-3144. There was no difference among 4 groups of SHRSP during the experiment (Fig 4). On the other hand, the H scores of WKY-0 were scarcely changed (Fig 4). The S scores which showed the sclerotic change of the retinal artery of SHRSP also were elevated with age, and the elevation was significantly depressed by treatment with ONO-3144 (20 and $40 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) at 44 and 48 weeks of age. However, the S scores of WKY-0 with normal blood pressure were hardly changed (Fig 4).

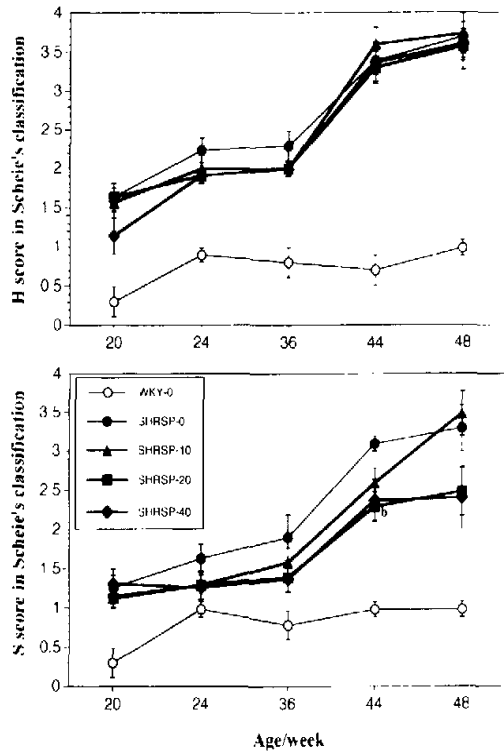


Fig 4. Retinal arterial findings of hypertensive changes (H: upper figure) and sclerotic changes (S: lower figure) during oral administration of ONO-3144 in SHRSP and WKY. $n = 8$. $\bar{x} \pm s$. $^b P < 0.05$ vs SHRSP control group.

Survival rates and average life-time

Each longest life-time of SHRSP-0, -10, -20, -40, and WKY-0 groups was 53 , 56 , 64 , over 68 , and 68 weeks, respectively (Fig 5). Average life-time of SHRSP-20 and -40 groups were elongated significantly in comparison with that of SHRSP-0 group (Fig 5).

Findings of autopsy

An autopsy was carried out

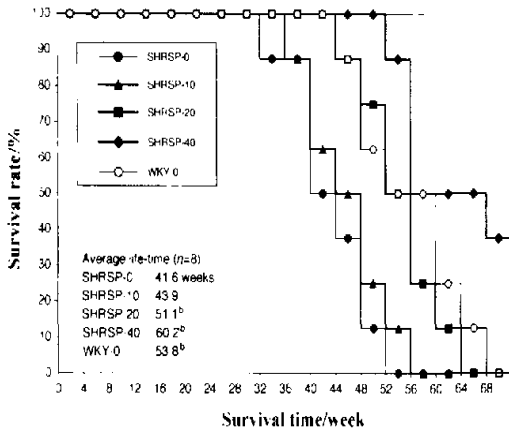


Fig 5. Survival rates during oral administration of ONO-3144, and average life-time in SHRSP and WKY. SHRSP-0 and WKY-0: no administration, SHRSP-10, -20, and -40: ONO-3144 10, 20, and 40 mg·kg⁻¹·d⁻¹ in due order. n = 8. $\bar{x} \pm s$. ^b*P* < 0.05 vs SHRSP control group.

on dead rats after 7-month-old and sacrificed rats at 16-month-old to know the pathophysiological changes. The incidences of brain lesion such as hemorrhage,

softening and edema formation were 6/8 cases (75.0 %), 4/8 cases (50.0 %), 3/8 cases (37.5 %), 2/8 cases (25.0 %), and 0/8 cases (0 %) in SHRSP-0, -10, -20, -40, and WKY-0 (*P* < 0.05 vs SHRSP-0), respectively. The results showed that long-term administration of ONO-3144 significantly inhibited the incidence of brain lesion in SHRSP. The appearance rates of mesenteric arterial aneurysms were 6/8 cases (75.0 %), 5/8 cases (62.5 %), 4/8 cases (50.0 %), 5/8 cases (62.5 %), and 0/8 cases (0 %) in SHRSP-0, -10, -20, -40, and WKY-0 (*P* < 0.05 vs SHRSP-0), respectively. It indicated that the incidence of aneurysms with hypertension and aging was inhibited by ONO-3144 20 mg·kg⁻¹·d⁻¹.

ONO-3144 inhibited an increase of tissue weight caused by edematous changes in the brain and kidney, and also inhibited an increase of tissue weight caused by hypertrophic changes of the heart and aorta (Fig 6). A weight increase of adrenal glands was inhibited by the administration of ONO-3144 as follows: (50.3 ± 1.7), (48 ± 4), (24.5 ± 2.0), (24.2 ± 2.2), and (34.6 ± 1.9) mg in SHRSP-0, -10, -20, -40, and WKY-0

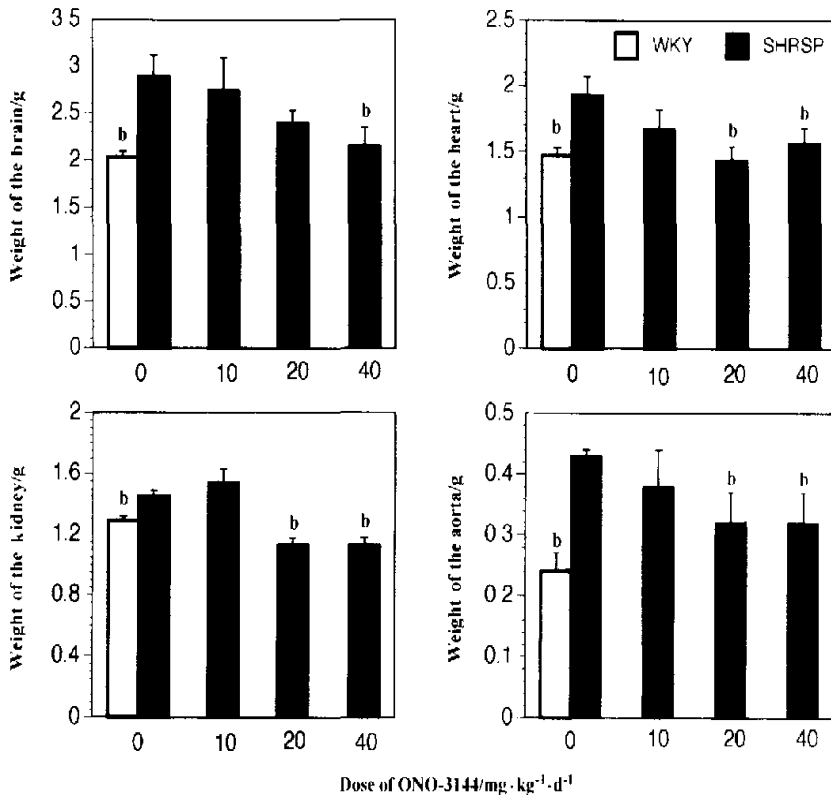


Fig 6. Tissue weights of the brain, heart, kidney, and aorta measured on the autopsy at the spontaneous death of SHRSP and WKY. n = 8. $\bar{x} \pm s$. ^b*P* < 0.05 vs SHRSP control group.

($P < 0.05$ vs SHRSP-0), respectively.

DISCUSSION

ONO-3144, a new type of basic nonsteroidal anti-inflammatory drugs (NSAID), possesses a scavenging effect to clutch peroxyradicals which are produced on a step of the conversion from PGG₂ to PGH₂^[1,3]. On this step, cytoprotective PGG₂ passively increases through the feedback inhibition to the PGH₂ production. Thromboxane A₂ (TXA₂) production, with an inflammatory action, is also declined through a prostacyclin (PGI₂) synthetase activation. As a result, the anti-inflammatory effects of ONO-3144 appear directly and indirectly through these steps.

SHRSP strain, which is over 250 mmHg in systolic blood pressure with a shorter life-span in comparison with normotensive Wistar rats (WKY), is a suitable animal model for studying on hypertensive disorders^[14]. It is supposed to be worth to elucidate the implication of active oxygen species such as peroxide free radicals, O₂⁻ and OH[·], in hypertension.

In this chronic experiments, every dose of ONO-3144 never changed the blood pressure, but showed an obvious prolongation of the average life-span of SHRSP by 23 % and 45 % at the doses of 20 and 40 mg·kg⁻¹·d⁻¹, respectively. Although this drug did not inhibit cyclooxygenase activity as common NSAID, it decreased the value of a higher rectal temperature and a higher oxygen consumption rate of SHRSP at the dose of 40 mg·kg⁻¹·d⁻¹, *po*, comparing to that of normotensive rats. ONO-3144 prevented a decrease of platelet number with age and hypertension, and maintained it at a normal level. Presumably, it suppressed the consumption of platelets in the peripheral vascular beds through an inhibition of aggregation caused by collagen or others. These three effects of antipyreticaction, a decrease of oxygen consumption rate, and anti-aggregative action without cyclooxygenase inhibition suggest that they are owing to the inhibition of TXA₂ production and acceleration of PGI₂ production at pathways of thermoregulation in the hypothalamus, and inhibition of platelet aggregation in the peripheral vessels.

It was reported that ONO-3144 clearly prevented reoxygenation injury in perfused heart^[2,4,5], brain edema due to regional ischemia^[6], and endotoxin-induced lung injury^[7] through antioxidant action on scavenging specific free radicals including O₂⁻ and OH[·]. And then, migration of eosinophils and neutrophils was inhibited,

and cell and mitochondrial membranes were maintained intact in the injured lung.

In this experiment using ONO-3144, sclerotic scores of the retinal arteries in the course of aging, edematous and hypertrophic changes in the parenchymal and circulatory organs such as brain, kidney, heart, and aorta, and appearance of aneurysms of the superior mesenteric artery were all significantly prevented. Meanwhile, total cholesterol and triglyceride levels, and thyroid hormone balance in the blood were not significantly changed. It is well known that SHR and SHRSP have disorders such as hyperthermia, higher oxygen demand, and earlier senile phenomenon in comparison with normotensive rats^[14]. As the results, ONO-3144 prevented the sclerotic changes of major organs, maintained their functions, and elongated the life span. These indicate that at least oxidative free radicals deeply relate to the development of hypertension-induced pathophysiological changes in SHRSP, and ONO-3144 prevent those disorders. This drug showed a beneficial result which might be able to achieve a desire for longevity.

REFERENCES

- 1 Aishita H, Morimura T, Obata T, Miura Y, Miyamoto T, Tsuboshima M, *et al*. ONO-3144, a new anti-inflammatory drug and its possible mechanism of action. *Arch Int Pharmacodyn Ther* 1983; 261: 316-27.
- 2 Kobayashi H, Kobayashi K, Hirohata Y, Aihara K, Ohtsuka T. Effect of ONO-3144, a free radical scavenger, on reoxygenation injury in anoxic myocardium. *Adv Prostaglandin Thromboxane Leukotriene Res* 1987; 17A: 388-91.
- 3 Cheeseman KH, Fomi LG. An investigation of the novel anti-inflammatory agents ONO-3144 and MK-447. Studies on their potential antioxidant activity. *Biochem Pharmacol* 1988; 37: 4225-33.
- 4 Kobayashi H, Ashraf M, Rahamathulla M, Kobayashi K, Schwartz A. Role of ONO-3144, a new cardioplegic agent, in the reoxygenation injury in the anoxic myocardium. *Jpn Cir J* 1987; 51: 421-30.
- 5 Ashraf M, Kobayashi H, Rahamathulla PM. Morphological and biochemical evidence of protective effect by ONO-3144, a free radical scavenger, on the reoxygenation injury in the anoxic myocardium. *Am J Cardiovasc Pathol* 1989; 2: 351-64.
- 6 Johshita H, Asano T, Hamamura T, Takakura K. Effect of indomethacin and a free radical scavenger on cerebral blood flow and edema after cerebral artery occlusion in cats. *Stroke* 1989; 20: 788-94.
- 7 Matsuda T, Onda MM, Miyashita M, Matsuda N. Endotoxin-induced lung injury. The role of leukocytes and

- oxidants, and the efficacies of steroids and antioxidants. Nippon Ika Daigaku Zasshi 1995; 62: 150-60.
- 8 Fehér J, Csomos G, Vereckei A, editors. Clinical importance of free radical reactions and their role in the pathogenesis of various human diseases. Free radical reactions in medicine. Berlin: Springer-Verlag; 1985. p 48-147.
- 9 Sohal RS, Agarwal S, Forster MJ, Lal H. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. Mech Ageing Dev 1994; 74: 121-33.
- 10 Barja G, Cadenas S, Rojas C, Lopez-Torres M, Perez-Campo R. A decrease of free radical production near critical targets as a cause of maximum longevity in animals. Comp Biochem Physiol B 1994; 108: 501-12.
- 11 Okamoto K, Yamori Y, Nagaoka A. Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circ Res 1974; 34 & 35 Suppl 1: 143-53.
- 12 Higashino H, Simeonova K, Lambev I, Markov M, Suzuki A. Proper measurement of blood pressure and heart rate in SHRSP and WKY by an indirect volume-oscillometric method. Clin Exp Pharmacol Physiol 1995; 22 Suppl 1: S292-3.
- 13 Aoki N, Horibe H, Ohno Y, Hayakawa N, Kondo R. Epidemiological evaluation of funduscopic findings in cerebrovascular diseases. III. Observer variability and reproducibility for funduscopic findings. Jpn Circ J 1977; 41: 11-7.
- 14 Higashino H, Suzuki A. Upper shift of the set point in the thermocenter causes hyperthermia in SHRSP. Acta Med Kinki Univ 1995; 20: 279-89.
- 长期服用一种自由基清除剂 ONO-3144 对易卒中自发高血压大鼠的影响¹
- R96 A
- 东野英明^{2,4}, 前田佳奈², 川本正纯^{2,3},
远藤 宏^{2,3}, 李文雄² (²近畿大学医学部药理学教室, 大阪 5898511; ³关西针灸短期大学针灸学临床教室, 大阪 5900482, 日本)
- 关键词** ONO-3144; 抗炎药; 自由基; 抗氧化剂; 近交SHR 大鼠; 长寿
- (责任编辑 韩向晖)

Corrigendum

Acta Pharmacologica Sinica 2002 Feb; 23 (2): 105-106. In Fig 2, 3;
 $P < 0.05$ should be $P > 0.05$.