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Mr-Proanp Rises during Exercise Even after Surgical Closure of the Left Atrial Appendage: A Sub-Study of the Laacs Randomized Study

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Abstract

Objectives: The atrial appendages play a regulatory role in the fluid homeostasis and in the secretion of atrial natriuretic peptide (ANP). ANP is a 28-amino-acid peptide synthesized in the cardiac myocytes, where it is stored as a pro-hormone in cardiomyocyte granules. When released, it participates in cardiovascular volume and pressure homeostasis.
We hypothesized that patients with surgical closure of the Left Atrial Appendage (LAA) – would have lower increase of Mid Region-pro-hormone ANP (MR-proANP) during exercise compared to patients with open LAA. Patients were randomized into two groups – one with surgical closure of the LAA during planned cardiac surgery and one with open LAA.

Methods: A group of 19 patients who had undergone elective open heart surgery, and who had been randomized to either the LAA-closure group (n=9), or to the LAA-open group (n=10) performed a standard bicycle exercise test, at least six weeks after the operation. Closure of the LAA was confirmed by Transesophageal Echocardiography (TEE). Venous blood samples were collected before the test, and within the first two minutes of recovery after maximum work-load.

Results: Baseline ANP showed (non-closed vs. closed LAA): 163.3pM (95% CI: 121.9 – 204.8) vs. 137.5 pM (95% CI: 69.5 – 205.6), p=0.4575.
Maximum work-load was similar for both groups. MR-proANP concentration rose significantly:
Max ANP (non-closed vs. closed LAA): 197.4pM (95% CI: 146.9 - 247.9) vs. 164.3pM (95% CI: 89.1 - 239.6), p=0.4061
MR-proANP increase was not different between the groups.
Delta ANP (non-closed vs. closed LAA): 34.1pM (95% CI: 22.1 – 46.1) vs. 26.8pM (95% CI: 15.3 – 38.3), p=0.3352

Conclusion: This study shows that plasma MR-proANP increases during exercise, and that surgical LAA closure did not reduce the increase of MR-proANP in response to physical exercise. These findings indicate that other areas of the heart elicit most of the MR-proANP production

Introduction

In this paper we present measurements of MR-proANP levels during exercise tests on two groups of patients who underwent open heart surgery, and who had been randomized to either having the Left Atrial Appendage (LAA) surgically closed as additional procedure or having it left open.

Background

The Natriuretic Peptide System (NPS) is an endogenous system consisting of natriuretic peptides, including atrial natriuretic peptide (ANP), brain-natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) [1]. ANP is a 28-amino-acid peptide synthesized in the atrial myocytes in response to atrial distension [1,2]. When synthesized ANP is stored in specific intracellular myocyte granules as a pro-hormone (pro-ANP) that undergoes final processing during release into the circulation, to yield the biologically active C-terminal and the inactive N-terminal fragment ANP [2]. N-terminal fragment of pro-hormone-ANP (NT-pro-ANP) derives from the proteolysis of pro-ANP, where pro-ANP is released both as an active hormone and an inactive N-terminal fragment [1]. NT-proANP has a much longer half-life than mature ANP and has therefore been suggested to be a more reliable analyte for measurement than mature ANP [3].

An assay for NT-proANP has been developed, utilizing antibodies against the mid-region of the molecule, thus termed mid-regional pro-atrial natriuretic peptide (MR-proANP) [4].

ANP is believed to protect the cardiovascular system from volume and pressure overload through two major pathways; a vasodilatory effect and a renal effect [1,2,5].

ANP directly dilates veins, resulting in a decrease in central venous pressure and thereby decreasing ventricular pre-load. It also dilates arteries, resulting in a decrease in systemic vascular resistance and arterial pressure. It increases glomerular filtration and decreases renin secretion, counter-effecting the renin-angiotensin-aldosterone system, thereby acting as a diuretic [2,5,6] In addition, ANP has a cardio protective role by regulating cellular growth, cellular proliferation, and cardiac hypertrophy [7]. Under normal circumstances the myocytes in the atria and the ventricles in the human heart will produce ANP to regulate fluid homeostasis [8,9] but when the ventricles and atria in the heart are stretched, the synthesis of these peptides increases. ANP gene expression is increased in the ventricular myocardium of the failing heart proportionally with the severity of congestive heart failure or ventricular haemodynamic overload [10]. Therefore resting levels of natriuretic peptides are higher in chronic heart failure and atrial fibrillation compared with normal [1,11,12].

ANP gene transcripts have been shown in both atrial and ventricular myocytes; however, the level of ventricular ANP transcription is generally lower than that found in the atria [1,9,13].

It has been reported that the most intensely staining granules are localized in the atrial appendages [13,14]. There is still a need for better mapping of the ANP producing areas,[8] and it is claimed that 30 % of the ANP in the heart is contained in the LAA [15].

During exercise circulating levels of ANP increase rapidly. After ending exercise ANP falls to pre-exercise values within 30 minutes [16]. Intriguingly, patients with heart failure and patients with ischemic heart disease experience a greater increase in plasma-ANP during exercise, which has been attributed to predominance of ANP pre-secretory atrial stores [17,18].

We hypothesized that patients in the LAA – closure group, randomized to surgical closure of the LAA during planned surgery, would have lower increases of MR-proANP during exercise compared to the LAA-open group, as a surrogate for ANP, due to its higher stability.

Method

The patients where recruited from the department of Thoracic surgery at the Region of Copenhagen, Denmark, and offered to participate in the LAACS project (Left Atrial Appendage Closure with Surgery) protocol (ID NCT02378116, at ClinicalTrials.org). Patients were randomized to surgical closure of the LAA in addition to their planned open heart surgery; (the LAA-closure group) or control (the LAA-open group). The study was approved by Regional Ethics Committee of Capital Region Denmark. All patients have signed informed consent. Exclusion- and inclusion criteria for the LAACS trial are presented in table I.

For the group of patients randomized to surgical closure, the surgeon closed the LAAs with a purse-string ligature secured with single stitches.

The main study aims to test whether surgical closure of the LAA protects the brain against thromboembolic events. The patients who accepted blood sample collection during an exercise test at least six weeks after surgery, provided the material for this sub-study. The exercise test was performed with a standard ergo-meter bicycle (Ergo line GMBH cycle, with Spacelab Healthcare cardio navigator v.2.604) with increasing load according to current guidelines [19,20] The patients were asked to refrain from physical activities on the day of testing.

After an initial physical examination, the patients rested for a minimum of 10 minutes, before collection of the first venous blood sample to determine resting levels of MR-proANP. A second blood sample was drawn within two minutes after reaching maximum tolerated exercise load.

We measured plasma MR-proANP as, unlike ANP, is well preserved in plasma [21]

For the measurements of plasma MR-proANP, we used an automated proANP immunoassay (BRAHMS, Hennigsdorf, Germany) with targeted epitopes in the mid-region of the precursor (MR-proANP) [22].

Selected results of the exercise test are listed in Table II.

Table I. Exclusion- and inclusion criteria for the LAACS trial.

Inclusion criteria	Exclusion criteria
Age > 18 years	Off-pump heart surgery
Planned CABG	Endocarditis
Planned valve surgery, alone or in combination with CABG	Patients with pacemaker or other metal implantations non- compatible with MRI
	Planned implantation of pacemaker.

Table II. Results of exercise test grouped according to randomization.

	LAA-closure goup	LAA-open group
Maximum heart rate, beats per minute mean, (SD)	127 (22)	121 (14) (p=0,53)
Start systolic blood pressure, mmHg, mean, (range),SD	140 (153-119),10,04	135 (189-105), 25,64 (p=0,59)
Max systolic blood pressure, mmHg, mean, (range),SD	198 (219-158), 22,75	190 (211-147), 21,52 (p=0,46)
BORG scale mean, (SD)	17 (0,83)	16 (0,96)
Time to max, minutes mean (SD)	11 (4.17)	11 (3.55)

Statistics

In healthy persons, plasma concentration of natriuretic hormones increases by 50% under maximum tolerated workload on an bicycle ergometer [23]. Patients who have undergone open heart surgery experience a similar increase, although they have higher pre-test levels [24]. Variation on natriuretic hormone measurements in a population of patients with heart disease is about 20% [25] According to these studies, we calculated that we could detect a 30% difference between the two groups (with expected lower increase in the group randomized to closure of the LAA) by including 42 patients with 90% power and a 5% risk of type 2 error. With 80% power and the same significance level, according to the variation observed in MR-proANP measurements [22], we calculated that we needed to include eight patients in each group and we planned to include 10 patients in each group.

All data analyses were performed using SAS9.4 (Cary, NC). Continuous data are expressed as mean ±SD and categorical variables as proportions. After inspection for normality, between group differences were compared using the t-test, Mann–Whitney U test and Fisher's exact test, as appropriate. For all hypothesis testing, a two-tailed $p<0.05$ was required for statistical significance.

Results

A total of 24 patients were included but five patients were not able to perform the test. One did not show up repeatedly to the appointments and four were too physically weak to perform the test.

Accordingly, data from a total of 19 patients were analyzed in this study: Nine patients randomized to closure of the LAA and 10 randomized to the control group. Patient characteristics are summarized in Table III.

Exercise test was performed in average 589 ± 116 days after surgery for the group with open LAA and 545± 210 days for the group with closed LAA. No tests were done earlier than six weeks from surgery.

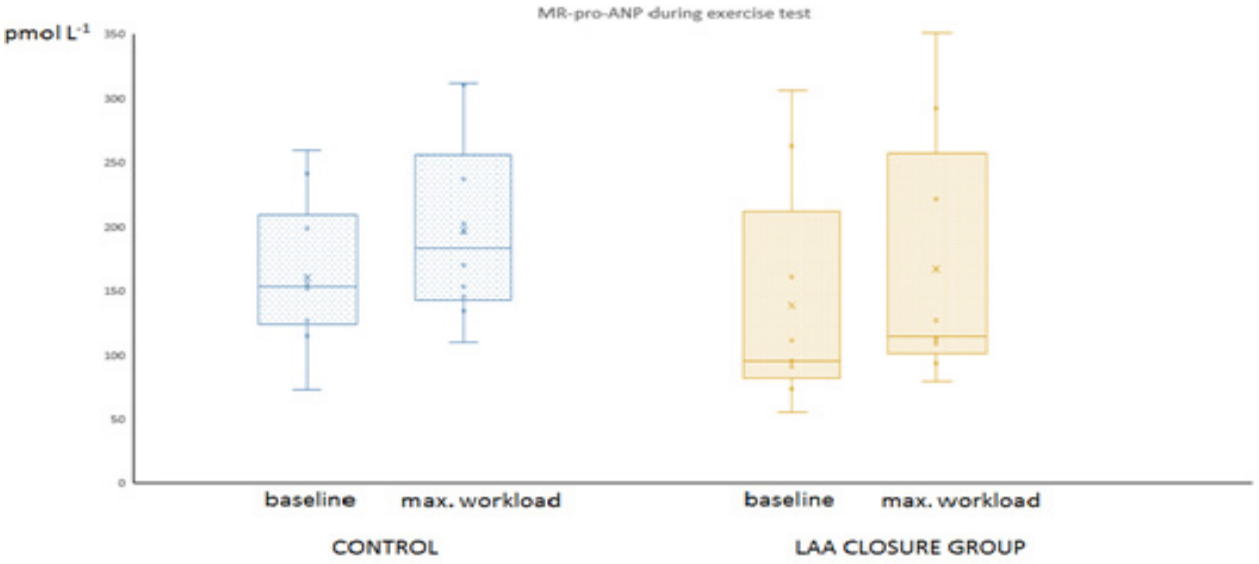
There were no significant difference in the exercise capacity of the patients in the two groups (Table II).

Plasma MR-proANP concentrations at baseline and after max workload for each individual in the two groups are depicted in figure 1 and can be seen in detail in Table III.

Table III. Patient characteristics and MR-pro-ANP levels.

	age (years)	sex	Left Ventricle Ejection Fraction (%)	Left Atrium volume/ BSA (ml/ m2)*	beta-blok-ker use	RAS blockade use	MR-pro-ANP baseline (pM)	MR-pro-ANP max. load (pM)	delta MR-pro-ANP (pM)
CON-TROL									
1	54	m	50	NA	yes	no	75	110	34
2	66	m	60	32.5	no	no	118	135	17
3	61	m	55	33	yes	yes	202	238	36
4	69	m	60	>40	yes	yes	158	196	38
5	56	m	60	>40	yes	no	263	311	48
6	80	f	55	NA	yes	no	245	312	68
7	66	m	60	NA	yes	no	130	146	16
8	76	m	60	32.2	yes	yes	155	171	16
9	74	m	60	NA	yes	no	159	203	44
10	73	m	45	NA	yes	yes	130	154	24
CLOSED LAA									
1	76	f	60	32.3	no	yes	160	219	59
2	53	m	50	27.9	yes	no	54	77	23
3	70	m	35	34.4	yes	yes	262	290	28
4	55	f	60	NA	yes	yes	90	110	20
5	72	m	60	28	no	yes	110	125	14
6	70	m	55	NA	yes	no	306	349	43
7	75	f	50	NA	no	no	94	112	18
8	64	m	45	37.6	yes	yes	72	91	19
9	74	m	50	28	no	no	90	106	16
p-values	0,96		0,14	0,08	0,10	0,52	0,46	0,41	0,32

Figure 1. "MR-ProANP during exercise test"



Baseline ANP showed (non-closed vs. closed LAA): 163.3pM (95% CI: 121.9 – 204.8) vs. 137.5 pM (95% CI: 69.5 – 205.6), p=0.4575.

Maximum work-load was similar for both groups. MR-proANP concentration rose significantly:

Max ANP (non-closed vs. closed LAA): 197.4 pM (95% CI: 146.9 - 247.9) vs. 164.3 pM (95% CI: 89.1 - 239.6), p=0.4061

MR-proANP increase was not different between the groups.

Delta ANP (non-closed vs. closed LAA): 34.1 pM (95% CI: 22.1 – 46.1) vs. 26.8 pM (95% CI: 15.3 – 38.3), p=0.3352

We made a sub analysis where we excluded a total of two patients with low ejection fraction (EF) and AF, which are known to give higher ANP levels.

Sub-analysis (excluding one patient with low EF and one patient with permanent AF):

Baseline ANP (non-closed vs. closed LAA): 163.3 pM (95% CI: 121.9 - 204.8) vs. 95.7 pM (95% CI: 64.7 - 126.7), p=0.0144

Max ANP (non-closed vs. closed LAA): 197.4 pM (95% CI: 146.9 - 247.9) vs. 120.0 pM (95% CI: 77.1 - 162.9), p=0.0229

Delta ANP (non-closed vs. closed LAA): 34.1 pM (95% CI: 22.1 – 46.1) vs. 24.3 pM (95% CI: 9.8 - 38.8), p=0.2403

Discussion

We hypothesized that the MR-proANP levels in the LAA-closure group would not rise as much as the LAA-open group. Our findings indicate that this is not the case, and that other areas of the heart may have important ANP secreting properties.

Even though LAA is thought to play a central role in MR-proANP release, [7,8] studies using immunohistochemically techniques have demonstrated a greater number of intensely granulated cardiomyocytes secreting MR-proANP in the right rather than in the left atrium [9]. If the myocytes outside the LAA produce most of the MR-proANP, it can be difficult to measure the impact of LAA closure on MR-proANP levels, thereby explaining our results. Older experimental studies on rats seem to support this dominant role of the right atrial appendage [26].

In humans, Omari et al. examined the effect of right atrial appendage removal during cardiac surgery, in patients with normal systolic function and concluded that preserving the right atrial appendage during cardiac operations significantly increased the release of atrial natriuretic hormone [27].

Two patients in the LAA-closure group had particularly elevated MR-proANP measurements. Trans-esophageal echocardiography demonstrated completely closed LAA in both patients, but they differed from the rest of the cohort as one of them was the only one with heart failure (EF 35%) and the other was the only patient in the cohort with permanent Atrial Fibrillation (AF). If we exclude these two patients in a sub-analysis, the LAA-open group had significantly higher pre-test MR-proANP levels (163.3 pM SD 54.95) compared to the group with closed LAA (95.7 pM, SD 42.94), p=0.0144, and significantly higher max ANP (non-closed vs. closed LAA): 197.4 pM (95% CI: 146.9 - 247.9) vs. 120.0 pM (95% CI: 77.1 - 162.9), p=0.0229.

Increase during exercise did not differ between the two groups 34.1% (SD 10.37) in the group with open LAA compared to 24.3% (SD 11.01) in the group with closed LAA, p=0.24.

Both hearth failure and AF are known to increase the levels of MR-proANP [12,11]. The only patient in the cohort with permanent AF had the highest levels of MR-proANP.

Possible clinical implications of affection of the ANP levels, especially if lower resting levels, could be disturbances in the blood pressure and electrolyte homeostasis, the areas mostly affected by ANP [5]. A study from 1998 looked at patients having Maze surgery. Maze procedure was performed in open heart surgery in order to treat AF and consists of surgical amputation of the LAA and the right atrial appendage and ablation of the pulmonary veins. This procedure is not commonly done in today's surgery and has largely been replaced by radio frequency ablation (RFA) together with LAA closure, where the right atrial appendage is left untreated.

A group of patients having maze surgery were compared with a control group. There was a tendency to fluid retention in the maze group and a significantly greater doses of furosemide and dopamine were administered to the maze group and the response of ANP secretion by exercise was significantly attenuated in the maze group (n = 12) compared with the non-maze group (n = 9) [28]. It has not been reported that patients with LAA closure have had problems with fluid retention and needing additional furosemide treatment. It is important to notice that the right atrial appendage in our cohort has been left untreated, furthermore supporting that the right atrial appendage is an area of importance involved in ANP production. Studies using immunohistochemically techniques have also demonstrated a greater number of intensely granulated cardiomyocytes secreting MR-proANP in the right rather than in the left atrium [9].

Much larger scale studies are needed to answer this question. We find this relevant to examine in future studies in regards to the safety of the LAA-closure which is becoming more and more common. Still we believe that the benefits of protection from stroke from closure of the LAA would by far surpass possible disturbances in the ANP levels.

The main strength of our study is that we had a unique opportunity to compare the effect of surgical closure of the LAA on MR-proANP circulating levels in a randomized study in humans. Our results could be explained by a more prominent role of the right atrium on MR-proANP changes during hemodynamic challenges, which could be addressed in future studies.

In a recently published study, Cruz Gonzales et al. [29] found a significant decrease in resting levels of BNP 45-60 days after device implantation for closure of the LAA. Unfortunately, we did not perform pre- surgery measurements of MR-proANP. Hence, we cannot discard that resting level differences could have been apparent before surgery in patients with conditions of volume overload where the diuretic effects of ANP are activated in the LAA, such as in heart failure. Furthermore, it is probable that other areas of the heart are able to "compensate" when the LAA is closed.

Conclusion

In our study, surgical LAA closure did not reduce the increase of MR-proANP in response to physical exercise, indicating that other areas of the heart elicit most of the MR-proANP production.

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