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The Role of Free Radical in the Pathogenesis of Impotence in Streptozotocin-Induced Diabetic Rats

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Diabetes is the most common cause of erectile dysfunction (ED). Oxidative stress has been suggested to be a contributory factor in vascular complications of diabetes in various organs. In the present study, we investigated whether oxidative stress is associated with erectile function in non- insulin dependant diabetes mellitus (NIDDM) rats. Fifty-four Sprague-Dawley rats were the subjects of this study. In each rat, NIDDM was induced by an intraperitoneal injection of 90mg/Kg of streptozotocin on the second day after birth. Based on the diabetic period, they were classified into either short-term or long-term diabetics (avg. 22 weeks, n=18 and avg. 38 weeks, n=20), respectively, and their age-matched controls (n=16). To evaluate the erectile function in each rat, the intracavernous pressure, and latency to maximal pressure, following cavernous nerve stimulation (frequency: 1 Hz, intensity: 3 - 6 V, pulse width: 1 msec, pulse duration: 1 min.) was analyzed. To evaluate both oxidative stress from reactive oxygen species, and antioxidant function as a defense against them, total malondialdehyde and glutathione levels were measured in the corpus cavernosum of the penis, using a spectrophotometric assay. The intracavernous pressure following cavernous nerve stimulation was significantly lower in the long-term (49.8 $\pm$ 9.4 cmH_2O) than the short-term diabetics (75.9 $\pm$ 14.8 cm H_2O), and markedly decreased in the diabetic rats, compared with their age-matched controls (long-term controls; 60.7$\pm$ 17.2 cmH_2O, short-term controls; 95.2 $\pm$ 20.4 cmH_2O). The malondialdehyde content in the corpus cavernosum was markedly increased in the diabetics (2.13 $\pm$ 0.27 nM/mg protein) compared to the controls (1.48 $\pm$ 0.22 nM/mg protein). Furthermore, the glutathione level was significantly decreased in the diabetics, compared to age-matched controls (short-term control; 218.3 $\pm$ 25.6 μM/mg protein, long-term control; 150.2 $\pm$ 9.8 μM/mg protein). In the diabetic groups, it was more significantly decreased in the long-term diabetics (134.8 $\pm$ 11.3 μM/mg protein) than in short-term diabetics (182.1 $\pm$ 18.8 μM/mg protein). NIDDM causes erectile dysfunction, which slowly progresses. Oxidative stress to cavernous tissue may be a contributory factor in erectile dysfunction in diabetics.

Key Words: Diabetes mellitus, erection, free radical, rat

INTRODUCTION

Erectile dysfunction (ED) is a prevalent condition affecting approximately 30 million men in the United States.[1] ED is most commonly associated with diabetes, affecting up to 75% of all men with the disease, and occurs at an earlier age than in the general population.[2,3] The Massachusetts Male Aging Study described, among other factors, diabetes and aging as the predominant predictors of erectile dysfunction.[1]

The pathogenesis of erectile dysfunction, as one of diabetic complications, remains to be completely understood. Diabetes in humans and rats has a known pathological effect on peripheral tissue innervation and vascularization, both of which are important for erectile function.[4,5]

In various organs, including blood vessel, oxidative stress has been reported to play an important role in the development of diabetes and diabetic complications.[6,7] Oxidative stress results from a disturbance in the balance between the formation of free radicals in the body, and their scavenging.[8] In diabetes, protein glycation and glucose auto-oxidation can lead to the formation of free radicals, and this can induce lipid peroxi-

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dation in several organs.[9,10]

However, there are few data on how it contributes to erectile dysfunction in diabetes, especially in non-insulin dependent diabetes mellitus (NIDDM), which is more prevalent in Asian areas than is insulin dependant diabetes mellitus (IDDM).

Therefore, we investigated the effect of oxidative stress on diabetic penile erection using a rat model with NIDDM.

MATERIALS AND METHODS

A total of 54 Sprague-Dawley rats, weighting 200 to 500g, were included in this study. They were classified into diabetic (n=38) and control (n=16) groups. After the initial induction of diabetes, both groups were further classified into short-term (rats with 22 wks. of age, n=18), and long-term diabetic (rats with 38 wks. of age, n=20), and short-term (n=10), and long-term control (n=6) groups.

Induction of diabetes

Pregnant Sprague-Dawley rats were checked twice daily for delivery of pups. Based on Wier's method,[11] 90 mg/Kg of streptozotocin (STZ), in 0.3 ml of citrate buffer (pH 4.5), was intraperitoneally injected into 2 day old male pups. At 4 days old, the glucose level was determined from blood collected by cardiac puncture. Animals were forwarded to further processing, only if the plasma glucose level, at 4 days after birth, was more than 275 mg/dl, as measured by a glucose analyzer (ReflouxR S, Typ 1172115, Boehringer, Ingelheim, Germany). After the initial confirmation of diabetes, the animals were weaned at approximately 24 days old, and than allowed to feed on standard laboratory chow. Between 6 and 15 weeks of age, plasma glucose determinations were additionally made on blood obtained from snipping the rats tails.

Evaluation of erectile function

The electroerection rat model has been well established, as demonstrated in our previous study.[12] Rats were anesthetized with 4% chloral hydrate (0.8 cc/100g), with supplemental doses of chloral hydrate administrated as required to maintain a uniform level of anesthesia.

The animal was placed in a supine position, and the bladder and prostate exposed through a midline abdominal incision. With the aid of a Zeiss dissecting microscope, the major pelvic ganglion and cavernous nerve were identified posterolaterally to the prostate on one side, and platinum wire electrodes were placed around these structures for electrical stimulation. The penis was denuded of skin, and a 25-gauge needle was inserted into one side of the corpus cavernosum for monitoring of the intracavernous pressure (ICP). The systemic arterial blood pressure was monitored via a 25-gauge cannula placed in the carotid artery. All fluid lines were connected to a Statham pressure transducer and a Macpacq system (Biopac Systems, Goleta, CA, USA), which were interfaced to a personal computer for recording and data analysis.

To produce a full erectile response, cavernous nerve stimulation was performed for 1 min. at 1 Hz, with 3 - 6 V square-wave pulses (1 msec pulse width). The maximal intracavernous pressure (cmH_2O) and latency to maximal erection (sec.) were the measured parameters of interest (Fig. 1).

Evaluation of lipid peroxidation and antioxidant status

Malondialdehyde (MDA), an end product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored substance. Immediately after induction of anesthesia, the corpus cavernosa of the rat were rapidly removed. Based on Ohkawa's method,[13] cavernous tissue was homogenized in 0.1 M Tris-HCl buffer 10% w/v. Tissue supernatant (0.2 ml) was added to test tubes containing 0.2 ml 8% SDS, 0.4 ml 0.8% TBA and 0.4 ml 20% acetic acid. The tubes were heated to 95℃ for 60 min. After cooling, 5.0 ml of 1-butanol: pyridine (15 : 1 mixture) was added to each tube. The tubes were vortexed for 20 sec., then centrifuged at 13000 rpm for 15 min. The supernatant was used for fluorometric measurement at 553 nm emission and 515 nm excitation. Tetraethoxypropane was used as a standard, and the results expressed as nmol of MDA/mg protein of cavernous tissue.

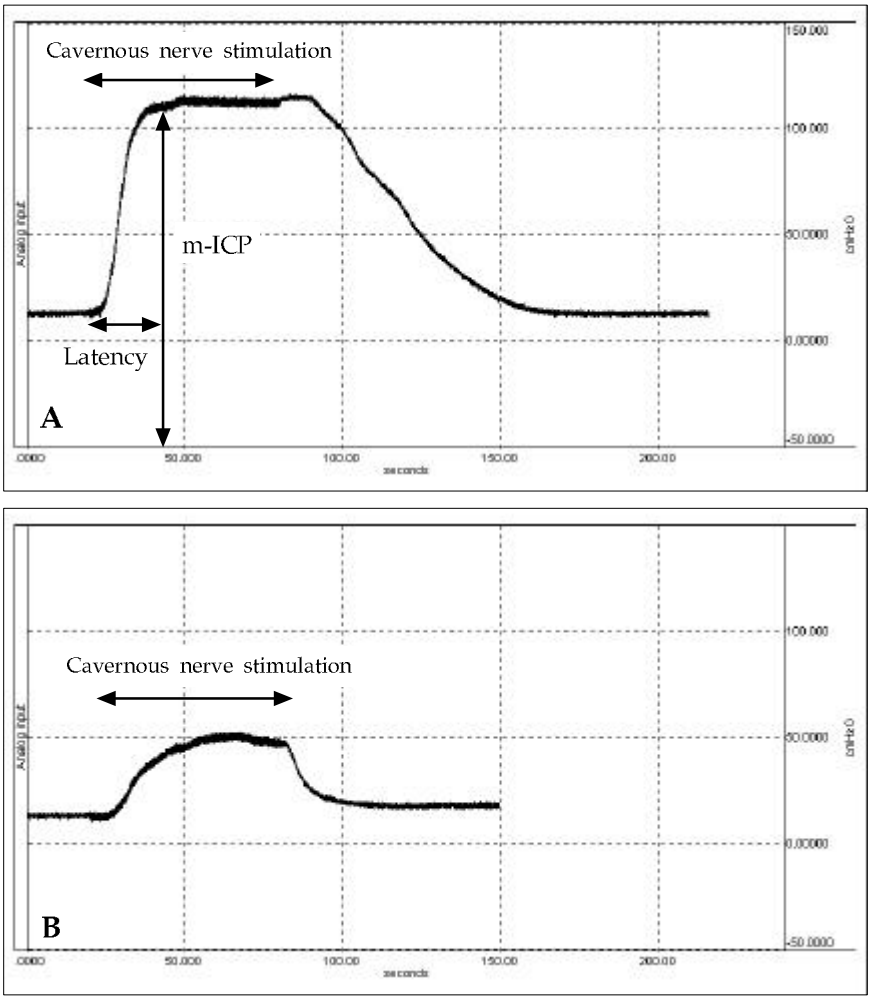

Fig. 1. Representative monitoring of nerve-induced erection in rats. Change of intracavernosal pressure is significantly decreased in a long-term diabetic (B) than a control rat (A). m-ICP: maximal intracavernous pressure following electrical stimulation of the cavernous nerve (frequency: 1 Hz, intensity: 3-6 V, pulse width: 1 msec, pulse duration: 1 min.), Latency: latency time to reach maximal erection from base line.

To measure the glutathione, as an antioxidant, tissue was homogenized in 5 volumes of cold 5% (w/v) 5-sulfosalicylic acid (Sigma, St. Louis, MO, USA). The homogenates were then centrifuged at 10,000 × g for 10 min between 0 to 4℃, and the resulting supernatant stored at -80℃ until required. The test sample (10 μl) was mixed with 0.1 ml of 6.0 mM DTNB (5′, 5-dithiobis-2-nitrobenzoic acid), 0.7 ml of 0.3 mM NADPH, and 0.18 ml of H_2O. The mixture was then incubated in a water bath for 10 to 50 min at 30℃. Glutathione reductase (10 μl) was added to the reaction mixture to initiate the reaction. The formation of TNB was monitored, against a sample blank, containing 5% 5-sulfosalicylic acid only, by recording the absorbance at 412 nm until it exceeded 2.0. The glutathione content in the test sample was determined from a standard curve of glutathione equivalents. The results are expressed as μmol/mg protein.

Statistical analysis

The results are expressed as the mean ± S.E., and the comparisons between the control, short-term and long-term diabetic groups were made using unpaired t tests. A p values less than 0.05 were considered to be significant.

RESULTS

Induction of diabetes

Transient hyperglycemia rapidly developed, and lasted for one week. Then the plasma glucose concentrations were maintained at normal levels until 6 weeks of age, when frank chronic hyper-

glycemia developed, with plasma glucose concentrations usually ranging between 200 - 500 mg/dl. The changing pattern of blood glucose level, according to age, was similar to that found by Wier.[11] Some rats, showing decreased glucose concentration of less than 200 mg/dl, were excluded from our study.

Evaluation of erectile function

The maximal ICP, representing full erectile status, was significantly lower in the long-term diabetics (49.8 ± 9.4 cmH_2O) than in the short-term diabetics (75.9 ± 14.8 cmH_2O, $p<0.05$). In addition, the maximal ICP in diabetics was also lower than those in their age matched controls (long-term controls; 60.7 ± 17.2 cmH_2O and short-term controls; 95.2 ± 20.4 cmH_2O, respectively, $p<0.01$) (Fig. 2). Also, there was a difference in penile erection between the long-term and short-term controls ($p<0.01$). However, no significant difference was observed in the latency to maximal erection between diabetics and their age-matched controls.

Evaluation of lipid peroxidation and antioxidant status

The malondialdehyde level was measured only in the short-term diabetic rats and controls, and was significantly increased in the diabetics (2.13 ± 0.27 nM/mg protein) compared to in the controls (1.48 ± 0.22 nM/mg protein).

The content of glutathione in the corpus cavernosum was more significantly decreased in the long-term (134.8 ± 11.3 μM/mg protein) compared to in short-term diabetics (182.1 ± 18.8 μM/mg protein, $p<0.05$). The glutathione level in the diabetics were also further decreased, compared to those in their age-matched controls (short-term; 218.3 ± 25.6 μM/mg protein, long-term; 150.2 ± 9.8 μM/mg protein, $p<0.05$) (Fig. 3).

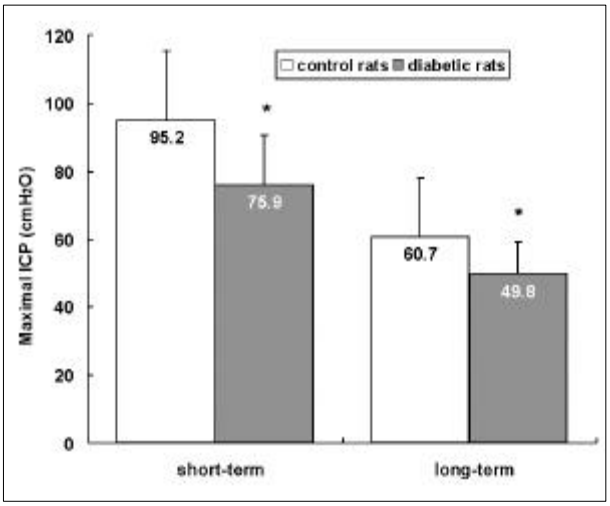

Fig. 2. Comparison of erectile function among the groups. Erectile function is significantly decreased in diabetics than their age-matched controls. Within the diabetic groups, it is also further markedly decreased in long-term diabetics than in short-term diabetics. *$p<0.01$ compared to their age-matched controls.

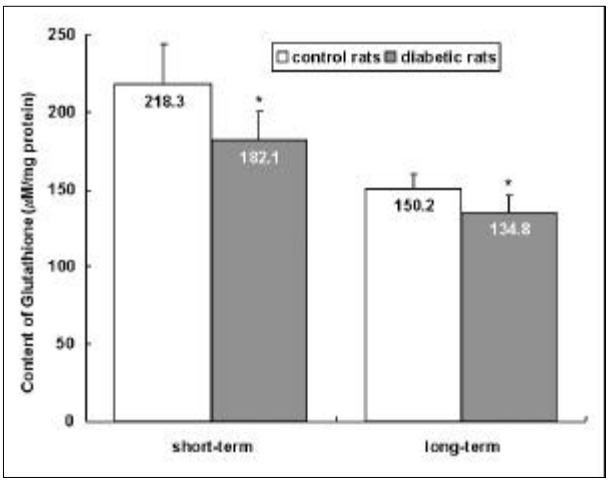

Fig. 3. Comparison of cavernous glutathione levels among the groups. Glutathione contents are significantly lower in long-term diabetics than in short-term diabetics, and are also lower than their age-matched controls. *$p<0.05$ compared to their age-matched controls.

DISCUSSION

In this study, the intracavernous pressure, following nerve stimulation, was significantly decreased in the diabetic rats, mimicking the erectile pattern of patients with vasculogenic ED. However, no difference in the latency to maximal erection was found between the diabetics and their age matched controls. Clinically, it is well established that large and small vessel vasculopathies are leading causes of morbidity and mortality in patients with diabetes. Alterations in the endothelial cell metabolism and function have

been described as major components of diabetic microangiopathy.[14] Likewise, vasculogenic deficiency is thought to be the major organic pathway leading to erectile dysfunction in diabetics.[15] Furthermore, studies in humans have demonstrated qualitative damage to the endothelial cells of the corpora cavernosa caused by diabetes.[16] It has also been reported that there is a reduction of intracavernous smooth muscle fibers in patients with vasculogenic impotence.[17] In addition, a recent study using chronic diabetic rats reported significant reductions in both smooth muscle and endothelial cell content in the corpora cavernosa, which may be important in the pathogenesis of ED in humans.[18]

The pattern of erectile dysfunction shown in the NIDDM rats of our study, along with the progression of diabetes, is likely to be gradual and slow in its progression. This is similar to that of NIDDM in humans, which is characterized by a gradual onset of clinical symptoms, including its complications. This implies the impact of hyperglycemia is not so strong, and its progression from initial microangiopathy to atherosclerosis is slow, and far ahead of organ failure. In contrast, IDDM rats in our previous study,[19] as well as others,[20,21] have shown severe erectile dysfunction, even in short-term diabetes.

The erectile function of the aged rats in this study, including both diabetic and the non-diabetic controls, was shown to be decreased, compared to that of young rats. This result indicates that aging may also contribute to erectile dysfunction. In fact, previous investigation have revealed a significant reduction of nerve fibers in the corpora cavernosa of aged rats,[22] as well as in neurogenic ED patients.[23]

To further investigate whether free radicals have an adverse effect on the endothelium and smooth muscle of corpora cavernosa in diabetic rats, assessment of malondialdehyde, a lipid peroxidation product, was used as an index of oxidative stress.[24] This method, although indirect, allows quantitative determination of the damage to cells induced by the radicals. We also measured the level of glutathione, which is well known to be involved in the glutathione peroxidase pathway that protects tissues from free radical damage.

In this study, a significant increase in the malondialdehyde concentration was shown in the erectile tissues of diabetic rats. Moreover, the glutathione level was decreased in the tissues of diabetic rats, and further decreased in the long-term diabetics. These confirm an imbalance between the excessive generation, and insufficient elimination, of free radicals in the corpora cavernosa of diabetic rats. Our results are consistent with other reports on the increase of lipid peroxides and/or decreased activity of antioxidant enzymes in serum, kidney, erythrocytes and corporal tissues of animals with experimental diabetes.[24,25] It is well known that oxygen free radicals exert their cytoplasmic effect by peroxidation of membrane phospholipids, which leads to a change in the permeability and the loss of membrane integrity.[26] Moreover, it is also known that the increase of lipid peroxidation in the erythrocytes decreases the lifespan of cells, and in turn, causes hypercoagulability, and increases their adhesiveness to the endothelium.[27] In addition, advanced glycation end products in diabetes have a tendency to accumulate on the vessel walls and tissues, and may result in a loss of endothelial cells following oxidative stress.[28,29]

Our study suggests that NIDDM causes erectile dysfunction, although its progression is slow. Excessive generation of free radicals, and decreased scavenging systems, may lead to diabetic angiopathy, including functional or structural impairment of corporal endothelium and smooth muscle, which is responsible for erectile dysfunction. Furthermore, these results may support the concept that treatment with either an antioxidant or a free radical scavenger has therapeutic potential for diabetics with erectile dysfunction.

In summary, the erectile function in NIDDM rats is more decreased, than that in their age-matched controls. The values for ICP in the short-term are more than those in the long-term controls and diabetes. In diabetic rats, the level of malondialdehyde was markedly increased and that of glutathione decreased, compared to the age-matched controls, in both the short-term and long-term rats. Therefore, non-insulin dependant diabetes mellitus causes erectile dysfunction, although it progression is slow. Oxidative stress to cavernous tissue may be a contributory factor in erectile dysfunction in diabetics.

REFERENCES

1. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 1994;151:54-61.
2. Newman HF, Marcus H. Erectile dysfunction in diabetes and hypertension. Urology 1985;26:135-7.
3. Hakim LS, Goldstein I. Diabetic sexual dysfunction. Endocrinol Metab Clin North Am 1996;25:379-400.
4. Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med 1989;320: 1025-30.
5. Colakoglu Z, Kutluay E, Ertekin C, Killi R, Alkis A. Autonomic nerve involvement and venous leakage in diabetic men with impotence. BJU Int 1999;83:453-6.
6. Wolff SP. Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the etiology of diabetes mellitus and complications. Br Med Bull 1993;49:642-52.
7. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257-67.
8. Feillet-Coudry C, Rock E, Coudry C, Grzelkowska K, Azais-Braesco V, Dardevent D, et al. Lipid peroxidation and antioxidant status in experimental diabetes. Clin Chim Acta 1999;284:31-43.
9. Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 1990;173:932-9.
10. Bayness JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405-12.
11. Wier GC, Clore ET, Zmachinski CJ, Bonner-Wier S. Islet secretion in a new experimental model for non-insulin dependent diabetes. Diabetes 1981;30:590-5.
12. Suh JK, Mun KH, Cho CK, Shin HC, Kim YS, Park DC. Effect of vasoactive intestinal peptide and acetylcholine on penile erection in the rat *in vivo*. Int J Impot Res 1995;7:111-8.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Clin Biochem 1979;85:351-8.
14. McMillan DE. Deterioration of the microcirculation in diabetes. Diabetes 1975;24:944-57.
15. Saenz de Tejada I, Goldstein I. Diabetic penile neuropathy. Urol Clin North Am 1988;15:17-22.
16. Aydos K, Baltaci S, Saglam M. Ultrastructural changes of corpora cavernosa in vascular erectile dysfunction. Int Urol Nephrol 1996;28:375-82.
17. Wespes E, Goes PM, Schiffmann S, Depierreux M, Vanderhaeghen JJ, Schulman CC. Computerized analysis of smooth muscle fibers in potent and impotent patients. J Urol 1991;146:1015-7.
18. Burchardt T, Burchardt M, Karden J, Buttyan R, Shabsigh A, de la Taille A, et al. Reduction of endothelial and smooth muscle density in the corpora cavernosa of the streptozotocin induced diabetic rat. J Urol 2000;164:1807-11.
19. Mun KH, Jung HC, Park DC, Lee YC, Huh K, Suh JK, et al. Erectile function and expression of neuronal nitric oxide synthase in diabetic rat. Korea J Urol 1997;38: 575-84.
20. Rehman J, Chenven E, Brink P. Diminished neurogenic but not pharmacological erections in the 2- to 3-month experimentally diabetic F-344 rat. Am J Physiol 1997; 272:1960-5.
21. el-Sakka AI, Lin CS, Chui RM. Effects of diabetes on nitric oxide synthase and growth factor genes and protein expression in an animal model. Int J Impot Res 1999;11:123-30.
22. Carrier S, Nagaraju P, Morgan DM, Baba K, Nunes L, Lue TF. Age decreases nitric oxide synthase-containing nerve fibers in the rat penis. J Urol 1997;157:1088-92.
23. Choi YD, Mah SY, Xin ZC, Choi HK. The distribution of nitric oxide synthase in human corpus cavernosum on various impotent patients. Yonsei Med J 1997;38:125-32.
24. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28.
25. Minhas S, Cartledge JJ. The effect of superoxide dismutase on nitric oxide-mediated and electrical field-stimulated diabetic rabbit cavernosal smooth muscle relation. BJU Int 2001;88:303-4.
26. Vane JR, Botting RM. Secretory functions of the vascular endothelium. J Physiol Pharmacol 1992;43:195-207.
27. Arduini A, Stern A, Storto S, Berfiglio M, Mancinelli G, Scurti R, et al. Effect of oxidative stress on membrane phospholipid and protein organization in human erythrocytes. Arch Biochem Biophys 1989;273:112-20.
28. Renard C, Chappey O, Wautier MP, Nagashima M, Lundh E, Morser J, et al. Recombinant advanced glycation end product receptor pharmacokinetics in normal and diabetic rats. Mol Pharmacol 1997;52:54-62.
29. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, et al. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 1994;269: 9889-97.