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CARDIAC LYMPHATIC OBSTRUCTION IMPAIRS LEFT VENTRICULAR FUNCTION AND INCREASES PLASMA ENDOTHELIN-1 AND ANGIOTENSIN II IN RABBITS

Y.-L. Wang, X.-H. Wang, Y.-L. Liu, X.-Q. Kong, L.-X. Wang

Department of Cardiology (Y-LW,X-HW,Y-LL,X-QK), Liaocheng People's Hospital, Liaocheng City, Shandong Province, PR China, and School of Biomedical Sciences (L-XW), Charles Sturt University, Wagga Wagga, NSW, Australia

ABSTRACT

The treatment of end-stage heart failure can include heart transplantation. During this procedure, cardiac lymphatics are disrupted, which has been demonstrated in animal models to alter left ventricular function, and this compromise itself can cause an increase in endothelin-1 and angiotensin II. We undertook a study in rabbits to assess the effect of cardiac lymphatic obstruction on left ventricular function and plasma levels of endothelin-1 and angiotensin II. Sixty-three New Zealand white rabbits were divided into study (n=41) and control (n=22) groups. Plasma levels of endothelin and angiotensin II were measured before, and at 3, 7, 14, 30, 90 and 180 days following the obstruction of cardiac lymphatic vessels. Left ventricular function was assessed by echocardiography. Six months following the surgery, 18 study and 6 control animals survived. In the study group, a significant decrease was seen in left ventricular ejection fraction within the first three months following the lymphatic obstruction (0.76±0.04 vs 0.72±0.01, p<0.01). Levels of plasma endothelin-1 and angiotensin II were elevated following ligation of cardiac lymphatic vessels with a peak between 3-7 days following lymphatic obstruction (all p<0.05). Plasma endothelin-1 and angiotensin II began to decline 14 days after lymphatic obstruction and returned to almost baseline levels in 6 months. The left ventricular ejection fraction, plasma endothelin-1 and angiotensin in the control group remained unchanged (all p>0.05). We conclude that cardiac lymphatic obstruction reduces left ventricular function in the first three months following obstruction. Cardiac lymphatic obstruction also increases plasma levels of endothelin-1 and angiotensin II. The clinical significance of these transitory changes requires further investigation.

Keywords: cardiac lymphatics, lymphatic obstruction, endothelin, angiotensin II, left ventricular function, rabbits

Cardiac transplantation is the most effective treatment for end-stage heart failure. The cardiac lymphatic system plays an important role in maintaining the balance of interstitial fluid, lipid metabolism, and immune response. After heart transplantation, cardiac lymphatics are inevitably disrupted, which may have a detrimental effect on the anatomy and function of the beating heart (1). In animals, subendocardial edema, myocardial ischemia, and various mitochondrial derangements may occur within several hours following cardiac lymphatic obstruction (2). Chronic cardiac lymphatic obstruction induces endocardial or subendocardial fibrosis, which may compromise left ventricular function (3-6).

Endothelin-1 and angiotensin II are important regulators of local and systemic circulation. The biosynthesis or release of endothelin-1 and angiotensin II are activated by a number of factors, one of which is ventricular dysfunction (7). The primary purpose of this study was to investigate the effects of chronic cardiac lymphatic obstruction on the left ventricular function and plasma levels of endothelin-1 and angiotensin II in a rabbit model.

Materials and Methods

Surgical preparation of the animal model

The study was approved by the Institutional Review Board of Liaocheng People's Hospital. Sixty-three New Zealand white rabbits of both sexes (body weight 2.5-3.5 kg, aged 12-18 months) were selected for the study. Forty-one animals were assigned to the study (lymphatic obstruction) group, and 22 underwent sham operation as control.

We followed our previously reported surgical techniques for cardiac lymphatic vessel ligation in the rabbit (2). In short, under general anesthesia, left thoractomy was performed in the fourth intercostal space, and the heart was suspended in a pericardial cradle. In the study group, 0.5 ml of 10% methylene blue was injected into the wall of the left and right ventricular apex, clearly marking the lymphatic vessels in the epicardium and the adjacent lymph nodes. The main lymphatic vessels seen in the epicardium were ligated, and the large lymph nodes between the aortic root and pulmonary artery and between the posterior aorta and right superior pulmonary artery were also destroyed by electric cautery. The chest was then closed by layers and animals returned to the animal housing center for standard care by investigators and staff. Penicillin (average 0.8 million units) was administered intramuscularly daily for one week after operation to prevent wound infection.

Control animals underwent the same open-chest surgery as the lymphatic obstruction group, but without the ligation of the lymphatic vessels or the destruction of lymph nodes.

Assessment of left ventricular function

Left ventricular ejection fraction (EF) was measured by echocardiography (HP SONOC-5500, Agilent Technologies, Andover, MA, USA) before and after operation.

Measurement of endothelin-1 and angiotensin II

Venous blood (4 ml) was obtained before, and 3, 7, 14, 30, 90 and 180 days following the lymphatic obstruction. Normal saline (5 ml) was intravenously injected after each blood collection. Blood was placed in a tube containing 30μl EDTA and centrifuged (3000r/min) at 4°C for 15 min. Plasma was separated and stored at -70°C for measurement of endothelin-1 and angiotensin II with ELISA (reagents from TPI, USA).

Statistical Analysis

Data was expressed as mean ± standard deviation. Numerical data were analyzed by ANOVA, and categorical data were analyzed using Chi-square test with $p<0.05$ considered statistically significant for all tests.

RESULTS

General Findings

Seven animals died within 3 days following lymphatic obstruction and by day 180, there were 18 experimental animals remaining. In the control group, all animals survived the initial operation and by day 180, 6 animals survived. The causes of the deaths were unclear but in a large proportion of the animals from study and control groups, severe diarrhea was present several days before the death.

TABLE 1
Left Ventricular Ejection Fraction Following the Surgery

	Study group	Control group	p
Before	0.76 ± 0.04 (41)	0.76 ± 0.04 (22)	NS
Day 3	0.64 ± 0.05* (34)	0.76 ± 0.02 (22)	<0.01
Day 7	0.64 ± 0.04* (31)	0.73 ± 0.05 (19)	<0.01
Day 14	0.68 ± 0.03* (27)	0.75 ± 0.05 (16)	<0.01
Day 30	0.71 ± 0.03* (21)	0.76 ± 0.03 (12)	<0.01
Day 90	0.72 ± 0.01* (21)	0.77 ± 0.05 (9)	<0.01
Day 180	0.74 ± 0.04 (18)	0.75 ± 0.04 (6)	NS

Note: The numbers in the bracket were the number of surviving animals at that time point.
*p<0.01 compared with the baseline value in the same group.

Pericardial fluid of 1-9 ml (between the left ventricular posterior wall and the pericardium) was detected by echocardiography in 55.9% (19/34) of the study and 22.7% (5/22) of the control group animals three days following the surgery (p<0.05). There was a gradual reduction in prevalence of pericardial fluid and at the end of the first four weeks, 4.2% (1/24) of the study group and none (0/12) of the control animals had pericardial fluid.

Changes in left ventricular function

Table 1 shows the average values of the left ventricular ejection fraction before and after operation. There was no significant difference in the ejection fraction between the study and control groups before the surgery (p>0.05). In the control group, average ejection fraction remained unchanged following operation.

Following operation, there was a significant reduction in the left ventricular ejection fraction in the study group between day 3 and 90. By day 180, the average ejection fraction returned to the baseline.

Changes in endothelin-1 and angiotensin II

As shown in *Table 2*, there was a moderate increase in the endothelin-1 levels on day 3 and 7 following the sham operation (p<0.05). The average values of angiotensin II in the control group remained unchanged (p>0.05, *Table 3*).

In the study group, endothelin-1 levels were elevated between day 3 and 30 (p<0.05, *Table 2*). By day 90, average endothelin-1 levels were not statistically significant different from baseline. There was a significant increase in the plasma levels of angiotensin II 90 days after the lymphatic obstruction (p<0.05, *Table 3*) and by day 180, the average levels of the angiotensin II had returned to baseline (*Table 3*).

DISCUSSION

The major findings of this study are: 1) Cardiac lymphatic obstruction results in a reduction in left ventricular ejection fraction within the first 8 weeks of the operation; and 2) Lymphatic obstruction is associated with a significant increase in the plasma endothelin-1 and angiotensin II within the first few weeks of obstruction. However, plasma levels of endothelin-1 and angiotensin II returned to baseline after three months.

TABLE 2
Changes in Endothelin-1 (ng/L) Following Cardiac Lymphatic Obstruction

	Study group	Control group
Before	28.3 ± 2.0 (41)	27.4 ± 2.0 (22)
Day 3	41.8 ± 4.2▲* (34)	32.2 ± 3.1▲ (22)
Day 7	45.4 ± 3.9▲* (31)	31.3 ± 2.6▲ (19)
Day 14	40.4 ± 4.6▲* (27)	29.4 ± 1.7 (16)
Day 30	34.2 ± 4.6▲* (21)	28.4 ± 3.2 (12)
Day 90	31.8 ± 3.3 (21)	27.5 ± 2.4 (9)
Day 180	31.4 ± 2.7 (18)	27.3 ± 2.6 (6)

Note: The numbers in the bracket were the number of surviving animals at that time point.
▲$p<0.05$ compared with baseline value in same group; *$p<0.05$ compared with control.

TABLE 3
Changes in Angiotensin II (ng/l) Following Cardiac Lymphatic Obstruction

	Study group	Control group
Before	505.1 ± 10.3 (41)	504.9 ± 18.3 (22)
Day 3	589.4 ± 34.8▲* (34)	506.9 ± 26.3 (22)
Day 7	630.8 ± 39.1▲* (31)	500.7 ± 27.8 (19)
Day 14	568.3 ± 24.8▲* (27)	492.0 ± 17.0 (16)
Day 30	529.5 ± 30.3* (21)	488.5 ± 21.2 (12)
Day 90	514.4 ± 20.2* (21)	487.7 ± 27.0 (9)
Day 180	510.6 ± 25.7 (18)	496.8 ± 19.3 (6)

Note: The numbers in the bracket were the number of surviving animals at that time point.
▲$p<0.05$ compared with baseline value in same group; *$p<0.05$ compared with control.

Endothelin-1 is a potent vasoconstrictor produced by vascular endothelial cells (8), and high levels of plasma endothelin-1 are seen in patients with heart failure (7). However, it is not clear whether the increase in endothelin-1 in heart failure patients is a cause or an effect. Hypoxia, ischemia, or shear stress induces the transcription of endothelin-1 messenger RNA (mRNA) and the synthesis and secretion of endothelin-1 within minutes (9). A previous study in ischemic heart disease showed upregulation of endothelin-1 receptors (10).

In the present study, ligation of large lymphatic vessels together with the destruction of large cardiac lymph nodes is expected to cause significant reduction or occlusion in lymph flow (9). Although the degree of lymphatic obstruction was not measured in the present study, the surgical procedures undertaken in this study have been found to result in significant myocardial edema and fibrosis within the first four weeks of operation (5). These factors may have contributed to the temporal reduction in left ventricular function and the increase in the

biosynthesis of endothelin-1. In addition, acute or chronic obstruction of lymphatic flow are known to cause ischemia and atherosclerotic changes in the coronary arteries (11-13), which may also stimulate the production of endothelin-1 from the vascular endothelial cells.

Angiotensin II is also a potent vasoconstrictor. It increases heart rate, cardiac contractile force, and induces ventricular hypertrophy (14). The signaling pathways for the angiotensin II elevation in the present study are unclear. However, the decline in the left ventricular function following lymphatic obstruction may be responsible, because ventricular dysfunction or heart failure is known to activate the renin-angiotensin system. Elevation in angiotensin II may be detrimental to the heart because angiotensin II-induced hypertrophy of heart muscle causes an increase in energy requirements and decline in efficiency (14). Angiotensin II and endothelin-1 have been found to precipitate heart failure by activating cardiac remodeling and causing ventricular hypertrophy (14).

Pericardial effusions were detected in more than 50% of the animals following lymphatic obstruction. This is likely caused by myocardial edema as our previous tissue examination using this animal model has shown a significant degree of edema in the ventricular myocardium (5). Another important finding is that the reduction in the left ventricular function following lymphatic obstruction in this rabbit model is transitory. In the first 90 days following the lymphatic obstruction, the average left ventricular ejection fraction in the study group was significantly lower than in the control group. However, there was no significant difference in the left ventricular ejection fraction between the two groups six months following the surgery. The reasons for the resumption of left ventricular function in the study group animals are unclear. It is possible that the cardiac lymphatic vessels have re-developed in these rabbits or alternatively, that the cardiac venous system may have taken up the function of the cardiac lymphatic vessels.

The cause of death for rabbits was not established since no autopsies were completed. In the majority of cases, animals exhibited severe diarrhea for several days before death indicating a likely cause other than the lymphatic obstruction. Supporting this conclusion are the many control rabbits that also died and the data demonstrating a return to baselines values by 180 days in experimental rabbits.

In conclusion, chronic cardiac lymph flow obstruction in this rabbit model impairs left ventricular function within the first three months of the surgery. The lymphatic obstruction is also associated with a significant elevation in plasma endothelin-1 and angiotensin II. The clinical significance of this transitory reduction in the left ventricular function and the elevation in endothelin-1 and angiotensin II as observed in this study requires further investigation.

REFERENCES

1. Kong, XQ, LX Wang, DG Kong: Cardiac lymphatic interruption is a major cause for allograft failure after cardiac transplantation. Lymphatic Res. Biol. 5 (2007), 45-48.
2. Sun, SC, JT Lie: Cardiac lymphatic obstruction: Ultrastructure of acute-phase myocardial injury in dogs. Mayo Clinic. Proc. 52 (1977), 785-792.
3. Kline, IK, AJ Miller, R Pick, et al: The relationship between human endocardial fibroelastosis and obstruction of the cardiac lymphatics. Circulation 30 (1964), 728-735.
4. Miller, AJ, R Pick, LN Katz: Ventricular endomyocardial pathology produced by chronic cardiac lymphatic obstruction in the dog. Circ. Res. 8 (1960), 941.
5. Kong, D, X Kong, L Wang: Effect of cardiac lymph flow obstruction on cardiac collagen synthesis and interstitial fibrosis. Physiol. Res. 55 (2006), 253-258.
6. Miller, AJ: The study of the lymphatics of the heart: An overview. Microcirc. Endothelium. Lymphatics 2 (1985), 349-360.
7. Konstam, MA, D DeNofrio: Endothelin expression and the progression of heart failure: Exemplifying the vagaries of therapeutic development. Circulation 109 (2004), 143-145.

8. Boulanger, C, TE Luscher: Release of endothelin from the porcine aorta. J. Clin. Invest. 85 (1990), 587-590.
9. Levin, ER: Mechanisms of disease: Endothelins. N. Engl. J. Med. 333 (1995), 356-363.
10. Wackenfors, A, M Emilson, R Ingemansson, et al: Ischemic heart disease induce upregulation of endothelin receptor MRNA in human coronary arteries. Eur. J. Pharmacol. 484 (2004), 103-109.
11. Tian, H, ZY Liu, Y Jiao: Impact of acute lymphatic obstruction on the structure of coronary arteries. J. Anatomy 203 (1997), 63-365.
12. Tia, H, ZU Liu, ZX Ding: Pathology of coronary arteries following chronic lymphatic obstruction. J. Shandong Med. Univ. 36 (1998), 271.
13. Miller, AJ, A De Boer, A Palmer: The role of the lymphatic system in coronary atherosclerosis. Med. Hypo. 7 (1992), 31-36.
14. Zolk, O, J Quattek, U Seeland, et al: Activation of the cardiac endothelin system in left ventricular hypertrophy before the onset of failure in TG (mREN2) 27 rats. Cardiovasc. Res. 53 (2002), 363-371.

Lexin Wang, MD, PhD
School of Biomedical Sciences
Charles Sturt University
Wagga Wagga, NSW 2678, Australia
Phone: +61 2 69332905
Fax: +61 2 69 332587
E-mail: lwang@csu.edu.au