The Effects of Enalapril Maleate and Cold Stress on Some Blood Parameters

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SUMMARY

Enalapril is a highly specific, competitive inhibitor of angiotensin converting enzyme (ACE) belonging to the category of ACE inhibitors. The beneficial effects of ACE inhibitors appear to result primarily the suppression of the plasma renin-angiotensin-aldesteron system. The pharmacological actions of enalapril maleate are related to hypotension. Enalapril maleate is an ACE inhibitor that controls high blood pressure by relaxing blood vessels.

Stress triggers important adaptive responses that enable an organism to cope with a changing environment. The release of catecholamine is a key initial event in responses to stressors and is followed by an increase in the expression of genes that encode catecholamine-synthesizing enzymes. This study is designed to detect the effects of enalapril maleate and cold stress on blood glucose, triglyceride, cholesterol, and albumin levels in rat sera.

Cold stress treatment has increased blood glucose, cholesterol and triglyceride levels but albumin level has decreased (P<0.05). Enalapril maleate treatment has decreased blood glucose and triglyceride levels (P<0.05). Cholesterol and albumin levels have not changed (P>0,05). Cold stress+enalapril maleate treatment has decreased blood glucose level (P<0.05). Triglyceride, cholesterol and albumin levels have not changed (P>0,05).

Key Words: *Cold Stress, Enalapril Maleate, Cholesterol, Glucose, Triglyceride, Albumin*

Bazı Kan Parametreleri Üzerine Soğuk Stresi ve Enalapril Maleate'ın Etkileri

ÖZET

Enalapril, ACE (Angiotensin Converting Enzyme) inhibitörleri kategorisine ait olup, anjiyotensin dönüştürücü enzimin kompetetif inhibitörüdür. ACE inhibitörlerinin faydalı etkileri, öncelikle plazma renin-anjiyotensin-aldesteron sisteminin baskılanmasıyla ortaya çıkar. Enalapril maleat'ın farmakolojik aksiyonları, hipotansiyonla ilişkilidir. Enalapril maleat, kan damarlarını gevşeterek, yüksek kan basıncını kontrol eden bir ACE inhibitörüdür.

Stres, organizmanın değişen çevreye uyumunu sağlayan önemli cevapları tetikler. Katekolaminlerin salınması, stres vericilere cevapta anahtar bir olaydır ve katekolamin sentez enzimlerini kodlayan genlerin ifadesinde artış ile takip edilir. Bu çalışma, sıçan kanında glukoz, trigliserit, kolesterol ve albumin seviyeleri üzerine enalapril maleat ve soğuk stresin etkilerini ortaya çıkarmak için düzenlenmiştir.

Soğuk stres uygulama grubunda kan glukoz, kolesterol ve trigliserit seviyesi artıp, albumin seviyesi azalmıştır (P<0,05). Enalapril maleat uygulama grubunda kan glukoz ve trigliserit seviyesi azalmıştır (P<0,05). Kolesterol ve albumin seviyesi değişmemiştir (P>0,05). Soğuk stres+enalapril maleat uygulama grubunda kan glukoz seviyesi azalmıştır (P<0,05). Trigliserit, kolesterol ve albumin seviyesi değişmemiştir (P>0,05).

Anahtar Kelimeler: *Soğuk Stres, Enalapril Maleat, Kolesterol, Glukoz, Trigliserit, Albumin*

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INTRODUCTION

TH is a key enzyme which plays a central role on neurotransmission and hormonal function of catecholamines (1). Different stress transmitters lead to increases in TH activity. It is known that certain factors such as prolonged cold exposure, hypertension, neurochemical

Epinephrine and norepinephrine have lots of metabolic effects. Epinephrine elevates blood glucose level and increase the formation of glucose from glucogene (glucogenolysis) in the livers. Epinephrine works as insulin antagonist in the adjustment of blood glucose level. Both hormones increase lipolysis in adipose tissue and free adipose acid level in blood rises (6-7).

Triglyceride synthesis in adipose tissue is just possible with glucose metabolism. Entrance of glucose to adipose tissue depends on insulin. If insulin exists, glucose can enter the adipose tissue. Triglyceride synthesis stops in the situations of starvation and diabetes mellitus. Because of hunger and lack of insulin, lipolysis and plasma free adipose acid secretion increase. Glucose enters into adipose tissue increases the formation of glycerophosphate glucolitically. Therefore, it increases synthesis of triglyceride (8).

Cholesterol is very widespread in all body cells especially nerve tissues and is main compound of plasma lipoprotein and plasma membrane. Having taken the dose of cholesterol is even high, liver keeps on the synthesis of cholesterol. Especially, this position appears in taking carbohydrate and poly unsaturated fatty acid. The level of the cholesterol is promoted and LDL increases (8). In the atherosclerosis, connective tissue of the artery walls, cholesterol esters of lipoproteins which include apo B-100 and the accumulation of the cholesterol are characterized (9).

In a study, it has been investigated whether epinephrine increases the plasma cholesterol or not in rats. After epinephrine had suspensed in sesame oil, it injected under skin. 12 hours later, it has been shown that plasma cholesterol has increased by epinephrine. Besides it has been observed that epinephrine increased plasma cholesterol in both normal rats and adrenolectomy rats (10).

changes and growing old increase TH activity in adrenal medulla and sympathetic neurons (2). TH mRNA and TH activity in adrenal glands of rats exposed to cold increases in plasma norepinephrine concentrations are observed (3-5).

The cardiovascular control system is a multivariate system, while changes in environmental conditions often result in alterations in system parameters and other non-linearities, in contrast to the fixed parameters of linear control systems. In blood pressure control these features are examplified by diurnal circadian fluctuations, alterations in lifestyle and psychosocial stress. Because the neurohumoral controllers are involved in virtually all aspects of homeostasis, they can be regarded as supercontrollers.

Neurohumoral mechanisms also play a key role in cardiovascular development. Increased sympathetic activity early in life causes hypertension in spontaneously hypertensive rats (SHR) and accounts for the differences in blood pressure and structural variables from corresponding values in Wistar-Kyoto (WKY) rats. In contrast, the renin-angiotensin system affects early cardiovascular development in the same way in each strain, so that it is unlikely to be a cause of hypertension in SHR. However, after drug withdrawal following treatment of young rats with the angiotensin converting enzyme inhibitor enalapril, there were between-strain differences in late cardiovascular development (11).

The renin-angiotensin system (RAS) helps maintain blood pressure and salt homeostasis and may play a role in the pathogenesis of aspects of the metabolic syndrome (12).

The renin-angiotensin system (RAS) is fundamental to blood pressure regulation; as such, each component is potentially involved in the etiology of the polygenic disorder known as primary hypertension. Treatment with ACE inhibitors (13,14) may reduce the risks from cardiovascular diseases, further implicating the involvement of the RAS in the development of vascular disease.

In addition to the regulation of blood pressure, the RAS may also affect other

aspects of the pathogenesis of the metabolic syndrome, such as the development of atherosclerosis and insulin resistance. ACE inhibitor therapy has been reported to reduce the rates of both of these disorders (15,16). Activation of the RAS through insulin resistance may promote the development of dyslipidemia and diabetes. This possibility is supported by the finding that ACE inhibitors reduced the increased lipolysis in adipose tissue associated with insulin resistance in centrally obese hypertensive subjects.

ACE inhibitor therapy has been shown to induce improvements in atherosclerosis and insulin resistance, which suggests that activation of the RAS may promote the development of dyslipidemia and diabetes (15,16).

Treatment with ACE inhibitors improves indexes of insulin resistance (15), possibly through a reduction in bradykinin degradation (17,18). Conditions in which ACE activity is reduced, such as in subjects carrying the ACE I allele (19), may increase bradykinin levels reducing insulin resistance, which would appear contradictory to the current findings. However, ACE is involved in the formation of the vasoconstrictor angiotensin II as well as the degradation of the vasodilator bradykinin. Low-dose angiotensin II infusion has been reported to reduce insulin resistance either through redistribution of renal blood flow to skeletal muscle, which increases glucose uptake and reduces insulin excretion (20,21) directly via diacylglycerol-mediated protein kinase C activation, or by increasing hepatic carbohydrate metabolism (22). Although the specific mechanisms by which this polymorphism may modulate these parameters remain to be determined (23), the physiological relationship between the polymorphism and diabetes is possible.

Insulin resistance has been reported to reduce insulin-mediated suppression of adipocyte lipolysis to which activation of the RAS in adipose tissue may contribute (23).

Angiotensin II exerts a number of harmful effects in patients with chronic heart failure (CHF) and, through an increase in oxidative stress, is thought to be critical in the development of endothelial dysfunction. Angiotensin II may be elevated in CHF despite treatment with angiotensin converting enzyme (ACE) inhibitors, producing a rationale for adjunctive angiotensin receptor blockade (24).

Angiotensin-converting enzyme (ACE) inhibitors effectively interfere with the renin-angiotensin system and exert various beneficial actions on vascular structure and function beyond their blood pressure-lowering effects. Data from experimental studies showed that angiotensin-converting enzyme inhibitors can attenuate the development of atherosclerosis in a wide range of species (25).

These data indicate that a short-term treatment with enalapril in healthy subjects is able to affect fibrinolysis response to a physiological stimulation such as physical effort. These results may be of help in the study of complex relationships among ACE inhibition, haemostasis and reduction in myocardial infarction (26).

It has been demonstrated that ACE inhibitors delay the progression of the diabetic nephropathy by normalizing glomerular capillary pressure independent of their antihypertensive effect (27). After starting captopril, an angiotensin converting enzyme inhibitor, the patient's proteinuria gradually decreased and her blood pressure normalized with the metabolic control of hyperglycemia.

In conclusion, the appearances of proteinuria and hypertension during the prepubertal period suggest that metabolic control of diabetes seems to be an important factor of diabetic nephropathy. Careful blood pressure screening is strongly recommended because blood pressure is often raised in microalbuminuric patients (28).

The purpose of the present study was to determine the effects of cold stress and enalapril maleate administration on blood glucose, triglyceride, cholesterol, and albumin levels in rat sera.

MATERIAL and METHODS

Three months old Fischer-344 rats are used in these experiments. They are given water and rat feed and moreover they lived in 12 hour light-dark period in room temperature during the study. Control, cold stress, enalapril

maleate and cold stress+enalapril maleate treatment groups are made with rats. These rats have been used in investigation of effects of enalapril maleate and cold stress treatments on glucose, cholesterol, triglyceride and albumin levels in blood. In cold stress treatment, rats are exposed to 8°C for 48 hours. Enalapril maleate is injected intraperitonally (IP) to rats as 10mg/kg. In cold stress + enalapril maleate treatment group, enalapril maleate is injected intraperitonally (IP) to rats as 10mg/kg. After the drug injection, rats are exposed to 8°C for 48 hours. After the treatments, rats are anesthetised by 75mg/kg sodium pentobarbital. After they are anesthesized, in order to determine glucose, cholesterol, triglyceride and albumin levels in blood, the processes below performed:

- 2 ml blood taken from the anesthesized rats by entering right ventricule from their hearts.
- The bloods are centrifuged in 3000 g 4°C for 5 minutes.
- After the centrifugation application, Olympus AU 600 machine is used in the analysis of biochemical parameters in blood plasma.

The data obtained from experiments are evaluated statistically and importance degree of the difference between treatment and control groups are made by ANOVA and LSD.

RESULTS

In this study, while blood glucose level of control group was found as 155.60±3.83 mg/ml (Table1), in cold stress treatment group, blood glucose level was found as 189.71± 3.07 mg/ml (Table1). In cold stress treatment group, increase of the blood glucose level was found to be statistically significant ($P<0.05$) (Figure1-a). Blood glucose level of enalapril maleate treatment group was found as 142.60 ±6.60 mg/ml (Table1). According to control group, decrease of blood glucose level of enalapril maleate treatment group was found to be statistically significant ($P<0.05$) (Figure1-a). In group which exposed to cold stress+enalapril maleate for 48 hours, blood glucose level was found as 94.75 ±4.11 mg/ml (Table1). According to control group, decrease of blood glucose level of the treatment group was found to be also statistically significant ($P<0.05$) (Figure1-a).

As blood cholesterol level of control fischer-344 rats was found as 66.40±4.64mg/ml (Table1), in cold stress treatment group, blood cholesterol level was found as 87.71±3.02 mg/ml (Table1). Increase of blood cholesterol level of cold stress treatment group was found to be statistically significant ($P<0.05$) (Figure 1-b). Blood cholesterol level of enalapril maleate treatment group was found as 71.80±4.28 mg/ml (Table1). According to control group, this level wasn't found to be statistically significant ($P>0.05$) (Figure 1-b). In cold stress+enalapril maleate treatment group, blood cholesterol level was found as 82.00±11.00mg/ml (Table1). According to control group, changing of treatment group cholesterol level was not found to be statistically significant ($P>0.05$).

Blood triglyceride level of control fischer-344 rats was found as 44.10 ±4.93 mg/ml (Table1) and in cold stress treatment group, blood triglyceride level was found as 92.71±11.84 mg/ml (Table1). This increase was found to be statistically significant ($P<0.05$) (Figure 1-c).

In enalapril maleate treatment group, blood triglyceride level was found as 74.80±16.78 mg/ml (Table1). According to control group, this blood triglyceride level was found to be also statistically significant ($P<0.05$) (Figure 1-c). Blood triglyceride level of cold stress+enalapril maleate treatment group was found as 31.80±5.48 mg/ml (Table1). According to control group, this level wasn't found to be statistically significant ($P>0.05$).

The control fischer -344 rats of blood albumin level was found as 3.84±0.12 mg/ml (Table1). In cold stress treatment group, blood albumin level was found as 2.95±0.14 mg/ml (Table1). This decrease of blood albumin level was found to be statistically significant ($P<0.05$). Blood albumin level of enalapril maleate treatment group was found as 3.72±0.22 mg/ml (Table1). Difference between control and enalapril maleate treatment groups wasn't found to be statistically significant ($P>0.05$) (Figure1-d). Blood albumin level of

cold stress+enalapril maleate treatment group was found as 3.60 ± 0.17 mg/ml. According to control group, this result was not found to be also statistically significant (P>0.05) (Figure 1-d).

Table 1. Glucose, cholesterol, triglyceride, and albumin levels in the blood of F-344 rats on which cold stress, enalapril maleate, cold stress + enalapril maleate treatment and control examined.

	Glucose (mg/ml)	Cholesterol (mg/ml)	Triglyceride (mg/ml)	Albumin (mg/ml)
Control	155.6 ± 3.83	66.4 ± 4.64	44.1 ± 4.93	3.84±0.12
Cold Stress	189.71±3.07	87.71 ± 3.02	92.71±11.84	2.95±0.14
Enalapril maleate	142.6 ± 6.69	71.8 ± 4.28	74.8 ± 16.78	3.72±0.22
Cold Stress+Enalapril maleate	94.75 ± 4.11	82.0 ± 11.00	31.8 ± 5.48	3.6 ± 0.17

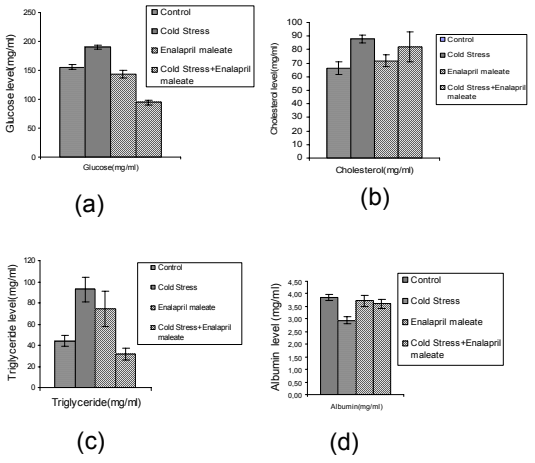

Figure 1. (a)glucose, (b)cholesterol, (c)triglyceride, (d)albumin levels in the blood of F-344 rats on which cold stress, enalapril maleate, cold stress + enalapril maleate treatment and control examined *(P<0.05).

DISCUSSION

It has been determined that blood glucose level in rats has increased because of cold stress. With that catecholamine synthesis increases because of the cold stress, the glucose level also rises. On the other hand; enalapril maleate application has caused lowers catecholamine synthesis because of its antihypertensive effect, so enalapril maleate lowers the blood glucose level.

If cold stress and enalapril maleate are applicated together, it was observed that the blood glucose level was declined. This result shows enalapril maleate application eliminates the negative effects such as hypertension and increases in the blood glucose level caused by cold stress.

Experiments demonstrated that epinephrine increased the plasma cholesterol. In our studies, cold stress has increased the cholesterol level in bloods of rats depending on their control groups. This is because cold stress increases TH activity and so epinephrine.

As a result of antihypertensive enalapril maleate and cold stress treatment enalapril maleate blocks increase in the TH enzyme activity. So epinephrine synthesis declines, there had not been an increase in cholesterol in blood. Therefore, it can be said that enalapril maleate blocks increase in the blood cholesterol level caused by cold stress.

In an experiment, exposing to cold didn't affect the plasma triglyceride level. The concentration of independent fatty acids in the plasma increased. As a result of exposing to cold for 3 hours, plasma triglyceride concentration has also increased. Exposing to cold has increased triglyceride level in rats' livers. Increase in the concentration of independent fatty acids causes accumulation of triglyceride in the livers (29).

In our study, cold stress has caused an increase in the triglyceride level in blood. Triglyceride level has affected because of increasing glucose level as a result of cold stress treatment.

Since enalapril maleate has an antihypertensive structure, catecholamine synthesis declines, so blood glucose level reduces and therefore triglyceride level reduces.

Enalapril maleate blocks increasing triglyceride level of cold stress when they are treated together. Decline in blood triglyceride level was not found to be statistically significant.

Cold stress treatment reduces albumin level in rats' blood. This is because triglyceride and

cholesterol levels increases in blood owing to cold stress. Endothelial dysfunction appears therefore to predate the development of microalbuminuria as a marker for the development of coronary artery disease (30). Since enalapril maleate is an antihypertensive drug, it can not affect blood albumin level.

Since cold stress and enalapril maleate treatment together have not so important influence on cholesterol and triglyceride level, they also can not affect albumin level.

To conclude, cold stress increases TH enzyme activity and biosynthesis and so biosynthesis of catecholamine by affecting adrenergic system in neural way. Enalapril maleate treatment eliminates negative effects such as increase in blood cholesterol, triglyceride and glucose levels caused by cold stress. The results of this study are parallel with literatures (15, 16, 31).

In addition, results showed decrease in blood pressure and drug score modifying risk factors, i.e. blood glucose, cholesterol and triglycerides decreased overall improvement in subjective well being and quality of life. There was decrease in catecholamine and sympathetic activity. The ACE inhibitor enalapril can play an important role in risk modification for cardiovascular diseases in mild to moderate hypertension.

REFERENCES

1. Blumenthal JA, Emery CF, Madden DJ, et al. Cardiyovascular and behavioral effects of aerobic exercise training in healthy older men and women. J. Geron,1989; 44:147-157.

2. Yürekli M. The Effects of Phenoxybenzamine of Tyrozine Hydroxylase (TH) and TH mRNA Level in Adrenal Medulla of Sprague Dawley Rats. Tr. J. of Med. Sci, 1998; 28: 35-40.

3. Reynolds JEF, Martindale. The Extra Pharmacopoeia. Thirty first Edition. The Royal Pharmaceutical Society, London Royal, 1996.

4. Miner LL, Baruchin A and Kaplan BB. Effect of Cold Stress on cholinergic receptors in the rat adrenal gland. Neuroscience Letters, 1989; 106:339-344.

5. Fregly MJ, Rossi F, Sun Z, et al. Effect of Chronic Treatment with Prazosin and L-Arjinine on the Elevation of Blood Pressure during Cold Exposure. Pharmacology, 1994; 49:351-362.

6. Noyan A. Fiztoloji Ders Kitabı. Sekizinci Baskı. Anadolu Üniversitesi Yayınları No: 2 Meteksan Ankara, 1993.

7. Roberts J, Tumer N. Age related changes in autonomic function of catecholamines. Rewiew of Biological in Aging, 1987; 3:27-298.

8. Klinik Biyokimya I Ders Notları, Çukurova Üniversitesi Tıp Fakültesi Yayınları, No:6 Klinik Biyokimya Anabilim Dalı, Adana 1997.

9. Vietor I, Rusnak M, Viskupik E, et al. Glucoprivation by insulin leads to trans-synaptic increase in rat adrenal tyrosine hydroxylase mRNA levels. Eur J Pharmacol, 1996; 313:119-27.

10. Kunihara M, Oshima T. Effects of epinephrine on plasma cholesterol levels in rats. J Lipid Res, 1983; 24: 639-44.

11. Korner PI. Circulatory control and the supercontrollers. Journal of Hypertension, 1995; 13: 1508-1521.

12. Thomas GN, Tomlinson B, Chan JC, at al. Renin-angiotensin system gene polymorphisms, blood pressure, dyslipidemia, and diabetes in Hong Kong Chinese. Diabetes Care, 2001; 24: 356-61.

13. Swales JD, Dzau VJ. ACE inhibition: research advances and clinical implications. Am Heart J, 1992; 123:1412-1414.

14. Pitt B, Segal R, Martinez FA, et al. On behalf of ELITE Study Investigators: Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan In The Elderly Study, ELITE). Lancet, 1997; 349:747-752.

15. Ferrannini E, Seghieri G, Muscelli E. Insulin and the renin-angiotensin-aldosterone system: influence of ACE inhibition. J Cardiovasc Pharmacol, 1994; 24: S61-S69.

16. Ambrosioni E, Bacchelli S, Degli ED, Borghi C. ACE-inhibitors and atherosclerosis. Eur J Epidemiol, 1992; 8:129-133.

17. Uehara M, Kishikawa H, Isami S, et al. Effect on insulin sensitivity of angiotensin converting enyme inhibitors with or without a sulphydryl group: bradykinin may improve

insulin resistance in dogs and humans. Diabetologia, 1994; 37:300-307.

18. Henriksen EJ, JacobS, Kinnick TR, et al. ACE inhibition and glucose transport in insulin resistant muscle: roles of bradykinin and nitric oxide. Am J Physiol, 1999; 277:R332-R336.

19. Young RP, Chan JCN, Critchley JAJH, et al. Angiotensinogen T235 and ACE insertion/deletion polymorphism associated with albuminuria in Chinese type 2 diabetic patients. Diabetes Care, 1998; 21:431-437.

20. Townsend RR, DiPette D. Pressor doses of angiotensin II increase insulin-mediated glucose uptake in normotensive men. Am J Physiol, 1993; 265:E362-E366.

21. Buchanan TA, Thawani H, Kades W, et al. Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. J Clin Invest, 1993; 92:720-726.

22. Morris AD, Donnelly R. Angiotensin II: an insulin-sensitizing vasoactive hormone? J Clin Endocrinol Metab, 1996; 81:1303-1306.

23. Hennes MMI, O'Shaughnessy IM, Kelly TM, et al. Insulin-resistant lipoloysis in abdominally obese hypertensive individuals: role of the renin-angiotensin system. Hypertension, 1996; 28: 120-126.

24. Ellis GR, Nightingale AK, Blackman DJ, et al. Addition of candesartan to angiotensin converting enzyme inhibitor therapy in patients with chronic heart failure does not reduce levels of oxidative stress. European Journal of Heart Failure, 2002; 4: 193-199.

25. Scribner AW, Loscalzo J, Napoli C. The effect of angiotensin-converting enzyme inhibition on endothelial function and oxidant stres. European Journal of Pharmacology, 2003; 482: 95-99.

26. Prisco D, Paniccia R, Bandinelli B, et al. Short-term ACE inhibition may influence exercise-induced changes in haemostasis in healthy subjects. Fibrinolysis & Proteolysis, 1997; 11:187-192.

27. Chiarelli F, de Martino M, Mezzetti A, et al. Advanced glycation end products in children and adolescents with diabetes: relation to glycemic control and early microvascular complications. J Pediatr, 1999;134:486-491.

28. Chiarelli F, Verrotti A, Mohn A, Morgese G. The importance of microalbuminuria as an indicator of incipient diabetic nephropathy: therapeutic implications. Ann Med, 1997;29:439-445.

29. Gorski J, Gorska M, Hryniewicz A. Effect of cold exposure on the concentration of triglyceride in the liver of the rat. Acta Physiol Pol, 1988; 39: 136-42.

30. Meeking DR, Cummings MH, Thorne S. Endothelial dysfunction in Type 1 diabetic subjects with and without microalbuminuria. Diabetic Medicine, 1999; 16:841.

31. Kodama K, Adachi H and Sonoda J. Beneficial Effects of Long-term Enalapril Treatment and Low-Salt Intake on Survival Rate of Dahl Salt-Sensitive Rats with Established Hypertension. Pharmacology, 1997; 283: 625-629.