ORIGINAL ARTICLE

THE EFFECT OF TRIMETAZIDINE ON C-REACTIVE PROTEIN, CYTOKINES AND ADHESION MOLECULES IN THE COURSE OF ACUTE MYOCARDIAL INFARCTION

Radek Pudil[1], Vladimír Pidrman[1], Jan Krejsek[2], Jaroslav Gregor[1], Miloš Tichý[1], Ctirad Andrýs[2], Marcela Drahošová[2], Václav Bláha[3]

Charles University in Prague, Faculty of Medicine in Hradec Králové: 2nd Department of Medicine[1]; University Teaching Hospital in Hradec Králové: Department of Clinical Immunology and Allergology[2]; Purkyně Military Medical Academy in Hradec Králové Department[3]

Summary: The aim of this randomised, double-blind, placebo controlled, parallel group study was to assess the effect of trimetazidine (TMZ), a potent antiischaemic drug, on plasma C-reactive protein (C-RP), cytokine and adhesion molecule levels. The study population consists of 18 patients (16 males, 2 females, average age 56.45 ± 10.97 years) with acute myocardial infarction admitted within 6 hours after onset of symptoms and treated with streptokinase. Blood samples were taken at 3-hour intervals during the time of treatment. All patients were randomised blindly using a centralised randomisation process, between trimetazidine (40mg bolus iv. then 60mg per day for 48 hours intravenously in glucose infusion) or placebo group. Plasma C-RP level was significantly lower in TMZ group (39.5mg/ml ± 9.7 mg/ml) as compared to placebo (75.7 ± 29.4 mg/ml, p≤0.001) and peaked 28 hours later in TMZ group. Plasma interleukin 6 (IL 6) level showed a sharp peak 9 hours after the onset of the symptoms in TMZ group (116.9 ± 180.2 pg/ml vs. 45.4 ± 37.9 pg/ml) and was increased up to 30 hours after the onset of the symptoms. Plasma interleukin 1 beta (IL 1β) was also higher in TMZ group notably 21 hours after the onset of symptoms (26.4 ± 9.3 pg/ml vs. 16.2 ± 2.4 pg/ml). TMZ group showed lower plasma E-selectin levels. Plasma IL 8, TNF α and ICAM 1 levels were without statistical significant differences. The present study demonstrates a significant reduction of plasma C-reactive protein level in the course of acute myocardial infarction treated with streptokinase and intravenous trimetazidine infusion compared with the group of patients without trimetazidine treatment.

Key words: *Trimetazidine; Acute myocardial infarction; C-reactive protein; Interleukins; Adhesion molecules*

Introduction

The association of inflammation with acute myocardial infarction (AMI) has been known for over a half of the century. Inflammatory lesions seen in tissue specimens many hours to days after the onset of infarction reflect, in part, a healing process. In recent years many investigators have studied the potential pathogenic role of inflammation in the myocardial ischaemia-reperfusion (MI/R) process (11).

Inappropriate inflammatory response can cause severe tissue destruction. During ischaemia-reperfusion, such as in acute myocardial infarction, the tissue damage may result not only from direct anoxic and hypoxic injury but also from other deleterious events occurring after the blood flow reestablishment to the occluded vascular bed. Reperfusion injury is partly caused by oxygen radicals, proteolytic enzymes and cytokines released by adhered and activated leucocytes that infiltrate into the affected area, because neutrophil depletion or prevention of neutrophil accumulation significantly diminishes tissue damage and enhances the recovery of cardiac function (5).

Recent studies have focused on the possible role of the soluble early mediators of acute phase response during MI/R process. Particular emphasis has been given to the study of acute response proteins (C-reactive protein, α_1 antitrypsin, serum amyloid) and cytokines.

Key role of interleukin 1beta (IL 1β), interleukin 6 (IL 6), interleukin 8 (IL 8), and tumor necrosis factor alpha (TNF α) in the process of lymphocyte, neutrophil, and platelet activation has been shown in previous studies (8,9,12,14). Elevation of plasma IL1β, IL 6, IL 8, TNF α, and soluble adhesion molecule levels (ICAM 1, E-selectin etc.) in the course of ischaemic event have been studied extensively. Based on these observations, new medications should have protective effect on myocytes, preserve myocardial cell function by decreasing ischaemic and reperfusion effect, and limit the area of necrosis.

Trimetazidine [TMZ, (1-(2,3,4-trimetoxybenzyl)] piperazine dichloride has cytoprotective properties and prevents metabolic disturbances that result from myocardial ischaemia. Its mechanism of action is, at least partially, directly related to the mitochondrial enzymatic systems. TMZ stimulates glucose oxidation by primarily reducing rates of β-oxidation (6), decreasing mitochondrial oxygen demand. TMZ has also been reported to have a protective effect in myocardial ischaemia-reperfusion process by increasing phospholipids turnover of the membrane (13). After periods of ischaemia it is able to improve cardiac function and reduce both the decrease in intracellular pH and ATP content (3,7). TMZ has been reported to reduce the neutrophil accumulation in myocardium during the ischaemia-reperfusion process (15).

The objective of our study was to assess the effect of TMZ on inflammatory response to acute myocardial infarction and to elucidate if TMZ can reduce interleukin and adhesion molecule production. Therefore, plasma C-reactive protein, IL1β, IL 6, IL 8, TNFα, and soluble adhesion molecule levels were measured in the course of 96 hours AMI.

Material and methods

1. Study population

The study subjects consisted of eighteen patients with AMI (16 men and 2 women; mean age 56.45 years, ranging from 39 to 71 years) who were admitted within 6 h after the onset of the symptoms. They were treated by thrombolytic (streptokinase) therapy and randomised blindly, through a centralized randomisation process, to a trimetazidine or placebo group. The TMZ group consisted of 5 men and 1 woman (mean age 55.06 years ± 11.11 years) the placebo group had 11 patients (10 men, 1 woman, mean age 57.18 years ± 11.57 years). There were no significant differences between the two groups. Table No.1 shows the characteristics of the study population. In the second week some of them underwent coronary arteriography.

Tab. 1: Characteristics of study group.

Variable	TMZ (n 7)	Placebo (n 11)	p
Age (years) (mean ± SD)	55 (11.10)	57,18 (11.78)	ns
Female gender (%)	14.3	9.1	ns
Diabetes mellitus (%)	42.8	36.4	ns
Arterial hypertension (%)	57.4	54.5	ns
History of MI (%)	28.6	27.3	ns
Dyslipidemia (%)	42.8	27.3	ns
Smoking (%)	57.2	36.4	ns
AMI location - anterior (%)	42.8	45.5	ns
- inferior (%)	28.6	18.2	ns
Peak CK level (μkat/L)	26.1 ± 15.1	31.5 ± 28.5	ns
(mean ± SD)			
Killip class >2 (%)	14.3	9.1	ns
BP on admission (mmHg)	137/86	132/75	ns
HR on admission (BPM)	84.6	88.8	ns

AMI - acute myocardial infarction, BP - systemic blood pressure, HR - heart rate, SD - standard deviation.

Inclusion criteria

The diagnosis of suspected myocardial infarction was based on the following two criteria:

- A typical thoracic pain occurring within the previous 6 hours: prolonged (more than 30 minutes) or for less than 30 minutes, but still present and resistant to sublingual nitrates

And:

- typical ECG tracing with 2 mm or greater ST segment elevation in at least two leads or atypical but with highly probable previous history of coronary disease.

All patients had a confirmed diagnosis of AMI by biochemical changes (elevation of the serum creatine kinase and MB iso-enzyme level more than twice the normal upper limit).

Exclusion criteria were serious renal or liver failure, pregnancy, patients having taken TMZ within the last 48 hours, patients treated with established or experimental antioxidants, patients treated with fibrinolytic therapy within the last 24 hours, successful resuscitation, serious systemic connective tissue diseases, acute and inflammatory disorders, refusal of the informed consent.

The average time from onset of the symptoms to admission was 146,76 min.

The study protocol was approved by the Ethic Committee of our institution.

2. Treatment of the patients

The TMZ treatment comprised:

TMZ group – intravenous bolus of 40 mg of TMZ followed by a continuous TMZ infusion 60 mg/24 hours for 48 hours. The bolus of a 40 mg TMZ was injected intravenously over 2 minutes before thrombolysis. For the continuous infusion, 6 ml (60 mg of TMZ), was diluted in a 5% glucose solution, 500 ml and was administered over 24 hours. A control group received intravenously a placebo with the same regimen.

3. Blood samples

The first blood sample was taken immediately after admission and then at 3 h intervals during the first 48 hours, at 6 h intervals during the next 48 h. The blood was immediately centrifuged; aliquot parts of plasma were stored at -20°C overnight and stored at -70°C until the assessment of the immune parameters the plasma samples. Plasma levels of circulating IL 1β, IL 6, IL 8, ICAM 1 and E-selectin were measured using commercial enzyme-linked immunosorbant assay (ELISA) developed by RD Quantikinine, Minneapolis, MN, USA. TNFα was measured by using the commercial ELISA set purchased from Immunotech, Marseille, France.

4. Biochemical measurements

Serum creatine kinase was measured by Hitachi 704 autoanalyser using diagnostic kits of activated CK-NAC by the enzymatic assessment method developed by Boehringer, Mannheim, Germany. The plasma C-RP levels were measured by single radial immunodiffusion using antiserum USOL Prague, Czech Republic and the Behring standard.

5. Statistical analysis

Data were expressed as mean and standard deviation (SD). Analysis of the differences between patients treated with TMZ and placebo was made by using the analysis of variance (ANOVA) design. The differences were considered to be significant at p values less than 0.05 ($p<0.05$).

Results

7 patients received TMZ (intravenous bolus of 40mg in 2 minutes, then 60mg TMZ daily in glucose solution for 48 hours), and 11 were treated with placebo. In-hospital management is shown in table No.2.

Tab. 2: In-hospital management.

Variable	TMZ (n 7)	Placebo (n 11)	p
Coronary angiography (%)	57.2	45.5	ns
PTCA in hospital (%)	28.6	27.3	ns
Beta blockers (%)	100	91	ns
ACE inhibitors (%)	57.2	55.5	ns
Acetylsalicylic acid (%)	100	100	ns
Nitrates (%)	100	100	ns
Diuretics (%)	42.9	45.5	ns
Ca blockers (%)	28.6	27.3	ns

Plasma C-RP level (fig. 1)

The plasma C-RP level was elevated in both groups. Statistically significant differences were found in the interval from 18 to 60 hours after the onset of the symptoms. TMZ group showed a 52% significantly less elevation of the plasma C-RP level than the placebo group, (75.7 mg/ml ± 29.4 mg/ml vs. 39.5 mg/ml ± 9.7 mg/ml, $p<0.001$). The peak plasma C-RP level (76.8 mg/ml ± 34.1mg/ml) of the placebo group occurred 54 hours after onset of the symptoms as compared peak plasma C-RP level of the TMZ group (52.6 mg/ml ± 23.1 mg/ml) which was measured 78 hours after the onset of the symptoms (i.e. 28 hours after the peak of the placebo group).

Plasma TNFα (fig. 2)

The plasma TNFα levels of both groups were elevated throughout the time of the observation, but neither group has shown significant differences. The peak plasma TNFα level of the TMZ group (40.6pg/ml ± 28.7pg/ml) was achieved 60 hours after the onset of the symptoms as compared to, the peak of the placebo group (71.1pg/ml ± 60.1pg/ml), which was achieved 90 hours after the onset of the chest pain.

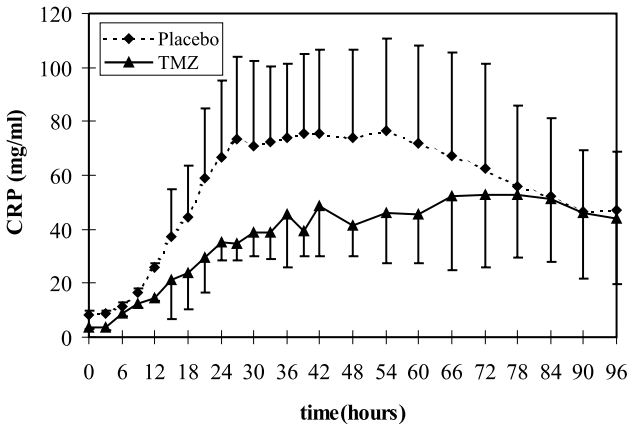

Fig. 1: Mean plasma CRP levels (mg/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

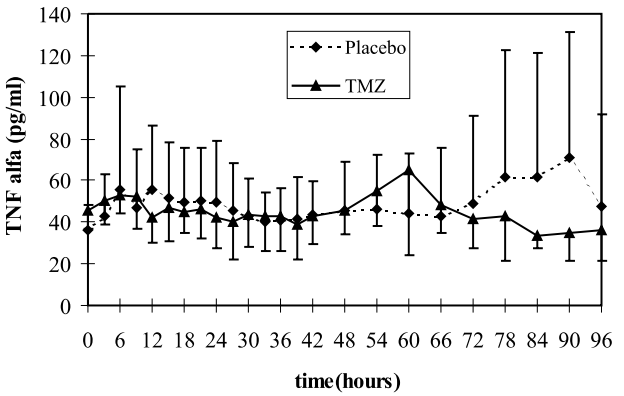

Fig. 2: Mean plasma TNF alpha levels (pg/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

Plasma IL 1β (fig. 3)

The plasma IL 1β levels were also elevated in both groups. Plasma IL 1β levels of the TMZ group were higher almost all of the time during the observation course (except at 96 hour after the onset of the symptoms, when plasma IL 1β level of placebo group was only slightly elevated). Plasma level elevation of the TMZ group was statistically significant 21 hours after the onset of the symptoms (26.4 pg/ml ± 9.3 pg/ml vs. 16.2 pg/ml ± 2.4 pg/ml $p<0.001$).

Plasma IL 6 (fig. 4)

Plasma IL 6 levels of the TMZ group showed a sharp peak 9 hours after the onset of the symptoms (116.9 pg/ml ± 180.2 pg/ml vs. 45.4 pg/ml ± 37.9 pg/ml, p=n.s.). The plasma IL 6 level was increased from the beginning of the observation to 30 hours after the onset of the symptoms.

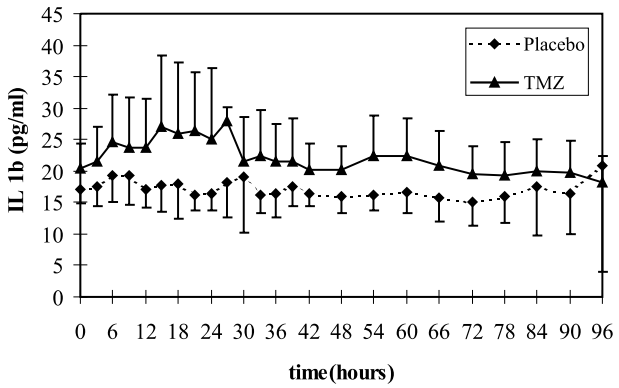

Fig. 3: Mean plasma IL 1beta levels (pg/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

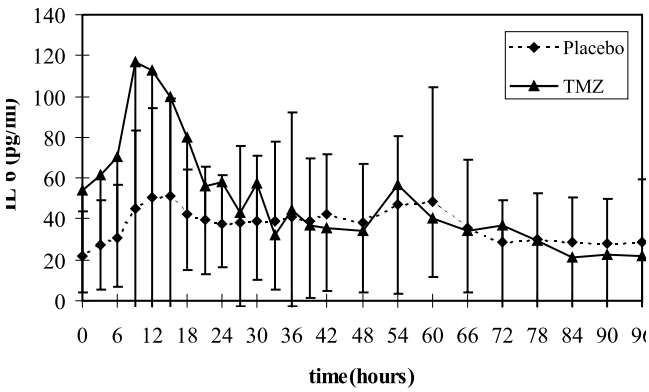

Fig. 4: Mean plasma IL 6 levels (pg/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

Plasma IL 8 (fig. 5)

Plasma IL 8 levels were elevated in both groups, but without any significant differences between them. While the peak plasma IL 8 level of the TMZ group was achieved 96 hours after admission (136.8 pg/ml ± 88.4 pg/ml), the peak plasma in the placebo group was achieved 72 hours after admission (136.5 pg/ml ± 75.2pg/ml).

Plasma adhesion molecule levels (figs. 6 and 7)

TMZ group showed a more rapid achievement of the peak plasma E-selectin level 6 hours after admission (117.8 ng/ml ± 35.9 ng/ml). Plasma level then decreased slightly until the end of the observation time course. Between 9 to 96 hours after onset of the symptoms E-selectin levels were much lower than in the placebo group, which achieved the peak level of 174.6 ng/ml ± 160.0 ng/ml 36 hours after the admission.

Plasma levels of ICAM 1 were non significantly different between the two groups. Plasma ICAM 1 level of the TMZ group was elevated for the first 24 hours. In the next two days, plasma ICAM 1 level was elevated in the placebo group.

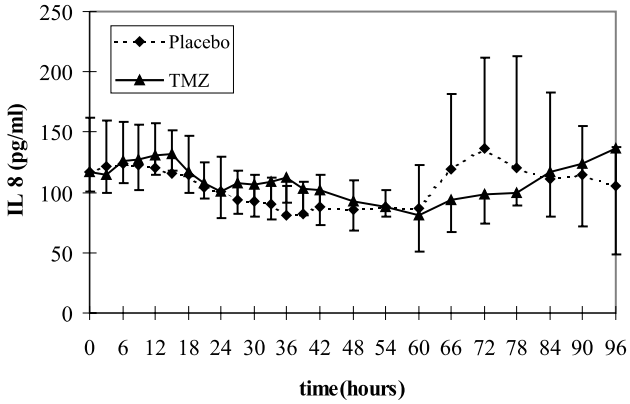

Fig. 5: Mean plasma IL 8 levels (pg/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

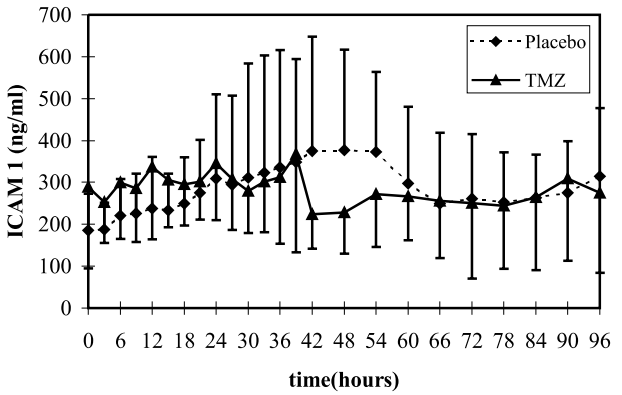

Fig. 6: Mean plasma E selectin levels (ng/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

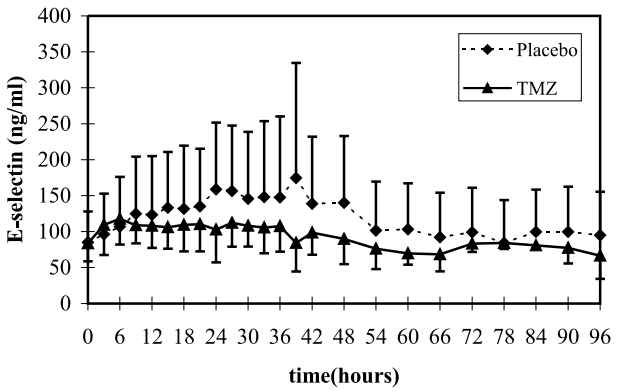

Fig. 7: Mean plasma ICAM 1 levels (ng/ml) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

Plasma CK levels (fig. 8)

Fig. No.8 shows the plasma CK levels in both groups of patients. Mean plasma CK levels of the placebo group were slightly more elevated compared to the TMZ group. Peak

plasma CK levels in both groups was achieved 9 hours after the onset of symptoms and reached 31.5 ± 28.5 μkat/ml in placebo group vs. 26.1 μkat/ml ± 15.1 μkat/ml in TMZ group, p non significant. There were no significant differences between the two groups.

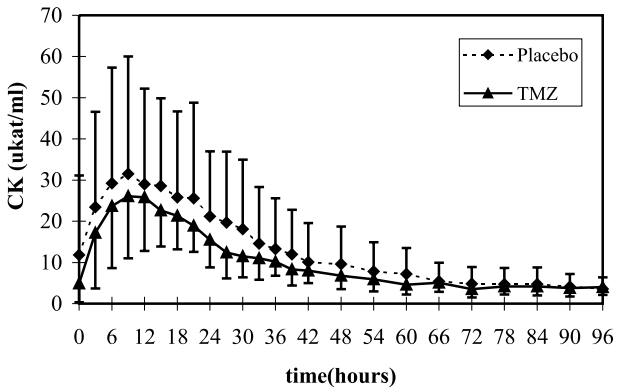

Fig. 8: Mean plasma CK levels ((kat/l) and SD in TMZ (–▲–) and placebo (- -◆- -) groups in the course of 96 hours AMI.

Discussion

Previous studies evidenced the cytoprotective effect of trimetazidine during ischaemia and reperfusion. In a placebo-controlled study, Fabiani et al. (4) showed myocardial metabolism and left ventricular function improved compared to placebo in patients receiving trimetazidine before and during cardiac surgery. Animal studies have shown that trimetazidine protects the myocardium from ischaemia and reperfusion during experimental myocardial infarction. Belcher et al. (1) showed that TMZ limits myocardial necrosis in rabbits, which received TMZ before a coronary artery ligation. Promising data from experimental studies have been encouraging regarding trimetazidine administration in acute ischaemia induced by coronary ligation in rabbits (15).

Recent studies have shown that trimetazidine is directly improving a metabolic status of the myocardium by inhibition of fatty acid oxidation (6), and reduction of the ionic imbalance induced by ischaemia. This metabolic modulation has been proved to indirectly reduce the ionic imbalance and improve myocardial function (3,7).

We examined the effect of trimetazidine on the cytokines, adhesion molecule levels and plasma C-RP levels in the course of AMI treated by streptokinase. We found a significant elevation of C-reactive protein in the placebo group as compared with the TMZ treated group. This observation supports the hypothesis that TMZ could reduce the inflammatory response to ischaemia also by improving a glucose metabolism in the ischemic area. This process can reduce an inflammatory response, and intercellular interactions. This observation is compatible with the experiment of Williams (15), showing that TMZ inhibits the neutrophil accumulation in the infarcted area.

Extravascular neutrophil migration depends on generation or release of chemoattractants agents and on initial adhesion of leukocytes to vascular endothelium. The latter process is mediated by adhesion molecules expressed on the surface of both neutrophil and endothelial cells. To determine whether trimetazidine affects release of plasma chemoattractant substances and the expression of adhesion molecules, plasma interleukin (IL 1β, IL 8, TNFα, IL 6) and adhesion molecules (E-selectin and ICAM 1) levels were examined. Although plasma levels of these cytokines have been elevated throughout the observation period, there was no significant difference in plasma levels of these interleukins and adhesion molecules of patients treated with TMZ compared with subjects on placebo.

In spite of the fact that plasma CK level can reflect the size of myocardial necrosis, we did not find a statistical difference in plasma CK level between the placebo and TMZ group. We also didn't find strong positive correlation between the plasma C-RP and CK levels.

Furthermore, it is known, that neutrophil accumulation in the ischaemic area is dependent on expression of CD 11/CD18 adhesion molecules levels (10) and interleukin 1 beta elicits neutrophil accumulation not through a direct action on the leukocytes, but more often by increased expression of endothelial adhesion molecule 1 (E selectin) and intercellular adhesion molecule 1 (ICAM 1). At this time we have no sufficient explanation for the inhibition of neutrophil accumulation in the infarct site.

Despite the limitations (small number of the patients, and inability to compare the size of the infarcted area), our study has shown the significant effect of trimetazidine on plasma C-RP level, which was lower as compared with the placebo group. The study has also shown that TMZ suppressed neutrophil accumulation is not dependent on plasma chemoattractants such as interleukin 1 beta, interleukin 8 and adhesion molecules.

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MUDr. Radek Pudil, Ph.D.,
Charles University in Prague,
Faculty of Medicine in Hradec Králové,
2nd Department of Medicine,
Pospíšilova 360, 500 05 Hradec Králové,
Czech Republic.
e-mail: pudil@fnhk.cz