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**The influence of Ivabradine and ω-3 polyunsaturated fatty acids on cytokines level in patients with ischemic heart failure**

S. V. Fedorov

SHHE “Ivano-Frankivsk National Medical University”

**Key words:** heart failure, treatment, ivabradine, ω-3 polyunsaturated fatty acids, cytokines

The aim of our studywas to evaluate of the possible effect of ivabradine and ω-3 polyunsaturated fatty acids on the levels of pro- and anti-inflammatory cytokines in patients with ischemic heart failure. 357 patients with ischemic HF and the sinus rhythm were observed. In accordance to treatment all patients were divided into four groups. The levels of interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10) in the serum were determined using commercial ELISA kits according to the manufacturer’s instructions. During treatment we observed decrease of the serum IL-1β level in all groups of patients. The medicines of the basic treatment decreased of the serum IL-6 level by 30%. Dynamics in the second group was higher – 37.6%. The more intensive changes were caused by additional use of PUFA (for 45.9%) or their combination with ivabradin (for 48.6%). All therapeutic schemes caused the increase of IL-10 levels in the HF patients blood. But more intensive changes were observed in groups with PUFA: by 26%; and 26.1%, respectively. In the first group this value increased by 21.4%; in the second group – by 20.6%. Conclusions:1. The PUFA medicines have the immunomodulatory effects: they decrease the levels of the serum pro-inflammatory cytokines (IL-1β, IL-6) and increase the level of the anti-inflammatory IL-10. 2. Ivabradine reduces the levels of proinflammarory IL-1β and IL-6 in the serum, but does not influence on the anti-inflammatory IL-10 concentration.

**Introduction.** Despite extensive evidence and recommendations from clinical trials, heart failure (HF) remains a substantial cause of morbidity and mortality.

The heart failure syndrome is characterized by impaired systolic and/or diastolic function and various clinical signs such as fatigue, dyspnea, ﬂuid retention, and cachexia. An inﬂammatory activation in CHF patients has long been recognized. Indeed, immune mechanisms modulate interstitial ﬁbrosis, cardiomyocyte apoptosis, and hypertrophy, all of which are central processes leading to maladaptive remodeling in response to a variety of stimuli [7].

Several reports have demonstrated enhanced expression and release of inflammatory cytokines such as tumor necrosis factor (TNF) -α , interleukin (IL)-1, IL-6, IL-18, cardiotrophin-1 and Fas ligand, as well as several chemokines [e.g. monocyte chemoattractant peptide (MCP)-1/CCL2, IL-8/CXCL8 and macrophage inflammatory protein-1 α/CCL3] in HF patients [8]. Plasma levels of inflammatory cytokines and chemokines appear to be elevated in direct proportion to deterioration of functional class (i.e. New York Heart Association classification) and cardiac performance (i.e. left ventricular ejection fraction (LVEF)) [9].

Pharmacological therapy of HF including administration of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor II blocker (ARB), beta-blocker (BB), and aldosterone receptor antagonist (AA) can reduce morbidity and mortality in patients with HF. Ivabradine is a new therapeutic agent designed to reduce heart rate at rest and during exercise by selective inhibition of a novel receptor (*I*f channel) located on the pacemaker-cell membrane within the sinoatrial node. As such, ivabradine joins a list of rate-limiting medications already available to prescribers for the control of heart rate in coronary artery disease (CAD) and HF with systolic dysfunction [5]. The ω-3 polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are known as anti-inflammatory factors, and are using for HF treatment [5].

The data for ivabradine influence for cytokine’ cascade are poor; but for PUFA – controversial.

**The aim of study** was to evaluate of possible influence of ivabradine and ω-3 polyunsaturated fatty acids for pro- and anti-inflammatory cytokines level in patients with ischemic heart failure.

**Material and Methods.** 357 patients with ischemic HF and sinus rhythm were observed. In accordance to treatment all patients were divided into four groups: I group – basic treatment (89 patients); II group - basic treatment and Ivabradine (Coraxan, Les Laboratoires Servier Іndustrie, France) – 5 or 7,5 mg twice a day (depends of heart rate); III group - basic treatment and PUFA (Omacor, Abbott Laboratories GmbH, USA) – 1000 mg per day; IV group – basic treatment with Ivabradibe and PUFA in similar doses. All patients were examined before and after 6 months of treatment. Control group – 30 practically healthy persons. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline [6]. The study was approved by the local ethics committee and written informed consent was obtained from all patients.

The interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10) levels in serum were determined using commercial ELISA kits (ProCon, Russia; Amersham Pharmacia Biotech, UK) according to the manufacturer’s instructions.

Statistical analyses were performed using the Statistica 12.0 (StatSoft, Tulsa, OK, USA). Statistical significance was assumed at p<0.05.

**Results and Discussion.** The average age of observed patients with HF was (67,98±12,06) years. Among all patients 278 persons (77,82 %) were males. HF FC

ІІ (NYHA) was verified in 63 patients (17,65 %); ІІІ – in 238 (66,67 %); IV – in 56 (15,68 %). 267 (74,78 %) patients with HF had history of myocardial infarction; 27 (7,56 %) had recurrent ones.

HF is characterized by pro-inflammatory cytokine levels increase. The middle levels of IL-1β was (49,29±3,78) pg/ml versus (25,31±3,71) pg/ml in control group (p<0,001); IL-6 – (15,47±0,52) pg/ml versus (7,19±0,67) pg/ml respectively (p<0,001). Their concentrations were increased due raise of HF class. The serum level of anti-inflammatory IL-10 was insignificant lower: (3,12±0,37) pg/ml versus (3,46±0,57) pg/ml in control group (p>0,05).

During treatment we observed decrease of serum IL-1β level in all groups of patients (see tab. 1). In particular, in first group this parameter was decreased for 25,5%: from (47,51±3,68) pg/ml to (35,41±3,11) pg/ml (p<0,01). More strong changes were observed in group with additional use of ivabradine, where the IL-1β level decreased for 32,6%: from (51,21±3,79) pg/ml to (34,49±2,97) pg/ml (p<0,001). Additional prescription of PUFA caused of IL-1 reduction for 35,9%: from (50,13±3,62) pg/ml to (32,12±3,14) pg/ml (p<0,001). In forth group this parameter decreased for 44,4%: from (48,92±3,81) pg/ml to (27,21±3,17) pg/ml (p<0,001), and was same like in control group (p>0,05).

The medications of basic treatment decreased of serum IL-6 level for 30%: from (14,71±0,71) pg/ml to (10,29±0,54) pg/ml (p<0,05). The its dynamics in second group was higher – 37,6%: from (16,31±0,49) pg/ml to (10,17±0,39) pg/ml (p<0,01). The more strong changes were caused by additional use of PUFA or their combination with ivabradin. In particular, in third group - for 45,9%: from (15,11±0,51) pg/ml to (8,18±0,47) pg/ml (p<0,01); in forth – for 48,6%: from (15,82±0,47) pg/ml to (8,13±0,41) pg/ml (p<0,01).

More than any other cytokine family, the IL-1 family of ligands and receptors is primarily associated with acute and chronic inflammation. The cytosolic segment of each IL-1 receptor family member contains the Toll-IL-1-receptor domain. This domain is also present in each Toll-like receptor, the receptors that respond to microbial products and viruses. Since Toll-IL-1-receptor domains are functional for both receptor families, responses to the IL-1 family are fundamental to innate immunity [3]. IL-6 plays a pivotal role during the transition from innate to acquired immunity, in the control of metabolism, in pain, acute and chronic inflammation etc. [12]. There are different experimental and clinical trials which showed the anti-inflammatory effects of statins and RAAS blockers (i.e. decrease of pro-inflammatory cytokines levels) [1, 4, 6]. The same results we observed in our research too.

All therapeutic schemes caused to raising of IL-10 levels in HF patients blood. But more strong changes were observed in groups with PUFA. In third group its growth was 26%: from (3,10±0,35) pg/ml to (4,19±0,29) pg/ml (p<0,01); in patients of Iv group –26,1%: from (3,11±0,35) pg/ml to (4,21±0,31) pg/ml (p<0,01). In first group this value increased for 21,4%: from (3,12±0,31) pg/ml to (3,97±0,27) pg/ml (p<0,05); in second group – for 20,6%: from (3,17±0,41) pg/ml to (3,99±0,21) pg/ml (p<0,05). For our opinion, this is result of basic treatment influences, but not ivabradine.

IL-10 is a regulatory cytokine with anti-inﬂammatory properties, potently inhibiting the capacity of innate immune cells to produce inﬂammatory mediators.

Established, that ω-3 PUFA concentrations are negatively correlated with several pro-inflammatory biomarkers including C-reactive protein, IL-6 and TNF-α, and positively correlated with anti-inflammatory markers, such as TGF-β and IL-10 [10]. However, there are reports that supplementation with PUFA has no effect on these cytokines [2]. In our case we observed of negative influence of PUFA for pro-inflammatory cytokine levels and positive – for IL-10 concentration.

**Conclusions.** 1. The PUFA medication has an immunomodulatory effects: they decrease of serum pro-inflammatory cytokines (IL-1β, IL-6) levels and increase of level of the anti-inflammatory IL-10.

2. Ivabradine is caused of reduction IL-1β and IL-6 in serum but not influence for IL-10 concentration.

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*Table 1*

**The Dynamics of Cytokine Levels in Serum of Patients with HF**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Value,  M±m | Patient with HF, n=357 | | | |
| I group, n=89 | II group, n=91 | III group, n=90 | IV group, n=87 |
| IL-1β, pg/ml  Before treatm.  After treatm.  ∆, abs. (%) | 47,51±3,68  35,41±3,11\*\*  -12,1 (25,5%) | 51,21±3,79  34,49±2,97\*\*\*  -16,72 (32,6%) | 50,13±3,62  32,12±3,14\*\*\*  -18,01 (35,9%) | 48,92±3,81  27,21±3,17\*\*\*  -21,71 (44,4%) |
| IL-6, pg/ml  Before treatm.  After treatm.  ∆, abs. (%) | 14,71±0,71  10,29±0,54\*  -4,42 (30,0%) | 16,31±0,49  10,17±0,39\*\*  -6,14 (37,6%) | 15,11±0,51  8,18±0,47\*\*  -6,93 (45,9%) | 15,82±0,47  8,13±0,41\*\*  -7,69 (48,6%) |
| IL, pg/ml  Before treatm.  After treatm.  ∆, abs. (%) | 3,12±0,31  3,97±0,27\*  0,85 (21,4%) | 3,17±0,41  3,99±0,21\*  0,82 (20,6%) | 3,10±0,35  4,19±0,29\*\*  1,09 (26,0%) | 3,11±0,35  4,21±0,31\*\*  1,10 (26,1%) |

Remarks: 1. HF – heart failure

2. Differences in values before and after treatment: \* -p<0,05; \*\* - p<0,01; \*\*\* - p<0,001.

Fedorov Sergiy – MD, PhD, MBA, Associate professor of Therapy and Family medicine Department of Postgraduate Faculty of Ivano-Frankivsk National Medical University

IFNMU

2, Galytska str

76000 Ivano-Frankivsk

serfed@i.ua