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THE INFLUENCE OF QUERCETIN ON PROINFLAMMATORY ACTIVITY OF MONOCYTES OF PERIPHERAL BLOOD OF WOMEN WITH METABOLIC SYNDROME IN MENOPAUSE

L.V.Glushko, A.H.Nasrallah, S.V.Fedorov

SHEE “Ivano-Frankivsk National Medical University”

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Metabolic syndrome (MetS) is becoming a worldwide epidemic as a result of the increased prevalence of obesity and a sedentary lifestyle, and the prevalence of MetS in the adult population is relatively high. Many studies showed the high prevalence of metabolic syndrome among postmenopausal women. The aim of our study was to investigate of spontaneous cytokines and chemokines production by macrophages in menopausal women with MetS (in vitro) and possible influence of quercetin. The mechanism behind the role of menopausal risk factors in initiating cardiovascular disease remains unclear. Several trials have recently been demonstrated that the chronic inflammatory condition associated with MetS is characterized by a continuous activation of the innate immune system. In vitro activity of monocytes/macrophages isolated from menopausal women with MetS blood were discovered. Spontaneous macrophage’s production of interleukin 1β (IL-1β), interleukin 6 (IL-6), and leukotriene B4 (Ltr B_4) were examined. The overproduction of both cytokines and chemokine by macrophages were established: IL-1β in 1,69 times (p<0,05); IL-6 – in 2,54 times (p<0,01) and Ltr B_4 – in 1,71 times (p<0,05). Some correlations between spontaneous production of IL-1β and IL-6 (r=0.52, p<0,05), IL-1β and Ltr B_4 (r=0.58, p<0,05), IL-6 and Ltr B_4 (r=0.75,

p<0,05) by macrophages of menopausal women with MetS were established. Quercetin in vitro normalized of IL-1β, Il-6 and Ltr B_4 production by monocytes/macrophages.

Metabolic syndrome (MetS) is becoming a worldwide epidemic as a result of the increased prevalence of obesity and a sedentary lifestyle, and the prevalence of MetS in the adult population is relatively high. The presence of MetS doubles the risk of developing CVD over the next 5-10 years and for 3-6 times increased risk of diabetes mellitus type 2. In addition, these patients have higher risk of mortality from CVD. According to the Framingham Heart Study, which included about 5,000 persons aged 18 to 74 years, a combination of 3 or more components of the metabolic syndrome increases the risk of coronary heart disease (CHD) in 2.4 times in men and 5.9 times in women [4].

Many studies showed the high prevalence of metabolic syndrome among postmenopausal women, which varies from 32.6% to 41.5% [3]. The mechanism behind the role of menopausal risk factors in initiating cardiovascular disease remains unclear. Several trials have recently been demonstrated that the chronic inflammatory condition associated with MetS is characterized by a continuous activation of the innate immune system [6]. It’s known, that inflammation take the crucial role in development of the atherosclerotic injure of arterial wall. Macrophages are the first inflammatory cells to invade atherosclerotic lesions, and they are the main component of atherosclerotic plaques [3]. Inflammatory cytokines produced by macrophages stimulate the generation of endothelial adhesion molecules, proteases, and other mediators, which may enter systemic circulation in soluble forms [3]. Cytokines and chemokines as inflammatory biomarkers, independent of cholesterol and regulators of blood pressure, could yield more information on different aspects of pathogenesis of atherosclerosis [6].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a member of the bioflavonoids family, is one of the most widely distributed dietary polyphenolic compounds in foods including vegetables, fruits, tea, and wine [10]. Like other members of the bioflavonoids, quercetin has been shown to have biological properties consistent with its sparing effect on the cardiovascular system. It has been shown that quercetin possesses, anti-atherogenic, anti-inflammatory, anti-coagulative, and anti-

hypertensive properties [10]. In addition, within the bioflavonoid family, quercetin is the most potent scavenger of reactive oxygen species (ROS). The anti-oxidant activity of quercetin has frequently been mentioned in connection with its pharmacological function in the cardiovascular system because oxidative modification of plasma low-density lipoprotein (LDL) was suggested to be involved in the initial event of atherosclerosis, leading to coronary heart disease [10]. In contrast, the influence of quercetin on monocytes/macrophages production of cytokines and chemokines in vitro in patients with MetS is unknown.

The aim of our study was to investigate of spontaneous cytokines and chemokines production by macrophages in menopausal women with MetS (in vitro) and possible influence of quercetin.

Materials and methods. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline. All patients gave written informed consent and the local ethics committee approved the study protocol. 18 menopausal women with MetS were observed. Control group consist of 16 practically healthy persons. Suspension of monocytes from blood received by H. Recalde method [8]. The isolated cells were labeled with a monoclonal antibody (Daco, Glostrup, Denmark) against the monocyte specific positive antigen CD14. The procedure yielded a population of 89-96% CD14-positive cells in the isolated fraction. Cell viability was confirmed by trypan blue test [1] and was 89-93%. Monocytes were suspended in 199 medium supplemented with 30% blood autoserum, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 µg/ml fungizone (Gibco, Grand Island, NY,USA). The cells were counted and the monocyte concentration was adjusted to 1×10^6 cells/ml. A constant number of monocytes (1×10^6 monocytes per well) was placed in a plastic 24-well microtiter plate (Becton-Dickinson, Franklin Lakes,NJ, USA) and left intact for 2 h to allow them to adhere. The medium was then changed, and the cultures were incubated for additional 24 h. Incubations were performed in triplicate at 37°C in a humidified atmosphere containing 5% CO_2 in air. After 24-h incubation, the supernatant was carefully aspirated and frozen at –80°C until it was assayed for basal cytokine and chemokine release. The removed medium was then

replaced with medium supplemented with a 0,517 mg/ml of quercetin (diluted in 0.9% solution of sodium chloride) or 0.9% solution of sodium chloride as control and the cells were incubated under the conditions mentioned for another 24 h. Interleukin 1β (IL-1β), interleukin 6 (IL-6), and leukotriene B4 (Ltr B_4) levels in culture supernatant were determined using commercial ELISA kits (ProCon, Russia, Cytimmune Sciences Inc., USA; Amersham Pharmacia Biotech, UK) according to the manufacturer's instructions. Statistical analyses were performed using the Statistica 6.1 (StatSoft, Tulsa, OK, USA). Statistical significance was assumed at $p < 0.05$.

Results and Discussion. In observed menopausal women with MetS the excessive activation of monocytes/macrophages were showed. This was manifested by high levels in cells supernatant of IL-1β: 93,50±15,30 pg/10^6 cells vs 55,45±9,54 pg/10^6 cells in control group ($p<0,05$); IL-6: 6,76±0,77 pg/10^6 cells vs 2,66±0,26 pg/10^6 cells ($p<0,01$) and Ltr B_4: 6,71±0,94 pg/10^6 cells vs 3,93±0,79 pg/10^6 cells ($p<0,05$) (see tab).

Some correlations between spontaneous production of IL-1β and IL-6 ($r=0.52$, $p<0,05$), IL-1β and Ltr B_4 ($r=0.58$, $p<0,05$), IL-6 and Ltr B_4 ($r=0.75$, $p<0,05$) by macrophages of menopausal women with MetS were established.

Driven by macrophage colony-stimulating factor (M-CSF) and other differentiation factors, monocytes differentiate into two major types of macrophages and/or dendritic cells [5,9]. M1 and M2 macrophages play opposite roles during inflammation, although both are present in atherosclerotic lesions. M1 macrophages, which are differentiated from $Ly6C^{high}$ monocytes and promote inflammation, are classically activated by lipopolysaccharide in the presence of IFN-γ, leading to the production of high levels of IL-2, IL-23, IL-6, IL-1, and TNF-α. In contrast, activated M2 macrophages, which are differentiated from $Ly6C^{low}$ monocytes and promote resolution inflammation, differentiate in the presence of IL-4, IL-13, IL-1, or vitamin D3 and tend to produce a large amount of IL-10 and express scavenger receptors, mannose receptors, and arginase [7].

Leukotriene B_4 (Ltr B_4) is a lipid mediator with potent chemoattractant properties and that is rapidly generated from activated innate immune cells such as neutrophils, macrophages, and mast cells. Elevated levels of LTB_4 have been reported in various inflammation diseases and atherosclerosis.

We established, that quercetin decreased of spontaneous production of IL-1β to 44,93±8,59 pg/10^6 cells ($p<0,05$), IL-6 – to 4,99±0,64 pg/10^6 cells ($p<0,001$), and Ltr B_4 – to 3,18±0,33 pg/10^6 cells ($p<0,001$).

Conclusions. Thus, monocytes/macrophages, isolated from peripheral blood of menopausal women with MetS are in chronic activation condition, what could stimuli inflammation in different stages of atherogenesis. Quercetin in vitro normalized of IL-1β, Il-6 and Ltr B_4 production by monocytes/macrophages.

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Table

The spontaneous production of cytokines and chemokines by monocytes/macrophages in vitro

Parameter	Menopausal women with MetS, n=18	Control group, n=16	Validity
IL-1β, pg/10^6 cells	93,50±15,30	55,45±9,54	p<0,05
IL-6, pg/10^6 cells	6,76±0,77	2,66±0,26	p<0,01
Ltr B_4, pg/10^6 cells	6,71±0,94	3,93±0,79	p<0,05