Journal of Cardiovascular Disease & Atherosclerosis

Open Access

Austin Publishing Group

Original Article

The Anti-Anginal Efficacy of Bosentan in the Coronary Slow Flow Phenomenon

Victoria Kopetz, BSc (Hons), PhD[1,3]; Rosanna Tavella, BSc (Hons), PhD[1-3]; Sivabaskari Pasupathy, BSc (Hons), PhD[1-3]; John Horowitz, BSc, MBBS, PhD[1,3]; John Beltrame, BSc, BMBS, FRACP, PhD, FESC, FACC, FAHA, FCSANZ[1-3]*

[1]School of Medicine, Faculty of Health Sciences, The University of Adelaide, Adelaide, SA 5000, Australia

[2]Central Adelaide Local Health Network, Adelaide, SA 5000, Australia

[3]Basil Hetzel Institute for Translational Health Research, Adelaide, SA 5011, Australia

***Corresponding author:** **John F Beltrame**
Faculty of Health Sciences, University of Adelaide Central Adelaide Local Health Network, 28 Woodville Road, Woodville South, Adelaide, SA, Australia.
Tel: +61-8-8222 6740; Fax: +61-8-8222 6042
Email: john.beltrame@adelaide.edu.au

Received: July 28, 2023
Accepted: September 07, 2023
Published: September 14, 2023

Abstract

Background: The Coronary Slow Flow Phenomenon (CSFP) is a coronary microvascular disorder that presents with recurrent angina for which there is no available effective therapy. The vasoconstrictor Endothelin-1 (ET-1) has been implicated in the pathophysiology of the CSFP. This study aimed to determine whether the ET-1 antagonist (Bosentan) is effective in ameliorating angina symptoms in CSFP patients.

Methods: Using a randomised, double-blind, placebo-controlled, cross-over trial methodology, the anti-anginal efficacy of Bosentan 125mg bd was assessed in 23 patients with symptomatic CSFP. Study endpoints were measured following 4 weeks of therapy with the primary endpoint being angina frequency. Secondary endpoints included (1) clinical measures of angina and health status, (2) endothelial function parameters, and (3) inflammatory and oxidative stress markers.

Results: There was no statistically significant reduction in angina frequency with Bosentan therapy compared to placebo (median angina episodes/4weeks [25-75% IQ]: Bosentan 14[5,36] vs Placebo 25[9,55]) although a trend for improvement in symptoms during the Bosentan phase was observed. Furthermore, Bosentan did not impact on any of the secondary endpoints compared with placebo.

Conclusion: Bosentan did not have a major impact on angina frequency in patients with the CSFP. A small anti-anginal effect cannot be excluded and requires a larger trial.

Introduction

The Coronary Slow Flow Phenomenon (CSFP) is a coronary microvascular disorder characterised by delayed distal vessel opacification in the absence of obstructive coronary artery vessel disease [1]. Clinically distinct from other microvascular disorders such as cardiac syndrome X [2], the mechanisms responsible for the microvascular dysfunction remain elusive, although a plethora of pathophysiological investigations have reported abnormalities in endothelial function [3-5], inflammatory markers [6,7], oxidative stress markers [5,8], and platelets [9]. However some studies have been unable to replicate these observed abnormalities [10-12] there by making it difficult to identify specific therapeutic targets. One line of pathophysiological investigations in the CSFP with a potential therapeutic target is Endothelin-1 (ET-1). This potent endogenous vasoconstrictor peptide is a combined Et-A and Et-B receptor antagonist and has been implicated in the pathogenesis of the CSFP based upon the following observations: (a) intracoronary ET-1 administration mimics the angiographic appearance of the CSFP in canine [13] and rabbit [14] studies; (b) ET-1 levels have been shown to be elevated in patients with the CSFP [15]; (c) intravenous ET-1 infusion into healthy individuals produces a fall in coronary sinus oxygen saturation [16] mimicking those observed in the CSFP [17]; and (d) a selective hyper-responsiveness to ET-1 in isolated subcutaneous microvessels of patients with the CSFP [18]. To provide confirmatory evidence that ET-1 plays a major role in the CSFP, the influence of a dual endothelin-1 receptor antagonist on clinical and pathophysiological measures should be investigated. Accordingly, the primary objective of this study was to examine if ET-1 blockade with Bosentan 125mg bd would influence the angina frequency in patients with symptomatic CSFP. Supporting secondary objectives examined the effects of Bosentan therapy on other clinical and pathophysiological endpoints including: (i) other clinical markers (prolonged angina episodes, sublingual nitrate consumption, health status measures); (ii) endothelial function assessed by pulse wave analysis and Asymmetric Dimethylarginine (ADMA) plasma levels; (iii) inflammation status as evaluated by High-Densitivity C-Reactive Protein (hsCRP) and Myeloperoxidase (MPO); and (iv) oxidative stress determined by Malondialdehyde (MDA) and homocysteine biomarkers.

Citation: Kopetz V, Tavella R, Pasupathy S, Horowitz J, Beltrame J. The Anti-Anginal Efficacy of Bosentan in the Coronary Slow Flow Phenomenon. Austin J Cardiovasc Dis Atherosclerosis. 2023; 10(1): 1057.

Methods

To achieve the above objectives, the study employed a randomised, double-blind, placebo-controlled, cross-over study design involving symptomatic CSFP patients. The study was approved by the local Hospital and University Human Research Committees. The active study medication utilised was Bosentan monohydrate 125mg tablet (Actelion Pharmaceuticals Australia Pty Ltd), administered orally twice daily. A corresponding placebo that was indistinguishable in appearance to the active drug was also supplied by the manufacturers.

Study Patients

Both recently diagnosed and patients with established CSFP were recruited if they fulfilled the following inclusion criteria: (1) stable recurrent angina episodes ≥3 times/week; and (2) angiographic evidence of the CSFP as defined by (a) requiring ≥3 beats to opacify pre-specified branch points in the distal vasculature of any of the three major epicardial coronary arteries (i.e. equivalent of TIMI-2 flow), and (b) the absence of obstructive CAD (i.e. <50% in any epicardial coronary artery). Exclusion criteria were based upon contra-indications to Bosentan therapy and included: (a) elevated plasma hepatic transaminases twice the upper normal limit; (b) concurrent use of cyclosporine A or oral contraceptives; (c) anaemia (haemoglobin ≤100g/L); and (d) pregnancy.

Study Protocol

Following an initial visit when clinical history and informed consent were obtained, patients were randomised to either twice-daily Bosentan (125mg) or matching placebo treatment using a computer-generated algorithm, with the sequence known only to the hospital clinical study pharmacist who had no contact with the patients. After two weeks of Bosentan/placebo therapy, patients were assessed for any adverse events and withdrawn from the study if transaminases demonstrated a ≥5-fold change from baseline. Patients continued with the assigned therapy for a further two weeks and at 4-weeks of therapy were clinically re-evaluated, venesection performed for biomarker determination and endothelial function assessed. Subsequently, patients commenced a one-week washout period and then crossed-over to the alternative Bosentan/placebo therapy with the above study protocol repeated. Throughout the study, the patient's maintenance medications were maintained constant.

Table 1: Baseline clinical characteristics of CSFP study patients.

Coronary Risk Factors	CSFP (n=23)
Age (years±SEM)	56±2.2
Gender	13M: 10F
Current Smoker	2(9%)
Hypertensive	10(45%)
Diabetic	5(23%)
Positive Family History	8(35%)
Hypercholesterolaemia	14(64%)
≥2 Risk factors	13(59%)
Current Medications	
Anti-platelet agents	19(86%)
Statins	14(64%)
Long-acting nitrates	15(68%)
Calcium-channel blocker	15(68%)
Beta-blocker	3(14%)
ACE inhibitor	6(27%)
Angiotensin II receptor blocker	4(18%)
ECG findings	
ST/T wave changes at baseline	2(9%)
CSFP Characteristics	
Recently diagnosed (angiogram < 1 month)	9(39%)
Time since initial diagnosis (months±SEM)	42±12
Baseline angina frequency (mean weekly±SEM)	15±3

Table 2: Median values (25%, 75% interquartile ranges) of total angina episodes, prolonged angina episodes (>20 min) and sublingual nitrate use during four weeks of placebo and Bosentan (125mg) twice daily therapy in 23 CSFP patients.

Parameter	Placebo	Bosentan	% Change (Median)	p Value
Total angina (episodes/month)	25(9,55)	14(5,36)	39	0.32
Prolonged angina (episodes/month)	5(0,19)	1.5(0,7)	67	0.31
Sublingual nitrate use (tablets or spray/month)	11(0,35)	7.5(0,19)	56	0.86

Table 3: Summary of secondary endpoints, including health status questionnaires, endothelial, inflammatory and oxidative stress biomarkers following four weeks of treatment with placebo or Bosentan therapy.

SF-36 Health Survey Concept	Placebo	Bosentan	p Value
Physical Health Concept			
Physical Functioning	58±5	61±5	0.3
Bodily pain perception	47±4	50±5	0.5
General health perception	46±5	46±4	0.9
Role limitation - physical health	39±10	40±9	0.9
Physical health summary score	38±2	38±2	0.9
Mental Health Concept			
Vitality	41±5	41±6	0.9
Social functioning	66±5	64±4	0.7
General mental health	60±4	67±5	0.1
Role limitation - emotional problems	56±10	55±10	0.9
Mental Health summary score	40±2	41±2	0.6
SAQ Questionnaire			
Physical limitation score	63±5	64±5	0.81
Angina stability	54±7	58±7	0.76
Angina frequency	42±5	49±7	0.19
Treatment satisfaction	89±2	88±3	0.57
Disease perception	51±4	57±6	0.37
Inflammatory Markers			
MPO (ng/mL)	24.2±7.1	17.9±3.2	0.43
CRP (mg/L)	5.75±1.78	4.28±1.08	0.39
Oxidative Stress Parameters			
MDA (ng/mL)	0.17±0.02	0.16±0.02	0.79
Homocysteine (mmol/L)	10.13±0.6	10.15±0.6	0.96
Endothelial Markers			
ADMA (mM)	0.58±0.02	0.53±0.01	0.03
SDMA (mM)	0.54±0.01	0.53±0.02	0.76
Arginine (mM)	104.3±8	104.7±9.1	0.93

Study Endpoints

The primary endpoint was the frequency of total angina episodes recorded during each 4 weeks of treatment. Secondary endpoints included (1) Clinical measures of angina and health status, (2) Endothelial function parameters, and (3) Inflamma-

tory and oxidative stress markers.

Clinical Measures

These included an angina diary and health status measures. Throughout the study period, patients were asked to maintain an angina diary, documenting the frequency of angina, duration of episodes (prolonged anginal episodes defined as >20 minutes) and sublingual nitrate consumption. During each visit, clinical observations (pulse and blood pressure) were recorded, the angina diary reviewed and any changes in medication or occurrence of adverse events documented. In addition to these clinical measures, in the last week of each treatment phase an electrocardiograph was performed and patients completed both a generic (Short Form- 36; SF-36) and disease-specific (Seattle Angina Questionnaire; SAQ) health status questionnaire. These instruments have been previously validated for patients with angina and provide important insights into the impact on health status [19,20]. Patient medication compliance was also assessed at the end of each phase by pill count.

Endothelial Function Parameters

For the assessment of endothelial function both a physiological functional test and a plasma biomarker were performed. The functional test employed pulse wave analysis using the method described and validated by Wilkinson et al [21]. ADMA is an endogenous inhibitor of nitric oxide and has been used as an indirect measure of endothelial function.

Pulse Wave Analysis for Endothelial Function

This technique involved placing a pressure transducer probe (Sphygmocor Software, Atcor Medical, Sydney, Australia) over the maximal arterial pulsation in the radial artery, so that adequate applanation of the vessel could be achieved to derive the radial pressure waveform. Aortic pressure waveforms were obtained from the peripheral pulse waveform utilising a validated generalised transfer function [22] in the integrated software. Systemic endothelial function was assessed through analysis of changes in aortic pressure waveforms and quantitative measurement of Augmentation Index (AIx) following serial administration of endothelium-independent (sublingual Glyceryl Trinitrate [GTN] 50mcg) and endothelium-dependent (salbutamol inhaler 400mcg) vasodilators. The AIx represented the difference in values between ventricular ejection (first systolic peak, P1) and systolic peak pressure (second systolic peak, P2), expressed as a percentage of pulse pressure and corrected for heart rate.

The following procedure was adopted for the applanation tonometry assessment of endothelial function. On arrival, subjects rested for 30 minutes in a supine position during which serial baseline blood pressure and pulse rate were non-invasively measured from the left brachial artery. Pulse wave analysis of the right radial artery waveforms was also measured during this resting period to ascertain the baseline AIx values. After establishing baseline parameters, 50µg GTN was administered sublingually and recordings of blood pressure, pulse and AIx values occurred at 3, 5, 10, 15 and 20-minute intervals. The protocol was repeated for 400µg salbutamol administration after AIx readings had returned to baseline values. For each time point, three AIx values were obtained and subsequently averaged. The arterial responses to GTN and salbutamol administration were defined as the maximum change in AIx over the total time interval compared to baseline as previously described [21].

Asymmetric Dimethylarginine Assay: Plasma ADMA, symmetric dimethylarginine (SDMA) and arginine concentrations were determined using a modified version of a previously described protocol [23]. Briefly, 150µL of plasma was combined with internal standard N- monomethylarginine [NMMA (5µg/mL)] and extracted on pre-conditioned Bond Elut Strong Cation Exchange (Varian) cartridges loaded on an automated solid phase extraction system (Gilson Aspec GX-274). Arginine and the olimethylargines were eluted and prepared for HPLC as per the published protocol. ADMA and SDMA were quantified by HPLC as described previously, from a 20µL injection volume. Arginine was quantified by injecting 2.5µL of mixture onto the HPLC.

Inflammatory and Oxidative Stress Markers

The inflammatory biomarkers assessed included hsCRP and MPO, while oxidative stress markers included MDA and homocysteine. Fasting plasma samples were obtained for these assays with homocysteine and hsCRP concentrations assessed at the time of sampling and the remaining plasma was stored at -80°C for subsequent batch analysis of ADMA, MPO, and MDA.

Homocysteine and high-sensitivity CRP assays. Plasma homocysteine was assessed using a well-established and validated chemiluminescence immunoassay [24]. Concentrations of plasma hsCRP were determined using a validated immuno-turbidimetric assay (Olympus OSR6199 Analyzer, Australia) [25].

Myeloperoxidase assay. MPO concentration was determined using the Zen™ Myeloperoxidase 96-well ELISA Kit (Invitrogen, Australia) as per the specified protocol. All antibody solutions, standards and patient samples were diluted with phosphate buffered saline/0.1%BSA. Briefly, individual ELISA wells: 1) were hydrated with PBS + 0.001% Tween (PBST) and incubated with 500ng/mL (100µL) solutions of mouse anti-MPO primary capture antibody; 2) underwent sequential washing steps with PBST; 3) incubated with standards, samples (100µL) and 1.0µg/mL of secondary rabbit anti-MPO capture antibody; 4) washed and incubated with 100µL goat anti-rabbit HRP conjugate; 5) and incubated with 100µL of Amplex UltraRed reagent mix (30 min) followed by addition of Amplex® stop solution. Fluorescence was measured using filters at $\lambda_{excitation}$ = 530nm and $\lambda_{emission}$ = 590nm.

Malondialdehyde assay. Plasma MDA levels were determined based upon a modified protocol that involved fluorescent detection of an extracted MDA-TBA acid adduct [26]. Briefly, plasma samples were thawed and underwent protein precipitation (2.3M perchloric acid) and lipid removal (200µL chloroform) protocols, respectively. For the adduct reaction to occur, 200µL of supernatant was aliquoted into conical glass tubes containing 0.15M H3PO4 (750µL), 42mM TBA (250µL) and made up to 1.5mL with MilliQ water. Samples were boiled for 60 minutes at 100°C and cooled on ice thereafter. Extraction of the MDA adduct was performed by addition of 70:30 chloroform:methanol (vol/vol) and the top aqueous layer was collected for detection of MDA fluorescence at $\lambda_{excitation}$ = 530nm, $\lambda_{emission}$ = 547nm.

Data Analysis

The study was undertaken using double-blind methodology and all analyses were conducted blinded to the study treatment. The clinical angina parameters were analysed using cross-over trial methodology with comparison between patients and respect to treatment order [27]. Large differences in angina fre-

quency between phases for individual patients were observed and therefore the data was log transformed and non-parametric (Mann Whitney U) tests used for comparison. The continuous, normally distributed health status questionnaires, endothelial function and biomarker assay endpoints were expressed as mean ± SEM and analysed by unpaired Student t-tests. Statistical values with α=0.05 were considered as significant.

The study sample size was calculated based upon the primary endpoint of total angina frequency. From a previous study examining the anti-anginal efficacy of mibefradil in the CSFP [28], the total angina frequency during placebo therapy was 28±31 episodes/month with 22 less episodes on active therapy. Thus, to detect a difference of 22 episodes or more with Bosentan therapy, a minimum of 22 patients would be required for 90% power at α=0.05 level.

Results

Over a thirty-month period, 26 patients were recruited into the study with 3 patients subsequently withdrawing, before completion of the study protocol. Two of these patients were withdrawn because of concerns with the study drug (mild elevations in hepatic transaminases and potential drug interactions with medications). A further patient was withdrawn due to non-compliance with study visits. Amongst the patients completing the study protocol, there were no major adverse effects and medication compliance was over 95% on pill count.

Patient Characteristics

The recruited patients were more often male, with over half having at least 2 atherosclerotic risk factors (Table 1). All of the patients were on conventional anti-anginal therapy, including long-acting nitrates (68%), calcium-channel blockers (68%) and/or beta-blockers (14%); yet their mean angina frequency at baseline was 15±3 episodes/week.

Clinical Endpoints

The primary study endpoint of total angina frequency did not statistically differ between active and placebo therapy although a median of 39% less angina episodes occurred on Bosentan therapy (Table 2). Similarly, there was no significant difference in prolonged angina episodes and sublingual nitrate consumption with Bosentan therapy although median reductions were 67% and 56%, respectively. Assessment of health-related quality of life with the SF-36 showed no improvement in physical or mental summary scores with the Bosentan therapy (Table 3).

Furthermore, SAQ-assessed health status did not show significant improvements although there was a non-significant 7-point improvement in angina frequency with Bosentan.

Endothelial Function Parameters

Pulse-wave analysis data was successfully obtained from sixteen CSFP patients. Baseline AIx values were found to be similar for each phase (placebo: 19.9±2.1% vs Bosentan: 15.7±2.3%, p=0.1). Comparison of maximum changes in AIx following GTN administration during placebo and Bosentan treatments demonstrated no significant differences (Placebo: -10.3±1.2% vs Bosentan: -10.0±1.1%, p=0.87). Peak changes in AIx following salbutamol administration tended to be larger with Bosentan therapy (Placebo: -1.4±1.2% vs Bosentan: -2.2 ± 1.3%, p=0. 61) despite not achieving statistical significance.

ADMA, SDMA and arginine plasma levels were assessed in eleven CSFP patients. As shown in Table 3, Bosentan therapy was associated with a significant increase in plasma ADMA levels but did not influence either SDMA or arginine concentrations.

Inflammatory and Oxidative Stress Biomarkers

Plasma inflammatory markers for MPO and hsCRP were obtained from seventeen patients and demonstrated no significant changes following Bosentan therapy (Table 3). Similarly, there were no changes in oxidative stress markers including plasma MDA and homocysteine concentrations.

Discussion

This novel study was designed to assess the anti-anginal efficacy of Bosentan in the CSFP based upon the accumulating evidence that ET-1 plays an important role in the pathogenesis of this disorder. Furthermore, the secondary endpoints were designed to explore potential mechanisms for any observed improvement as well as examining the impact of the therapy on health status. Unfortunately, the study demonstrated no benefit in the clinical endpoints of total angina frequency, prolonged angina episodes, nitrate consumption or health status with Bosentan therapy. Moreover, there was no change in inflammatory/oxidative stress biomarkers and endothelial function parameters, except for a small improvement in ADMA.

Although previous clinical and pathophysiological studies have implicated ET-1 in the pathogenesis of the CSFP, ET-1 blockade with Bosentan has not ameliorated the symptoms or associated pathophysiological abnormalities in this small but comprehensive study. The logical interpretation of these findings is that ET-1 may play a role in the pathophysiology of the CSFP but it is not a major role since its blockade has not impacted on clinical or pathophysiological markers. Another factor to take into consideration is that Bosentan is a dual endothelin receptor antagonist and blockade of ET-B receptors could have impaired NO release, thereby potentially off-setting any beneficial effect produced by ET-A receptor blockade. It can be speculated that a selective ET-A receptor blocker may have produced different results. An alternative explanation is a Type-2 error in the study findings due to sample size, study drug or endpoints. Each of these potential study design limitations resulting in an inappropriate study conclusion, are discussed below.

The sample size calculations were based upon a previous study that utilised similar methodology and demonstrated a reduction in angina frequency from 34 episodes/month (IQR: 11, 56) on placebo to 8 episodes/month (IQR: 3, 25) on mibefradil[28]. The corresponding values for this study are 25 episodes/month (IQR: 9, 55) and 14 episodes/month (IQR: 5, 36) on placebo and Bosentan respectively. Hence compared with the mibefradil study, the study populations were less symptomatic on placebo and the effect size with Bosentan was not as large as mibefradil. Importantly, maintenance anti-anginal medications were ceased in the mibefradil study but maintained in the current study, which may account for the difference in angina frequency on placebo. Although a larger study or ceasing the maintenance anti-anginal medications may have revealed a significant difference in angina frequency with Bosentan, the effect is not as remarkable as that observed with mibefradil and therefore its clinical and pathophysiological importance likely to be less significant.

In this study, ET-1 blockade was achieved with Bosentan 125mg bd. This drug blocks both endothelin-A and endothelin-

B receptors, and is an important therapeutic agent in the treatment of pulmonary hypertension. This is the first clinical study where an ET-1 blocker has been used in the treatment of angina. The dosage was empirically based upon those utilised for the treatment of pulmonary hypertension and whether higher doses would have been effective is unknown. Moreover, selective endothelin-A blockade may have been more effective than dual endothelin receptor blockade considering the inhibition of endothelial nitric oxide release by endothelin-B receptor blockade. Thus whether a higher dose or alternative ET-1 blocking agent to Bosentan would have resulted in different findings is open to speculation.

Although the clinical endpoints used in this study are valid clinical targets, the pathophysiological endpoints were chosen to provide insights into the potential mechanisms of any benefits observed with Bosentan. Since the pathophysiological endpoints essentially showed no difference with Bosentan therapy, it may be interpreted that ET-1 blockade has no impact on the pathophysiological mechanisms of the CSFP; consistent with the clinical findings. However, the pathophysiological endpoints were chosen on the basis of contemporary literature at the time of study design and subsequent studies have questioned the role of these pathophysiological abnormalities in the CSFP. For example, endothelium-dependent vasodilation has been reported as being impaired in the CSFP [3-5], however it was intact in our study patients, as shown in our previous studies [10] and by others [12]. Furthermore although ADMA levels have been reportedly raised in the CSFP [4,29], during the placebo phase of this study they were similar to those we have reported in control patients [10]. The fall in ADMA levels with Bosentan therapy is interesting but difficult to resolve in light of the clinical responses.

Previous reports have suggested that inflammation [6,7] and oxidative stress [5,8] play important role in the pathogenesis of the CSFP, prompting the inclusion of these markers in this study. However as in our previous publication [10], these were not elevated in these study patients nor were they influenced by Bosentan. In contrast to these patients with chronic angina, we have reported increased inflammatory and oxidative stress biomarkers during an acute coronary syndrome presentation with the CSFP [30], which resolve within 4 weeks of the acute presentation. Hence the pathogenesis of the acute and chronic phases of this disorder may differ.

Conclusion

In conclusion, this study has not demonstrated a large anti-anginal benefit of administering Bosentan 125mg twice daily in CSFP patients. However, a small benefit cannot be excluded and requires a larger, more appropriately powered study. Thus continued investigations are required into this intriguing disorder, which still does not have an effective therapy available for its associated recurrent angina.

References

1. Beltrame JF, Limaye SB, Horowitz JD. The coronary slow flow phenomenon - a new coronary microvascular disorder. Cardiology. 2002; 97: 197-202.

2. Di Fiore DP, Beltrame JF. Chest pain in patients with 'normal angiography': could it be cardiac? Int J Evid-Based Healthc. 2013; 11: 56-68.

3. Sezgin AT, Sigirci A, Barutcu I, Topal E, Sezgin N, Ozdemir R, et al. Vascular endothelial function in patients with slow coronary flow. Coron Artery Dis. 2003; 14: 155-61.

4. Arı H, Arı S, Erdoğan E, Tiryakioğlu O, Huysal K, Koca V, et al. The effects of endothelial dysfunction and inflammation on slow coronary flow. Turk Kardiyol Dern Ars. 2010; 38: 327-33.

5. Tanriverdi H, Evrengul H, Enli Y, Kuru O, Seleci D, Tanriverdi S, et al. Effect of homocysteine-induced oxidative stress on endothelial function in coronary slow-flow. Cardiology. 2007; 107: 313-20.

6. Turhan H, Saydam GS, Erbay AR, Ayaz S, Yasar AS, Aksoy Y, et al. Increased plasma soluble adhesion molecules; ICAM-1, VCAM-1, and E-selectin levels in patients with slow coronary flow. Int J Cardiol. 2006; 108: 224-30.

7. Barutcu I, Sezgin AT, Sezgin N, Gullu H, Esen AM, Topal E, et al. Increased high sensitive CRP level and its significance in pathogenesis of slow coronary flow. Angiology. 2007; 58: 401-7.

8. Enli Y, Turk M, Akbay R, Evrengul H, Tanriverdi H, Kuru O, et al. Oxidative stress parameters in patients with slow coronary flow. Adv Ther. 2008; 25: 37-44.

9. Sen N, Basar N, Maden O, Ozcan F, Ozlu MF, Gungor O, et al. Increased mean platelet volume in patients with slow coronary flow. Platelets. 2009; 20: 23-8.

10. Kopetz V, Kennedy J, Heresztyn T, Stafford I, Willoughby SR, Beltrame JF. Endothelial function, oxidative stress and inflammatory studies in chronic coronary slow flow phenomenon patients. Cardiology. 2012; 121: 197-203.

11. Yazici M, Aksakal E, Demircan S, Sahin M, Sağkan O. Is slow coronary flow related with inflammation and procoagulant state?. Anadolu Kardiyol Derg. 2005; 5: 3-7.

12. Shirani S, Darabian S, Jozaghi S, Hamidian R. Correlation between endothelial dysfunction in normal coronary patients with slow flow and aortic ectasia: the first report. Cardiol J. 2009; 16: 146-50.

13. Larkin SW, Clarke JG, Keogh BE, Araujo L, Rhodes C, Davies GJ, et al. Intracoronary endothelin induces myocardial ischemia by small vessel constriction in the dog. Am J Cardiol. 1989; 64: 956-8.

14. Hirata K, Matsuda Y, Akita H, Yokoyama M, Fukuzaki H. Myocardial ischaemia induced by endothelin in the intact rabbit: angiographic analysis. Cardiovasc Res. 1990; 24: 879-83.

15. Camsarl A, Pekdemir H, Cicek D, Polat G, Akkus MN, Döven O, et al. Endothelin-1 and nitric oxide concentrations and their response to exercise in patients with slow coronary flow. Circ J. 2003; 67: 1022-8.

16. Pernow J, Ahlborg G, Lundberg JM, Kaijser L. Long-lasting coronary vasoconstrictor effects and myocardial uptake of endothelin-1 in humans. Acta Physiol Scand. 1997; 159: 147-53.

17. Beltrame JF, Limaye SB, Wuttke RD, Horowitz JD. Coronary hemodynamic and metabolic studies of the coronary slow flow phenomenon. Am Heart J. 2003; 146: 84-90.

18. Turner SP, Beltrame JF. Microvascular hyperreactivity to endothelin-1 in patients with the coronary slow flow phenomenon. Eur Heart J. 2004; 25: 342-8.

19. Ware JE, Jr., Sherbourne CD, The MOS. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992; 30: 473-83.

20. Spertus JA, Winder JA, Dewhurst TA, Deyo RA, Prodzinski J, McDonell M, et al. Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. J Am Coll Cardiol. 1995; 25: 333-41.

21. Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol. 2002; 22: 147-52.

22. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur Heart J. 1993; 14: 160-7.

23. Heresztyn T, Worthley MI, Horowitz JD. Determination of l-arginine and NG, NG - and NG, NG' -dimethyl-L-arginine in plasma by liquid chromatography as AccQ-Fluor fluorescent derivatives. J Chromatogr B Analyt Technol Biomed Life Sci. 2004; 805: 325-9.

24. Tewari PC, Zhang B, Bluestein BI. Analytical and clinical evaluation of the Bayer Advia Centaur homocysteine assay. Clin Chim Acta. 2004; 342: 171-8.

25. Marie Dupuy A, Boutet A, Paul Cristol J. Evaluation of the high-sensitivity, full-range Olympus CRP OSR6199 application on the Olympus AU640. Clin Chem Lab Med. 2007; 45: 402-6.

26. Arstall MA, Yang J, Stafford I, Betts WH, Horowitz JD. N-acetylcysteine in combination with nitroglycerin and streptokinase for the treatment of evolving acute myocardial infarction. Safety and biochemical effects. Circulation. 1995; 92: 2855-62.

27. Jones B, Kenward MG. Design and analysis of cross-over trials. London: Chapman & Hall; 1989.

28. Beltrame JF, Turner SP, Leslie SL, Solomon P, Freedman SB, Horowitz JD. The angiographic and clinical benefits of mibefradil in the coronary slow flow phenomenon. J Am Coll Cardiol. 2004; 44: 57-62.

29. Selcuk MT, Selcuk H, Temizhan A, Maden O, Ulupinar H, Baysal E, et al. Asymmetric dimethylarginine plasma concentrations and L-arginine/asymmetric dimethylarginine ratio in patients with slow coronary flow. Coron Artery Dis. 2007; 18: 545-51.

30. Kopetz VA, Penno MA, Hoffmann P, Wilson DP, Beltrame JF. Potential mechanisms of the acute coronary syndrome presentation in patients with the coronary slow flow phenomenon - Insight from a plasma proteomic approach. Int J Cardiol. 2012; 156: 84-91.