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Extralipid effects of hypolipidemic drugs – why do clinical trials weakly support experimental data?

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Abstract:
There are many experimental and clinical data confirming the inflammatory cause of atherosclerosis. It is in agreement with the commonly accepted, pathomorphological theory described as: "reaction to injury". Connections between the concentration of proinflammatory cytokines, chemokines and serum lipoproteins are under current investigations. There are also attempts to compare amount of the above-mentioned molecules with atherosclerotic plaque dimentions and increased artery wall thickness. Much more promissing seems to be describing the role of inflammatory cell products in vascular risk stratification.
Carefully planned, prospective observations of patients are of greatest value for reliable information. The role of multicenter clinical studies is smaller because of their strict end-points and methodological restrictions. Hence, the question has arisen whether adherents of large population trials and evidence-based medicine trust in value of single-center studies. How to reconcile the effectiveness of therapy based on large population trials with complicated methods of determination of proinflammatory factors? Restrictive inclusion criteria requiring accurate diagnosis of inflammation raise doubts. For some investigators, it is only preselection, which reduces the real value of achieved results.
Anti-inflammatory and vasoprotective influence of hypolipidemic and hypotensive drugs is considered to be an important clinical supplementation to their basic mechanism of action. It cannot be ruled out that these additional effects of drugs are responsible for better outcomes in the treated patients.
Generally, precise distinguishing the effects of different groups of drugs is usually impossible in circumstances of clinical trials. However, we can measure different molecules, hs-CRP assay represents the best choice at this time.

Key words:
atherosclerosis, proinflammatory cytokines, adhesion molecules, hs-C-reactive protein (hs-CRP), hypolipemic therapy

Abbreviations: HOMA – Homeostatic Model Assessment; HMG-CoA – 3-hydroxy-3-methylglutaryl-coenzyme A, hs-CRP – hs-C-reactive protein, ICAM-1 – intercellular adhesion molecule-1, IFN-γ – interferon gamma, IL – interleukin, LDL – low-density lipoproteins, MCP-1 – monocyte chemoattractant protein-1, MONICA – Multinational Monitoring Trends and Determinants in Cardiovascular Disease, PRINCE – The Pravastatin Inflammation/CRP Evaluation, PROVE-IT – The Pravastatin or Atorvastatin Evaluation and Infection Therapy, TIMI 22 – Thrombolysis in Myocardial Infarction Study Group 22, TNF-α – tumor necrosis factor-α

Introduction

In the 19th century Virchow described inflammatory reaction within the vascular wall that had occurred before atherosclerosis progress. Aniczkow proved in 1913 the presence of lymphocyte and monocyte cells in atherosclerotic changes.

The endothelial injury theory; according to which injury leads to vessel myocyte proliferation and mi-

gration, was presented in 1973 by Glomset and Ross. That is why from a pathological point of view; all stages of atherosclerosis might be considered as an inflammatory response to injury. Reported lymphocyte/monocyte cell infiltration is characteristic not only of atherosclerosis but also of long-term inflammatory diseases, like: liver cirrhosis; rheumatoid arthritis; glomerulotubular kidneys sclerosis; chronic pancreatitis and fibrothorax [31]. The fibrination process and the production of glycoprotein as well as collagen fibriles by fibroblasts is a type of response to the chronic inflammation and plays a special role in all above-mentioned disorders. The fibroblasts and transformed phenotypically smooth muscle cells of vascular wall have the same role in chronic inflammation as in atherosclerosis process. They produce the fibrous cap of atherosclerotic plaque. There is also very similar location of immunoglobulin deposits and complement activity in subintimal space of arteries both in atherogenesis and autoimmunologic diseases. Many factors that promote atherogenesis, like smoking, hyperlipidaemia, hyperglycemia have been established. Proinflammatory cytokines or their lipopolisaccharides seems to be also dangerous. These risk factors give rise to a variety of triggers that elicit secretion of both: leukocyte soluble adhesions molecules, which facilitate the attachment of monocytes to endotheliocytes, and chemotactic factors, which facilitate the migration of monocytes into the subintimal space. The transformation of monocytes into macrophages and the uptake of lipoproteins are thought to initiate the formation of fatty streak. Oxidized or glycated low density lipoproteins (LDL) take part in that early infiltration of vascular wall. There is a suspicion that some modified LDL may be one of several factors that contribute to loss of smooth muscle cells through apoptosis in plaque cap.

Proinflammatory cytokines released by activated macrophages, T cells and mastocytes may up-regulate process of collagen breakdown, and weakening and rupture of the cap of atherosclerotic plaque. This damage of the plaque cap is followed by exposure the plaque core containing tissue factor, which induces thrombosis. On the other hand, interferon gamma (INF-γ) released by T cells is one of the powerful inhibitors of collagen fiber biosynthesis and myocyte apoptosis initiation [12]. These processes lead to enhancement of plaque disruption.

Therefore, every stage in the process of atherosclerosis is believed to involve cytokines, other bioactive molecules and cells that are characteristic of inflammation [3]. Some investigators defend the hypothesis that *Helicobacter pylori* and *Chlamydia pneumoniae* also initiate the atherosclerotic process. Many presented pro-atherosclerotic molecules provide potential targets for diagnostic purposes. The risk factors (oxidized or glycated LDL), proinflammatory cytokines (interleukin-1 – IL-1, tumor necrosis factor α – TNF-α), adhesion molecules (intercellular adhesion molecule-1 – ICAM-1, selectins), cytokines with hepatic action (IL-6) as well as acute-phase reactants – C reactive protein (CRP) and fibrinogen can be measured in order to assess the intensity of inflammatory reaction. [5].

Unfortunately, these substances have only potential value. Plasma markers of arterial injury should be measured by standard, reproducible methods. Oppositely to commonly known risk factors, there is a need to discover new independent methods. The next problem is the estimation of standardized normal ranges for correct interpretation of the obtained results without the seasonal fluctuations for different populations.

Up to date, only hs-C-reactive protein (hs-CRP) assay seems to have predictive value under described conditions in cardiovascular events risk stratification [28]. However, hs-CRP has not been a good predictor of the extent of atherosclerotic disease, showing poor correlation with results of Doppler ultrasound scans of carotic arteries and coronary calcium score assessed by electron beam computerized tomography [26]. There are some data suggesting positive correlation between inflammatory markers and atherosclerotic mass [15]. Hence, more clinical trials are required to finally define the relationship.

A lot of studies have established hs-CRP as a predictor of recurrent coronary heart disease and risk of revascularization following restenosis. Although several markers have been studied, the strongest association with prognosis has been established for fibrinogen and hs-CRP [13]. Elevated hs-CRP levels also seem to be a predictor of recurrent events in patients with stroke and peripheral artery disease. The metabolic syndrome is consistently associated with the elevated hs-CRP and that is why some authors concluded that hs-CRP was merely a marker for obesity and insulin resistance [10, 14].

According to other suggestions proinflammatory state in the metabolic syndrome is an important component of that combined pathology [6].

Inflammatory markers are useful in the identification of patients who ought to be considered for

hypolipidemic, hypotensive or antiplatelet drug therapies, as well as reinforcement in therapeutic life style changes [32]. The last update of Adult Treatment Panel III described hs-CRP as an additional risk factor causing the increase in LDL level in high-risk patients [18]. Extralipid effects of 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statin therapy) include lowering of hs-CRP level. Unfortunately, that response to statins is very heterogeneous with many non-responders. The results of trials with statin therapy initiated the studies where that drugs were administered as immunosupressants (e.g. in rheumatoid arthritis, ulcerative colitis, after transplantations, septic shock, osteoporosis, glomerular nephritis) [1, 17].

In multicenter randomized clinical trials, the inflammatory markers are very seldom classified even as secondary endpoints. Of large population trials, only Multinational Monitoring Trends and Determinants in Cardiovascular Disease (MONICA), The Pravastatin Inflammation/CRP Evaluation (PRINCE), The Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) and Thrombolysis in Myocardial Infarction Study Group 22 (TIMI 22) trials confirmed the prognostic value of hs-CRP and statin therapeutic effectiveness in lowering activity of hs-CRP [2, 7]. It probably happened because of choosing only one central laboratory as a place where assays were performed. The sample of blood for measurement of concentrations of proinflammatory cytokines must be frozen and sent in dry ice to the central laboratory (except hs-CRP). Conditions for the transportation are not always fulfilled.

The "hard" endpoints are of major interest in clinical trials. Their results are used to formulate the algorithms and guidelines for therapy. The next problem is the restrictive procedure of patients inclusion to the investigations with measurement of cytokine activity. At first elimination of all possible inflammatory disorders have to be performed. Unfortunately, procedures sometimes differ between medical centers participating in a trial. On the other hand, the restrictive patients enrolment leads to preselections. The protocols with such procedures limit the value of the obtained results for the whole population.

In addition, the results from one site, though of the largest cognitive value, do not reflect global data for statistical analyses. However, the studies are absolutely comparable in terms of inclusion procedures and the control of course of investigation. Some assays are possible to be performed immediately in local laboratory. The next limitations in investigations of proinflammatory factors are associated with applied laboratory procedures. Cytokine activity and concentration of other molecules in plasma do not identify the cells actually producing them [16]. The isolation of inflammatory cells from blood is indicative of the circulating population without information specifically about plaque cells. On the other hand, isolation of cells from damaged vessel, destroys them and complicates estimation of their activity. The best method for confirmation of cellular source of cytokine production is the parallel measurement of the gene expression and cytokine level in the same sample [20]. The measurement of both the gene expression and its products (cytokine release) could be performed at basal conditions as well as after the stimulation with a standard factor, usually lipopolysaccharide [35]. The obtained so-called basal results are difficult to quantify and analyze statistically because of borderline values obtained with usually used standard kits.

The proinflammatory cytokines may be studied in fresh drawn blood samples, in short-term cultures or in supernatant derived from isolated *ex vivo* and then incubated monocytes [19].

Patients and procedures

In our investigations, we have used two procedures: the estimation of activity of proinflammatory cytokines in plasma and supernatant and the assay of monocytes isolated from the patients with IIa and IIb hyperlipidemia [8]. The activity of chemotactic factor of monocytes contributes to the unfavorable profile of two variants of hyperlipidemia. Treatment of the patients with statins and fenofibrate reduces the activity of monocyte chemotactic peptide. Similarly, therapy with statins or fenofibrate decreases plasma MCP-1 concentration in supernatant of incubated monocytes.

Pleiotropic mechanisms of action of statins includes also their impact on coagulation and fibrynolysis processes [30]. Estimated in our studies proinflammatory activity of fibrinogen, IL-1, CRP, complement, and the therapeutic effect of statins and fibrates were described previously [11, 23].

Immunosuppressive activity of statins and fibrates on T cells in prospective long- term clinical trial was established [25]. Significant decrease in IFN-γ and IL-2 released by T cells was observed after treatment.

Anti-inflammatory effect of statin and fibrate therapy was demonstrated by methods of TNF-α and IL-1 monocyte release in patients with hyperlipidemia IIa and IIb [21]. The monocyte gene expression confirmed the above-mentioned results of cytokines release [24].

In our studies in patients with impaired glucose tolerance, the relationship between insulin resistance (index HOMA) and the activity of plasma inflammation markers as well as hemostasis has been established. The improvement of metabolic parameters and decrease in cytokine levels by micronized fenofibrate has been shown [22, 34].

Angiotensin converting enzyme inhibitors (ACEI) are responsible for blocking of numerous angiotensin II-induced processes which are crucial in the atherosclerotic damage of coronary arteries. We assessed the hypotensive, antiinflammatory, antioxidant and fibrinolytic effects of plasma and tissue type ACEI in normotensive patients with stable coronary heart disease (CHD), who were given optimal treatment (aspirin, beta-adrenolytic, statin). The anti-inflammatory action affects the balance between pro- and anti-inflammatory cytokines (IL-10), and this impact was more pronounced for tissue ACEI. The same tissue type ACEI was also shown to have antioxidant, antithrombotic and profibrinolytic activity.

The inflammation source in atherosclerosis became a basis for the *in vitro* investigations. This concept requires the long-term prospective studies. Large multicenter clinical trials are not so unmistakable.

Such drugs as: statins, fenofibrates and ACEI have strong attenuating effect on the release of proinflammtory cytokines [9]. It could not be ruled out that these additional effects of drugs are responsible for better outcomes in the treated patients [33].

In a majority of reports, the anti-inflammatory effect of hypolipemic drugs is independent of lipidogram correction, and for hypotensive drugs, it is independent of decreasing blood pressure [29]. It is possible to estimate the prognostic reduction of cardiovascular events after therapy with hypolipidemic and hypotensive drugs. However, clinical benefits for treated patients are much greater than prognostic values [27].

The correlation between basal drug effects and their additional mechanisms of action has not been clearly confirmed.

Parallel groups of patients received a standard therapy; hypolipemic diet or placebo. The study provided evidence of the additional direct anti-inflammatory mechanism of action of the investigated drug.

Although we can measure different types of molecules, hs-CRP assay represents the best choice at this time.

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