Cardiovascular disease - related inflammatory markers in prisoners of war

- ***Anđelko Vidović (1),*** *Katja Gotovac (2), Ante Sabioncello (2), Ilija Kuzman (3), Neven Henigsberg (4), Mladen Lončar (5), Elvira Čeljuska (3), Ivana Dijanić Plašć (5), Sabina Rabatić (2), Dragan Dekaris (2)*
- *(1) University Hospital Dubrava, Department of Psychiatry, Zagreb, Croatia; (2) Institute of Immunology, Department for Cellular Immunology, Zagreb, Croatia; (3) University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia; (4) Croatian Institute for Brain Research, Zagreb, Croatia; (5) Ministry of Family Affairs, War Veterans and Intergenerational Solidarity, Zagreb, Croatia*

Introduction

There is growing evidence that individuals with posttraumatic stress disorder (PTSD) are at higher risk of developing coronary heart disease. PTSD can be regarded as a prolonged stress reaction with associated neuroendocrine alterations which could contribute to initia-tion and development of atherosclerosis, thereby leading to cardiovascular disease (CVD). Studies have shown that PTSD is linked with the chronic low-grade inflammation which could be associated with systemic vascular inflammation and atherogenesis. In line with these notions, we have measured seven inflammatory markers involved in pathogenesis of atherosclerosis in detainees that were imprisoned in war camps during the war in Croatia. Two of these markers are primarily related to platelet activation (sCD40L, sP-selectin) while MCP-1 and IL-8 represent chemokines responsible for recruitment of inflammatory cells to vessel walls. sVCAM-1 is a member of adhesion molecules responsible for firm adhesion of the cells to the vessel walls and tPA reflects fibrinolytic activity. IL-6 is one of the major pro-inflammatory cytokines that plays a significant role in virtually all stages of the inflammatory response (for details about these markers and recent findings in human chronic stress states please refer to the box at the bottom "Inflammatory markers").

Table 1. Sociodemographic, psychometric and medical data of the participants

		No PTSD (N=13)		PTSD (N=18)		Statistics	
		Mean	SD	Mean	SD	t	p
Age		46,8	10,0	49,8	14,1	0,657	0,516
Days in detention		126	119	147	117	0,509	0,614
Impact of Event Scale (IES)		50,0	21,1	61,3	9,9	2,003	**0,050**
Avoidance		16,3	8,8	18,8	5,3	0,971	0,339
Reexperiencing		18,9	8,0	24,9	6,4	2,311	**0,028**
Hyperarrousal		14,8	8,7	17,6	5,9	1,067	0,294
Los Angeles Symptom Checklist (LASC)		45,0	28,8	62,9	31,1	1,633	0,113
WHO-Five Well-being Index (WB)		13,7	4,7	9,6	5,0	2,327	**0,027**
The Major (ICD-10) Depression Inventory (MDI)		15,7	10,3	24,1	8,5	2,488	**0,018**
		N		N		χ^2	p
Married		13	100,0%	13	72,2%	4,306	**0,038**
Education	Elementary	1	7,7%	4	22,2%		
	Secondary	10	76,9%	13	72,2%	1,764	0,414
	University	2	15,4%	1	5,6%		
Work status	Retired	7	53,8%	15	83,3%		
	Employed	5	38,5%	1	5,6%	5,239	0,073
	Un-employed	1	7,7%	2	11,1%		
Economic status	Above average	6	46,2%	4	22,2%		
	Below average	2	15,4%	2	11,1%	2,542	0,281
	Average	5	38,5%	12	66,7%		
Major depression		0	,0%	3	16,7%	2,399	0,121
Classical CVD risk factors		4	30,8%	5	27,8%	0, 033	0,856
Medication use		7	53,8%	17	94,4%	7,117	**0,008**

Subjects and methods

A total of 31 male detainees imprisoned in war camps during 1991 and 1992 (detention lasting from 6 to 332 days) were examined in 2007 as a part of special medical program led by Croatian Government. All the participants underwent thorough clinical examination including psychiatric examination and psychometric testing. All the relevant demographic data were recorded and every participant was examined (including laboratory testing) for the presence of traditional CVD risk factors (smoking, hypertension, hyperlipdaemia, obesity, diabetes). For this purpose body mass index, blood preassure, blood glucose, and serum lipids levels were determined.

Citrated whole blood was collected by venipuncture followed by immediate plasma separation. Plasma concentrations of soluble CD40 ligand (sCD40L), soluble P-selectin, monocyte chemoattractant protein 1 (MCP-1), tissue-type plasminogen activator (t-PA), vascular cell adhesion molecule 1 (VCAM-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) were determined by FlowCytomix Human Cardiovascular 7plex kit (Bender MedSystems, Vienna, Austria), bead array system for simultaneous determination of multiple analytes by flow cytometry..

Results are summarized in tables 1-3.

Table 2. Comparison of inflammatory markers between groups

	No PTSD (N=13)		PTSD (N=18)		Statistics	
	Mean	SD	Mean	SD	t	p
sCD40L	643,89	115,59	742,40	167,30	1,827	**0,077**
sP-selectin	308,78	103,82	268,47	91,78	1,142	0,262
MCP-1	397,67	96,70	383,23	102,38	0,396	0,694
IL-8	69,23	148,18	7,89	19,29	1,747	0,091
sVCAM-1	1437,68	725,03	1302,92	429,73	0,648	0,521
tPA	1281,28	301,82	1099,34	251,89	1,820	**0,078**
IL-6	2,52	4,79	3,03	5,16	0,280	0,781

Table 3. Correlations between psychometric and biological data

	sCD40L	sP-selectin	MCP-1	IL-8	sVCAM-1	tPA	IL-6
Days in detention	,129	-,093	,048	-,105	-,220	-,121	-,297
	p=,488	p=,616	p=,795	p=,571	p=,234	p=,516	p=,104
IES (avoidance)	,198	-,032	,137	-,412	-,098	-,072	,170
	p=,284	p=,863	p=,461	p=,021	p=,598	p=,699	p=,360
IES (reexperiencing)	,211	-,067	-,199	-,289	-,321	-,142	-,097
	p=,253	p=,718	p=,282	p=,115	p=,078	p=,445	p=,603
IES (hyperarrousal)	,0222	-,078	-,221	,111	-,097	-,020	-,059
	p=,906	p=,674	p=,231	p=,552	p=,604	p=,915	p=,752
IES (total)	,189	-,085	-,129	-,262	-,230	-,106	-,001
	p=,307	p=,649	p=,486	p=,153	p=,212	p=,569	p=,997
LASC	,030	-,264	-,355	,010	-,349	-,201	-,248
	p=,869	p=,150	p=,050	p=,954	p=,054	p=,276	p=,177
WB	-,074	,087	,289	-,043	,220	,178	,280
	p=,692	p=,641	p=,114	p=,817	p=,233	p=,335	p=,126
MDI	,091	-,283	-,173	,070	-,279	-,124	-,196
	p=,625	p=,122	p=,352	p=,705	p=,128	p=,505	p=,290

INFLAMMATORY MARKERS

All of the below mentioned biomarkers have been shown to be elevated in acute coronary syndromes and independent risk markers of cardiovascular disease.

CD40 ligand (CD40L, CD154) is a 39-kD transmembrane glycoprotein structurally related to tumor necrosis factor-α (TNF-α), which was originally identified on stimulated CD4 positive T cells, mast cells, and basophils. Subsequently, it was demonstrated that also platelets carry preformed CD40L, which is rapidly translocated onto the cell surface following activation. CD40, the receptor for CD40L, is constitutively expressed on endothelial cells (ECs),monocyte/macrophages, smooth muscle cells (SMCs) and platelets. Platelets contribute at least 90% of the sCD40L in circulation, and in addition to traditional activation by ADP, thrombin, and collagen, platelets can also be activated through ligation of CD40 (Parikh and de Lemos 2006; Ferroni et al. 2007). It has been shown that patients with major depression had elevated sCD40L (Leo et al. 2006).

P-selectin is a C-type lectin that mediates adhesion (rolling and tethering) of platelets and leukocytes to the vascular surface. It is expressed on the surface of stimulated platelets and endothelial cells. It seems that the primary contributors to the formation of soluble P-selectin in the physiological state are platelets and thus baseline concentrations are mostly determined by platelet P-selectin shedding (Kappelmayer et al. 2004). Binding of platelets to dysfunctional endothelium results in platelet activation, and rapid translocation of the adhesion molecule P-selectin from platelet granules to the cell surface. Platelet P-selectin binds to the leukocyte receptor, P-selectin glycoprotein ligand-1 (PSGL-1), leading to the formation of PLAs, thus promoting leukocyte recruitment to the endothelium (Brydon et al. 2006). Elevated plasma levels of sP-selectin have been demonstrated in major depression (Leo et al. 2006; Wirtz et al. 2008).

Monocyte chemoattractant protein-1 (MCP-1) is the primary chemokine responsible for the recruitment of monocytes into the developing atheroma. MCP-1 is produced by multiple types of cells in the atheroma, including smooth muscle cells, monocytes/macrophages, and endothelial cells. Monocytes/macrophages are an integral component in the initiation, progression, and complications of coronary atherosclerosis. CCR-2 receptors expressed on monocytes recognize MCP-1, initiating a chemoattractant signal for monocytes/macrophages to the vessel wall in the atherogenic process(Parikh and de Lemos 2006). High levels of MCP-1 have been reported previously in patients with major depression (Rajagopalan et al. 2001; Sutcigil et al. 2007; Piletz et al. 2008) and women under psychosocial stress (Asberg et al. 2009).

Interleukin 8 (IL-8, CXCL8) activates chemokine receptors CXCR1 and CXCR2 and contribute considerably to monocyte recruitment. Although IL-8 has initially been linked to neutrophil activation and migration, it also appeared to be a potent mediator of firm adhesion of monocytes to the vascular endothelium under flow conditions and thus is deemed to be a key determinant in the initiation of atherogenesis (Kraaijeveld et al. 2007). Some studies show higher circulating levels of IL-8 in depressed patients (Song et al. 1998) and lower levels in PTSD patients (Song et al. 2007).

Vascular cell adhesion molecule-1 (VCAM-1) is a member of immunoglobulin superfamily adhesion molecules. It mediates firm leucocyte adhesion on the vascular endothelium through binding to activated leucocyte integrins. Under appropriate pro-inflammatory conditions where the endothelium is exposed to inflammatory cytokines such as tumour necrosis factor-a or IL-1b and becomes activated, gene expression is rapid leading to expression of VCAM-1 by the vascular endothelium. VCAM-1 is eventually released from the endothelial cell surface by proteolytic cleavage allowing measurement of soluble VCAM-1 (Preiss and Sattar 2007). Studies in patients with major depression are inconclusive (Rajagopalan et al. 2001; Dimopoulos et al. 2006; Thomas et al. 2007). Nevertheless, lowering of sVCAM-1 was associated with the treatment of depressed patients with SSRIs (Lekakis et al. 2008).

Tissue-type Plasminogen Activator (t-PA) is a serine protease that can convert the inactive proenzyme plasminogen to the active protease plasmin. Plasmin can degrade fibrin, the matrix of a blood clot in a process known as fibrinolysis, leading to dissolution of the clot. t-PA is primarily synthesized and secreted by the vascular endothelium. It has a short half-life (6 minutes) in the blood and circulates in trace concentrations in plasma (Ridker et al. 2004). t-PA antigen concentrations indicate an activation of the fibrinolytic system or a complex formation with the inhibitor (PAI-1). Therefore, increased levels of t-PA can indicate enhanced as well as decreased fibrinolytic activity (if t-PA/PAI-1 complex is elevated). t-PA tends to be elevated after acute stress without change in its inhibitor PAI-1, consistently with findings of increased fibrinolytic capacity triggered by acute mental stressors (von Känel et al. 2001). Impaired fibrinolysis was found in chronic stress models (i.e. low activity of t-PA in job stress) (von Känel et al. 2001). tPA-plasminogen system may be implicated in MDD pathogenesis through the BDNF/proBDNF pathway (Hou et al. 2009). Patients with depression showed significantly lower plasma tPA concentrations than healthy persons (Pietraszek et al. 1991).

Interleukin-6 (IL-6) is a multi-functional cytokine that regulates immune responses, acute phase reactions and hematopoiesis and is produced by a variety of immune cells. As much as third of total circulating concentrations of IL-6 originate from adipose tissue. IL-6 might play a key role in the development of coronary disease through a number of different mechanisms; metabolic, endothelial and coagulant. In parallel to increased plasma cortisol levels, increased plasma concentrations of IL-6 have been reported in depression (Alesci et al. 2005; Maes et al. 1993). Similarly, the majority of the studies in adults with PTSD have reported increased levels of circulating pro-inflammatory cytokines including IL-6 (Maes et al. 1999; Pervanidou et al. 2007; Rohleder et al. 2004; Sutherland et al. 2003; von Kanel et al. 2007), as well as increased levels of CSF IL-6 (Baker et al. 2001).

Summary and conclusions

- Plasma concentrations of sCD40L tend to be higher, while concentrations of tPA tend to be lower in PTSD subjects indicating higher platelet activation and decreased fibrinolytic activity. Possibly decreased tPA levels may be important because the recent evidence suggests that the tPA-plasminogen system is important for neuronal plasticity and survival through the BDNF/proBDNF pathway.
- Negative correlation of the PTSD-related symptoms with the plasma concentration of chemokines (IL-8, MCP-1) suggests possible involvement of psychological factors in regulation of chemotaxis during inflammation.
- Although not all of the subjects met criteria for PTSD, a high levels of PTSD related symptoms were present in all participants. Participants with PTSD had more pronounced symptoms of depression including 3 participants with major depression. Inclusion of the healthy control group, without war camp experience and major depression, is needed to test the specific influence of war camp-related experience on measured markers. This would also possibly explain our finding of generally high levels of sVCAM in all participants (much higher than reference values or those found in other studies).
- In this preliminary study we have identified all the problems related to the method used for determination of inflammatory markers (sample collection, processing and storing, flow cytometry). We have also roughly identified expected changes in measured markers to determine sample size for future analysis. Final study will include 70 participants with the inclusion of the healthy control group. The analysis will be based on regression model identifying predictors associated with CVD inflammatory markers in prisoners of war.