Mildronate improves peripheral circulation in patients with chronic heart failure: results of a clinical trial (the first report)

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Received 14 March 2005; accepted 29 April 2005

Summary

Objectives: The main aim of the study was to compare the efficacy of combined treatment of chronic heart failure (CHF) with mildronate and angiotensin-converting enzyme inhibitor lisinopril and the treatment of CHF with lisinopril used alone.

Design and Methods: The study was designed as a controlled, parallel-group, double-blind, randomised phase IV clinical trial. The study was carried out in Latvia and Lithuania. The study group comprised 119 patients (men and women; aged 30–80 years) with CHF (NYHA I–III) due to coronary heart disease. The first group (ML20) received mildronate (M) 1000 mg and lisinopril (L) 20 mg daily; the second group (ML5) received M 1000 mg and L 5 mg, the third (L20) received L 20 mg daily.

Results: In the ML20 group, dyspnoea decreased in 48.7% of patients, whereas in the L20 group only in 33.3% of patients. As to the NYHA functional class, the decrease was observed in all groups. During bicycle ergometry, the greatest increase in exercise time was recorded in the ML20 group patients. The changes in parameters of reactive hyperemia in forearm muscles after the treatment were shown to differ greatly in the ML20 and ML5 groups as compared to the L20 group. The analysis of blood flow data of a handgrip test showed statistically significant increase in maximal and summary blood flow in the ML20 group.

Conclusions: This study reveals the advantage of the combined treatment with "lisinopril 20 mg/daily and mildronate 1000 mg/daily" and "lisinopril 5 mg/daily and mildronate 1000 mg/daily" over the treatment with "lisinopril 20 mg/daily" on the leading symptoms of CHF. The combined treatment is associated with the improvement of the quality of life, exercise capacity and mechanisms of peripheral circulation. The additive beneficial effect of mildronate on the vasodilation capacity of the magistral arteries and the resistance vessels at rest and during the static muscle load has been demonstrated.

Seminars in Cardiology 2005; 11(2): 56–64

Keywords: mildronate, heart failure, regional blood flow, exercise tolerance

Chronic heart failure (CHF) is a clinical syndrome which is caused by myocardial ischaemia induced by coronary heart disease (CHD) in up to 70% of cases [1]. For the last 15 years advances have been experienced in the treatment of CHF where the leading role is attributed to the introduction of angiotensin-converting enzyme inhibitors (ACEI) [2]. There is compelling evidence that ACEI decrease cardiac pre-load and after-load by dilating arteries and veins. Consequently, this results in an augmented stroke volume. Besides, ACEI have demonstrated a positive effect on the ejection fraction, degree and speed of myocardial fibre contractility, as well as the reduction of the heart rate and arterial blood pressure. Improvement in haemodynamic parameters promotes an increase in exercise tolerance and a decrease in sympathetic nerve system acti-

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vation [2–4]. Patients with CHF, however, have a very poor prognosis with a 5-year mortality rate of about 50% [5,6]. Thus, an efficient CHF treatment is still a topical issue in all countries and regions [7]. Ongoing studies are conducted to improve this situation. Newer pharmacological therapies are emerging along with the hope that these agents may address some of the currently unmet treatment needs. One such prospective method is believed to be related with the development of the so-called "cytoprotective" agents. The accumulation of the results of this treatment is still being continued. As an example of this approach, the history of introducing of trimetazidine (Preductal, Servier) in the CHF treatment can be mentioned. Trimetazidine is known to modulate mitochondrial energetic metabolism under ischaemia providing antianginal effect and preserving myocardial contractile properties [8,9]. The "cytoprotective" agent group also includes mildronate (M; 3-(2,2,2-trimethylhydrazinium) propionate). A number of animal studies as well as clinical trials of the effect of mildronate on the cardiovascular system and cardiac function in ishaemic CHF have been performed. Mildronate was demonstrated to improve the myocardial contractile function and hemodynamic profile, favour the regression of ischaemic cardiac remodelling during ishaemia and reperfusion. The efficiency of mildronate is shown to be similar to that of ACEI captopril [10–13]. Mildronate was found to improve symptoms of CHF, quality of life of the patients, exercise tolerance, systolic function and a decrease in peripheral arterial resistance [14–16]. Moreover, experimental studies have also substantiated mildronate as an agent possessing a vasodilating and an antispasmic action [17,18].

The aim of this study was two-fold: to compare the efficacy of the combination of ACEI (lisinopril) with mildronate and ACEI (lisinopril) used alone in the CHF treatment, and to assess the effect of both regimens on peripheral circulation and function of skeletal muscles.

Design and Methods

The study was designed as a controlled, parallel-group, double-blind, randomised phase IV clinical trial. The study was carried out in 2 medical centres: in Latvia and Lithuania. The study group comprised 119 patients (men and women; aged 30–80 years) with CHF (NYHA I–III) due to coronary heart disease (CHD). Written informed consent was obtained from all the patients before the enrolment. The study was performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the Ethics Committee of the Latvian Institute of Cardiology. Patients were randomly divided into three groups receiving different treatment during a 3-month period. Patients of the first study group (ML20) received mildronate (M) 1000 mg and lisinopril (L) 20 mg daily; the second study group (ML5) received M 1000 mg and L 5 mg daily; patients of the third (control) group (L20) received L 20 mg daily.

Primary end points of the study were:

1. Regression of the symptoms (dyspnoea, fatigue, palpitation);
2. Improvement of exercise tolerance;
3. Assessment of quality of life.

The effect of the therapies was evaluated by the surrogate outcomes: the improvement of peripheral circulation and the related increase of the function of the target-organ (skeletal muscles).

Symptoms of coronary heart failure were considered determining CHF functional class: before a double-blind treatment (3 times), during a coded treatment (6 times) and once after the treatment. Additionally, dyspnoea, fatigue, palpitation were assessed during each visit using a 5 point scale.

The quality of life was assessed according to the Minessota Living With Heart Failure questionnaire and the Health Satisfaction Score.

Exercise tolerance was assessed by bicycle ergometry (BE) according to a standard protocol. The power output was increased stepwise, starting at 25 W with subsequent increments of 25 W every third minute. BE was performed before and after the treatment. A pre-treatment test was validated if two measurements did not differ by more than 15%.

The main criteria were: exercise time, min (ET); maximal load, W (MaxL); performed work (PW), PW = Load (W) × ET (s)/1000 in each step of BE; total performed work, kJ ($\sum$PW); heart rate recovery index (HRRI); myocardial reserve (MR). HRRI was calculated according to the equation: HRRI = MaxL × HR_{max}/body weight (kg) × $HR_{2nd\ min}$. The double product (DP) of systolic pressure (P_s) and the heart rate (HR) was calculated before the exercise ($DP_F = P_s \times HR \times 10^{-2}$) and during the maximal load ($DP_{max} = P_s \times HR \times 10^{-2}$). MR was estimated as the relationship between calculated parameters: $MR = DP_{max}/DP_F$.

A six-minute corridor-walking test was performed twice: before and after the coded treatment.

The changes in the tone of peripheral (forearm) vessels during the treatment were assessed by measuring forearm blood flow before and af-

ter a 7-minute arterial occlusion using the venous occlusion plethysmography (VOP). After the restoration of free blood flow, forearm blood flow was measured during the first two seconds, then with the interval of 10 seconds. The following parameters were evaluated: blood flow at rest (I_F, ml/100 cm^3/min), dynamics of blood flow and maximal blood flow during reactive hyperemia (I_{max}, ml/100 cm^3/min); mean arterial blood pressure (P_M, mm Hg); pulse pressure (ΔP, mmHg), haemodynamic resistance of forearm blood vessels at rest R_F (measured in peripheral resistance units, PRU) [$R_F = P_M/I_F$] and minimal resistance during reactive hyperemia (R_{min}) [$R_{min} = P_M/I_{max}$]. The compliance of the magistral arteries of the forearm (D) was calculated as the relationship between the amplitude of pulse volume ΔV and pulse pressure ΔP: D (units) = $\Delta V/\Delta P$.

Changes in the tone of precapillary vessels of the forearm were evaluated comparing the values of I_F, R_F, and R_{min} before and after the treatment.

The changes in the tone of the magistral arteries of the forearm was estimated by D before and after the treatment.

Central and peripheral haemodynamic parameters were detected during the isometric contraction of the forearm muscles (the handgrip test) as well. The handgrip test was performed in the following manner: maximal voluntary contraction (MVC) was defined by recording forearm blood flow at rest. Forearm arterial occlusion was produced by inflating an upper arm cuff to 30–50 mmHg above P_s. In parallel, the participant was asked to perform a handgrip with 10% of MVC for 90 s. After the cessation of a handgrip, the arrest of blood circulation was still retained for 30 seconds. After the restoration of free blood flow, forearm blood flow was measured by VOP during the first two seconds ($I_{2\ sec}$), then with the interval of 10 seconds. Systemic arterial pressure was recorded with an interval of 15–20 seconds in the resting forearm and electrocardiogram was registered throughout the test. Dynamics of the heart rate and arterial pressure was assessed during a handgrip, after the cessation of a handgrip while the arrest of muscle blood circulation was retained and after the restoration of free blood flow. The dynamics of forearm blood flow was assessed after the restoration of circulation.

Results

Symptoms and NYHA functional classes

Figure 1 represents the number of patients (% of the total number) with the improvement in dyspnoea and fatigue after the treatment.

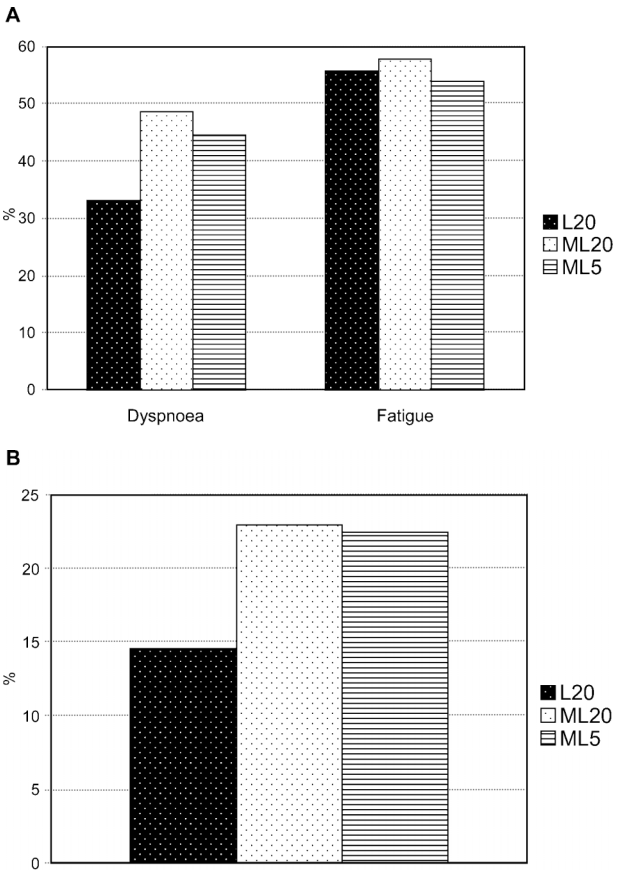

Figure 1. A, Regression of the intensity of symptoms after the treatment (% of patients). B, Improvement of chronic heart failure functional class (% of patients).

Improvement in dyspnoea was stated in 48.7% of patients of the ML20 group and in 44.7% of patients of the ML5 group while only in 33.3% of the L20 group patients. Worsening dyspnoea was stated in two patients of the L20 group and in one patient of the ML5 group but none in the ML20 group. Decrease in fatigue was found in 57.9% of the ML20, 53.8% – in the ML5 and 55.6% – in the L20 group patients. Increase in fatigue was found in one patient from the L20 and the ML5 groups, but it was not found in any of the patients of the ML20 group. Although the number of cases with the improvement in dyspnoea (the main symptom of CHF) in both combined therapy groups exceeded the number of cases in the L20 group, the difference was not statistically significant for the given number of patients.

NYHA functional class improvement was found in 23.7% of patients of the ML20 group, in 23.1% – of the ML5 group but only in 13.9% of patients of the L20 group. NYHA functional class worsening was not stated in any of the study groups. In both study groups receiving the combined treatment, functional class improvement was found in twice as many patients as in the L20 group. This

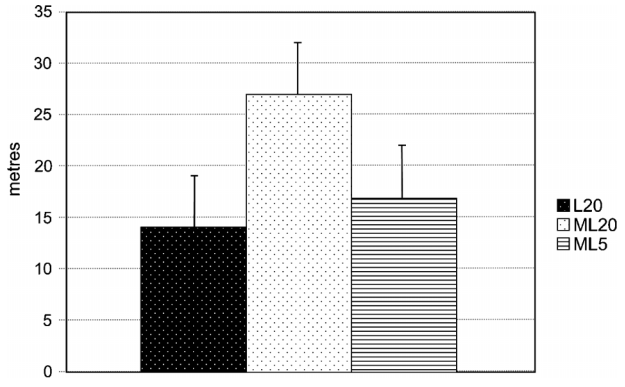

Figure 2. A six-minute walking test: the increase of the covered distance after the treatment (m ± SE).

difference, however, is not statistically significant for the given number of patients.

Exercise tolerance

The most pronounced increase in the covered distance was found in the patients of the ML20 group (Figure 2). Worthy of note, a statistically significant increase was found in the covered distance after the therapy in comparison with that before the treatment in all the study groups.

All the study groups showed a statistically significant increase in ET. Although the difference in the increase of ET among the groups did not reach statistical significance, the greatest increase, however, was found in the ML20 group (Figure 3A).

Although all the study groups manifested increase in MaxL after the treatment, it was statistically significant in the ML5 and the ML20 groups (Figure 3B).

Increase of $\sum$PW was found in all the study groups (the ML20 group, $p = 0.0004$; the ML5 group, $p = 0.009$; the L20 group, $p = 0.006$) after the treatment.

HRRI increased in all the study groups after the treatment, but statistically significant increase was stated only in the ML20 group ($p = 0.0002$).

Myocardial reserve increased in all the study groups, but statistically significant increase was stated only in the groups receiving the combined treatment: $p = 0.02$ – in the ML5 group; $p = 0.046$ – in the ML20 group.

Quality of life

The score calculated according to the responses obtained by the Minessota Living with Heart Failure Questionnaire decreased in all the study groups. However, it reached statistical significance only in the groups receiving the combined treatment ($p = 0.049$ – in the ML5 group; $p = 0.003$ – in the ML20 group). All the study groups manifested the improvement in general health according to the Health Satisfaction Score System

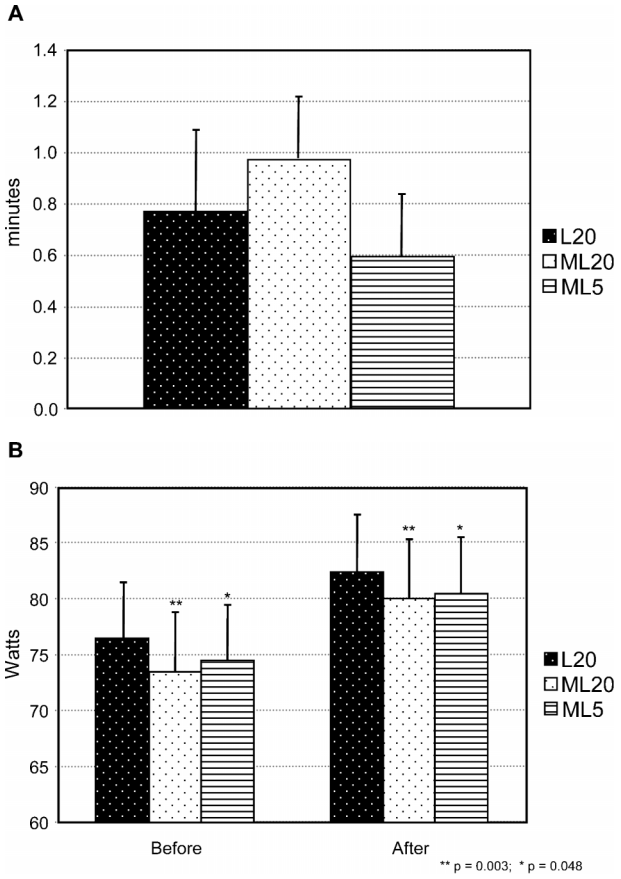

Figure 3. A, Bicycle ergometry: the increase of exercise time (ΔET) after the treatment (min ± SE). B, Maximal load before and after the treatment (W ± SE).

($p = 0.0002$ – in the L20 group; $p < 0.0001$ – in the ML5 and the ML20 groups).

Peripheral circulation

Characteristics of reactive hyperemia differed in all the study groups before and after the treatment: in the L20 group, I_{max} decreased from 37.47 ± 4.03 ml/100 cm^3/min to 23.58 ± 2.85 ($p = 0.005$). Increase in R_F was not statistically significant but R_{min} increased from 3.11 ± 0.28 PRU to 5.11 ± 0.55 ($p = 0.003$). Both study groups receiving the combined therapy showed increase in I_{max}. It rose from 27.71 ± 2.17 to 36.72 ± 3.92 ml/100 cm^3/min ($p = 0.02$) after the treatment in the ML20 group and from 33.16 ± 3.43 to 42.99 ± 5.26 in the ML5 group ($p = 0.007$). In the ML20 group, decrease in R_{min} was from 4.22 ± 0.36 to 3.07 ± 0.23 PRU ($p = 0.008$), in the ML5 group – from 3.72 ± 0.42 to 2.70 ± 0.26 ($p = 0.008$). Statistically significant increase in the compliance of the forearm magistral arteries was found only in the ML20 group: from 5.69 ± 0.79 units to 7.19 ± 0.67. The comparison of dynamics of changes in the parameters of reactive hyperemia is presented in Figures 4 and 5.

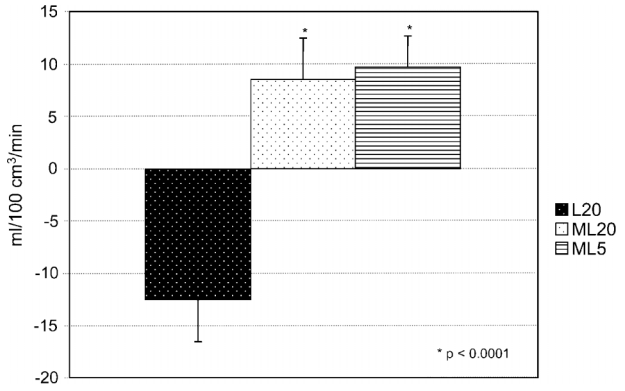

Figure 4. Reactive hyperemia before and after the treatment (ΔI_{max}).

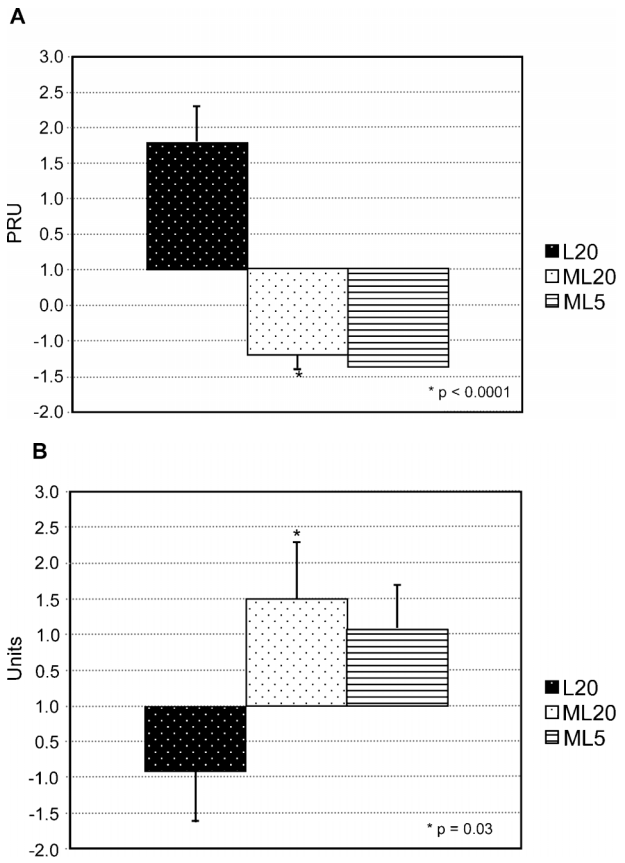

Figure 5. A, Reactive hyperemia before and after the treatment (ΔR_{min}). B, Reactive hyperemia before and after the treatment (ΔD).

Analyses of the changes in HR characteristics during the handgrip test showed that the treatment results in statistically significant decrease in HR was found only in the ML20 group. It was lowered during the contraction period (p = 0.003), after the cessation of a handgrip while muscle ischaemia was still retained (p = 0.03), as well as during the recovery period (p = 0.005) (Figure 6A). The treatment showed insignificant increase in HR in the L20 group but virtually no changes occurred in the ML5 group.

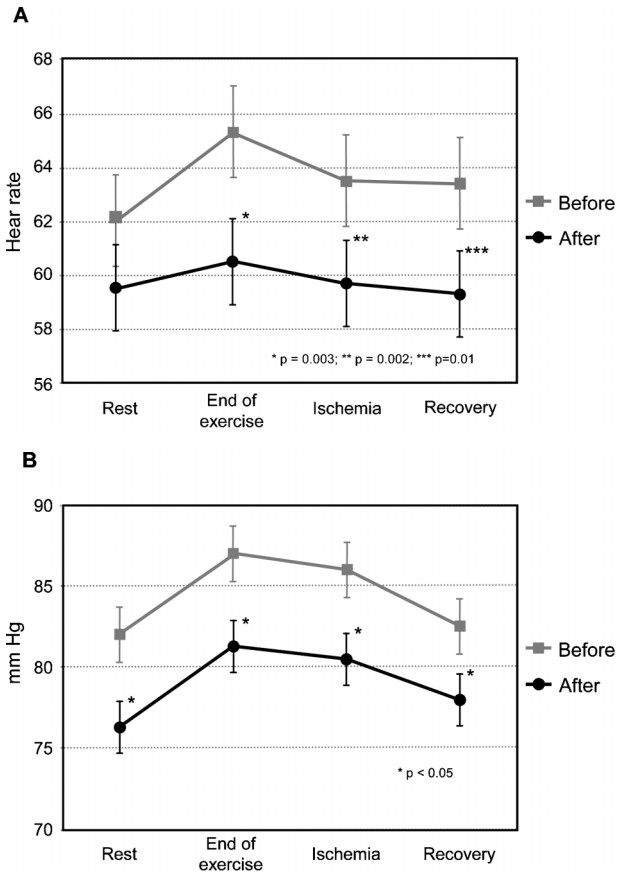

Figure 6. A, Heart rate before, during and after a handgrip (ML20) before and after treatment. B, Diastolic blood pressure before, during and after a handgrip (ML20) before and after treatment.

Arterial blood pressure (BP) values during the handgrip test were compared before and after the treatment in all study groups. Systolic BP, diastolic BP and mean BP lowered in all the groups while statistical significance this decrease reached in systolic BP (p = 0.01) and mean BP (p = 0.02) during the muscle contraction phase after the treatment. However, diastolic BP lowered in all investigation phases in the ML20 group patients (Figure 6B).

Blood flow measurements (I_F, I_{max}, I_{sum}) during a handgrip before and after the treatment revealed a trend to decrease in all investigation phases in the ML20 group patients. In the ML20 group patients statistically significant increase in I_{max} and I_{sum} was found, whereas in the ML5 group patients, the increase was observed in all blood flow measurements but the difference did not reach statistical significance. Comparing the pre- and after-treatment measurements in all the three study groups, the increase in $I_{2\ sec}$ was statistically more significant in the ML20 and the ML5 groups than in the L20 group (p = 0.004 and p = 0.007, respectively). Moreover, increase in I_{max}, I_{sum} was also found to reach statistically

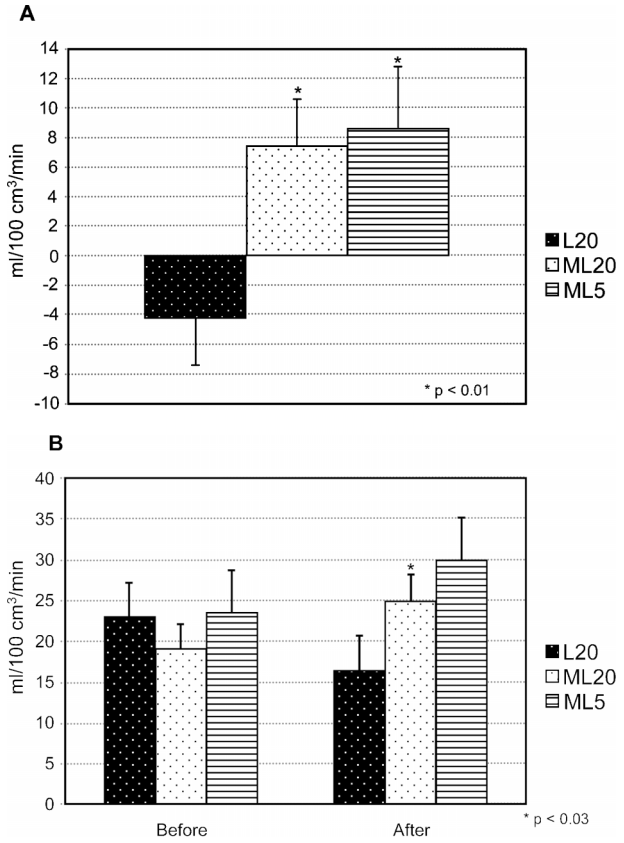

Figure 7. A, Handgrip before and after the treatment: changes of I_{max} (ml $\pm$ SE). B, Handgrip before and after the treatment: summary blood flow (ml $\pm$ SE).

greater significance in the ML20 and ML5 groups than in the L20 group (Figure 7).

Discussion

The major finding of the study was that comparing both treatment regimens, the combination of ACEI (lisinopril) with mildronate was found to be more effective in a potential regression of the main symptoms of CHF as well as in the improvement of the NYHA functional class and the quality of life. More importantly, the improvement in the NYHA functional class was not ACE inhibitor dose-dependent. A different mechanism of mildronate activity on tissue energetic metabolism, presumably, widens the beneficial effect alongside with that of the ACEI (lisinopril). Nonetheless, the questions whether the combined treatment with mildronate can reduce the frequency of side-effects and whether the dose of ACEI can be reduced are still open. Large clinical trials (for instance, ATLAS) have shown a positive correlation between the increase in the ACEI dose and the frequency of side-effects while the mortality rate, risk for hospitalisation and a number of hospitalisations were found to be reduced [19]. Thus, trials with the drug combination of ACEI and mildronate seeking for the hard end point such as mortality are needed. Mildronate was shown to be significantly effective on exercise tolerance. This has been confirmed by the results obtained during a 6-minute walking test and bicycle ergometry. The combined treatment demonstrates more pronounced increase in physical load capacity (MaxL, $\sum$PW) and adaptability of the cardiovascular system (HRRI, MR) in comparison to the treatment with the standard dose of lisinopril. This was convincingly proved in the ML20 group by a statistically significant increase in HRRI and MR, as well as in MaxL and $\sum$PW. We believe that HR performance in the after-load period is an important factor affecting the ultimate outcome of the treatment because HR recovery is recognized to indicate the recovery in vagal tone. Moreover, HR dynamics is considered an independent marker of mortality. There is the overwhelming evidence of a close relationship between a decrease in HRRI and an increase in the mortality of CHD patients [20,21]. Clinical studies following a patient's outcome using a myocardial scintigraphy method have constantly found that a decrease in HRRI is substantially associated with myocardial ischaemia [22]. In our study, the statistically significant increase of HRRI in the ML20 group confirms our assumption of the prospects of the beneficial affect of the combination of lisinopril (20 mg daily) with mildronate on the survival of CHR patients.

Over the past 15 years, much attention was given to "the peripheral syndrome" of the CHF development. It is characterized by (1) an increase in vascular tone in different target organs including the skeletal musculature; (2) a decrease in vasodilating capacity in skeletal muscles which greatly affects exercise tolerance; (3) endothelial dysfunction. A particular role of endothelial function in the regulation of vascular tone should be emphasized in the context of our study. Numerous studies have provided evidence that endothelial cells can produce and release at least three smooth muscle relaxing factors: nitric oxide (NO), prostacycline and endothelium-derived hyperpolarizing factor(s) (EDHF) [23]. Several different specific endothelial mechanisms at the endothelial membrane level can trigger the synthesis of those endogenous vasoconstrictors in the endothelium [24]. One of those mechanisms is related to the peculiarity of the endothelial membrane to respond to an increase in flow velocity. In physiology, this mechanical effect, which can influence endothelial function, is called "shear stress", the term generally used in physics [25]. Recently, the assessment of the vasodilation evoked by endothelial cell response to

increased flow velocity known as "flow-mediated vasodilatation" or "endothelium-dependent vasodilatation" is widely used in order to evaluate the endothelial function in different vascular districts including the peripheral circulation [26]. This is confirmed by evidence that endothelial dysfunction of the peripheral circulation is observed in patients with cardiovascular diseases including CHF. This endothelial dysfunction is manifested as decrease in vasodilating capacity [27,28]. These changes are considered to be due to the inhibition of NO synthetase and, thus, deficiency of endothelial NO [28]. Factors contributing to such endothelial NO deficiency could be shear stress failure as well as the effect of some other factors (superoxides, circulating dimethylarginine). Analysing the results obtained from peripheral circulation in the view of the possible endothelium-dependent vasodilation of skeletal muscles, blood flow peculiarities could not be missed at the initial phase of reactive hyperemia. The comparison of values of blood flow and hemodynamic resistance at the 2nd second and during 10–20th seconds clearly revealed that a number of patients with vasodilation in both study groups receiving the combined treatment amounted to 95–100%, whereas in the L20 group it was only 66%. Thus, consistent with our presumption, the differences in dynamics of reactive hyperemia strongly points towards the beneficial activity of mildronate accounting for endothelium-dependent vasodilatation. In addition, the results of the dynamics of flood flow, peripheral resistance and compliance of the magistral arteries and the differences among the study groups show clear additive benefits of mildronate in the combined treatment with ACEI in the improvement of vasodilatation under conditions with increased demand of blood supply in skeletal muscles. Our findings support the concept of the stimulating effect of mildronate on vascular smooth muscles through activating NO production. Mechanisms affecting vascular tone under conditions of increased demand of blood supply in skeletal muscles were investigated by means of a static load tolerance test. During the static load, the dynamics of blood flow parameters were determined by integral interaction of central (cortex efferentation, activation of baroreflex, vegetative nervous system, etc.) and peripheral mechanisms (afferentation from active muscles). Development of peripheral mechanisms in CHF patients is a heterogeneous process and is associated with skeletal muscle atrophy, changes in the proportion of fast and slow muscle fibres and decrease in their oxidative capacity. Thus, a similar external work evokes greater metabolic changes in CHF patients than in healthy subjects. At the same time, the data about decreased sensitivity of metabolic receptors in CHF patients cannot be ignored [29]. Thus, the intensity of the peripheral signal and its physiological significance can differ in each definite case. This is especially worthy of note in the context of our study because mildronate possesses both neurotropic and metabolic activity [30]. Thus, peripheral signal response and intensity can be affected by antagonistic mechanisms. If the positive effect of mildronate on muscle metabolism is accepted, a decrease in the intensity of the peripheral signal can be expected after the treatment in comparison to the basal measurement, whereas accepting neurotropic effect of mildronate, an increase in the sensitivity of metabolic receptors and the intensity of the peripheral signal in hemodynamic processes can be expected. All this emphasize the complexity of interpreting the results obtained and the significance of each hemodynamic factor. In our study, dynamics of HR and BP were analysed in all phases of a handgrip in all the study groups. After the treatment in the ML20 group, systolic BP and mean BP were found to reach statistically significant decrease in the after-load period, while a decrease in HR and diastolic BP was statistically significant during all phases of a handgrip. This allows proposing a hypothesis that the combined therapy of mildronate with ACEI has reduced the activity of the mechanisms responsible for handgrip performance. Whereas analysing the dynamics of blood flow parameters in the after-load period in the study groups receiving the combined treatment, an increase in all parameters was stated after the treatment in comparison with the pre-treatment period. This refers to all blood flow parameters in the after-load period. Of main importance is the understanding that in our load test, all blood flow values comprise two components, namely, that of after-load and reactive hyperemia. The former component mainly represents the compensation of accumulated energetic debt during a handgrip, while reactive hyperemia reflects endothelium-dependent vasodilator capacity. Less pronounced increase in HR and BP in the after-load ishaemia phase in the study groups receiving the combined therapy indirectly suggests an improved metabolism in working muscles. Thus, it could be speculated that the pronounced increase in blood flow during the recovery period in these patients more refers to reactive hyperemia than to the after-load hyperemia. Accordingly, this confirms the above-mentioned hypothesis about the additive beneficial effect of mildronate in the treatment of CHF patients affecting endothelial function represented by an increase in vasodilation evoked by shear stress.

Conclusions

1. This study reveals the advantage of the combined treatment with "lisinopril 20 mg/daily and mildronate 1000 mg/daily" and "lisinopril 5 mg/daily and mildronate 1000 mg/daily" over the treatment with "lisinopril 20 mg/daily" on the leading symptoms of chronic heart failure and the improvement of the NYHA class.
2. The combined treatment is associated with the improvement of the quality of life, exercise capacity and mechanisms of peripheral circulation.
3. Our results confirm the additive beneficial effect of mildronate on the vasodilation capacity of the magistral arteries and resistance vessels at rest and under conditions of increased demand of blood supply.
4. The data obtained suggest the favourable influence of mildronate on the optimisation of energetic metabolism and vasodilatory capacity of static muscle load.

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