Serum levels of TRAIL OPG and RANKL in patients with severe heart failure

Nivele serice ale TRAIL, OPG ŞI RANKL la bolnavi cu insuficiență cardiacă severă

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Abstract

Background. *Inflammation and apoptosis are associated with the progressive deterioration of left ventricular function. Osteoprotegerin (OPG) is a soluble member of the TNF receptor superfamily. The activity best characterized for OPG is inhibition of receptor activator of nuclear factor-kappa B ligand (RANKL). OPG can also interact with TNF-related apoptosis-inducing ligand (TRAIL). OPG may exert direct biological activities independent of its neutralizing effect towards TRAIL and RANKL. The physiologic role of TRAIL may be tissue specific and more complex than initially assumed. TRAIL – mediated apoptosis may be involved in cardiomyocyte apoptosis in chronic heart failure. Both soluble RANKL and OPG may induce the activation of matrix metalloproteinases (MMPs) involved in ventricular remodeling. TRAIL has also endothelial protective properties. The role of the interaction between OPG and TRAIL with potential antiapoptotic effects has not been well studied. The objective of the study was to investigate the relationship between OPG, RANKL and TRAIL in patients with ischemic cardiomyopathy and chronic heart failure.* ***Material and methods.*** *In 23 patients with ischemic cardiomyopathy and severe heart failure (NYHA III and IV, LVEF = 0.25 ± 0.1), 10 patients with Killip II class acute myocardial infarction (AMI), and 11 controls (healthy volunteers) we measured TRAIL (ELISA assay), OPG (ELISA assay), RANKL (ELISA assay), NT proBNP (ELISA assay). Values (pg/ml) are expressed as mean ± SEM.* ***Results.*** *As compared with controls, in patients with ischemic cardiomyopathy serum levels of TRAIL are significantly decreased (82.6 ± 7.53pg/ml vs 103 ± 7.98; p < 0.05), while serum levels of OPG are increased in both groups (in ischemic cariomyopathy: 137.74 ± 19.81pg/ml vs. 60.18 ± 5.06; p < 0.006; in AMI: 129.8 ± 20.2 pg/ml vs. 60.18 ± 5.06; p < 0.0008). No differences in serum levels of OPG, RANKL and TRAIL were found between the two study groups. Positive correlations between serum levels of OPG and the age of the patients, and negative correlations between serum levels of TRAIL and OPG (r = - 0.574; p < 0.01) and serum levels of TRAIL and RANKL (r = - 0.458; p < 0.05) were found.* ***Conclusions.*** *In patients with heart failure in the same etiological context, ischemic cardiomyopathy and acute myocardial infarction, the patterns of TRAIL, OPG and RANKL serum levels are different. No correlations between serum levels of TRAIL, OPG and RANKL or with the LVEF were found.*

Keywords: *TRAIL, OPG, heart failure*

Rezumat

Inflamaţia şi apoptoza sunt asociate cu deteriorarea progresivă a funcţiei ventriculului stâng. Osteoprotegerin (OPG) este un membru solubil al superfamiliei receptorilor TNF. Inhibiţia RANKL (receptor activator of nuclear factor-kappa B ligand) este activitatea cea mai bine caracterizată a OPG. OPG are şi alte activităţi biologice independent

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de efectele de neutralizare ale TRAIL şi RANKL. Se apreciază că rolul fiziologic al TRAIL este specific pentru ţesut şi mult mai complex decât s-a crezut iniţial. În insuficienţa cardiacă cronică TRAIL poate media apoptoza în cardiomiocite. Formele solubile ale RANKL şi ale OPG pot induce activarea metaloproteazelor membranare (MMP) implicate în remodelarea ventriculară. TRAIL are şi proprietăţi de protecţie endotelială. ***Pacienţi şi Metode.*** *S-au determinat nivelele serice ale TRAIL (ELISA), OPG (ELISA), RANKL (ELISA), NT-proBNP (ELISA) la 23 bolnavi cu cardiomiopatie ischemică şi insuficienţă cardiacă severă (NYHA III şi IV; FEVS = 0,25 ±0,1), la 10 bolnavi cu infarct miocardic acut şi insuficienţă cardiacă (FEVS = 0,4 ±0,03) şi la un lot de control (11 voluntari sănătoşi). Valorile sunt exprimate ca medie ±SEM.* ***Rezultate.*** *Comparativ cu lotul de control, în grupul bolnavilor cu cardiomiopatie ischemică nivelele serice ale TRAIL sunt semnificativ statistic mai scăzute (82,6 ±7,53 vs 103 ±7,98; p < 0,05), în timp ce nivelele serice ale OPG sunt semnificativ mai crescute (137,74 ±19,81 vs 60,18 ±5.06; p < 0,006). Am găsit o corelaţie inversă între nivelele serice ale TRAIL şi OPG (r = - 0,574; p < 0,01) şi între nivele serice ale TRAIL şi RANKL (r = - 0,458; p< 0,05).*

Cuvinte cheie: *TRAIL,OPG, insuficienţă cardiacă*

Introduction

It has been shown that calcification in intimal atherosclerotic lesions is not merely a passive consequence of chronic vascular inflammation but an active process that may lead to the progression of the atherosclerotic plaques (1).

The link between bone and vascular biology begins in the embryo and remains in the late adulthood. Many of the genes activated during hematopoietic development are also expressed in the vascular endothelium. In the adult, bone-marrow-derived circulating endothelial progenitors contribute to postnatal neo-vascularization and enhance vascular repair following ischemic injury. Osteoporosis occurs simultaneously with atherosclerosis, raising important questions about the mechanisms involved (2).

Proteins initially characterized in bone, including osteopontin, osteocalcin, RANKL, OPG are also present in atherosclerotic plaque. Soluble RANKL induced MMP activity in vascular smooth muscle cells (3). In addition to its enhancing effects on RANKL, OPG also had MMP-inducing effects on its own (4). OPG expression in higher levels in symptomatic compared to asymptomatic plaques of carotid arteries could potentially reflect its ability to enhance MMP activity with and without RANKL co-stimulation. High serum levels of OPG are associated with symptomatic vascular stenosis compared with stable atherosclerotic disease and long term prognosis (4 - 7).

Chronic heart failure progresses because of activation of neurohormones and pro-inflammatory cytokines following an initial cardiac injury or a mutation of the genetic program (8). Apoptosis plays a crucial role in the pathogenesis and progression from left ventricular dysfunction to heart failure (9,10). Ischemic cardiomyopathy is associated with apoptosis (11). TRAIL is a specific inducer of apoptosis. The balance between pro- and anti-apoptotic effects of TRAIL is influenced by the presence of OPG. A strong inverse association of sTRAIL with mortality underlines the clinical importance of TRAIL. In patients with heart failure sTRAIL appears to be protective (12). OPG and TRAIL are more studied in patients with acute myocardial infarction.

The objective of this study was to investigate the relationship between OPG, RANKL and TRAIL in patients with ischemic cardiomyopathy and chronic heart failure.

Material and methods

Patients:

- 23 patients with ischemic cardiomyopathy and severe heart failure. The admission criteria: NT-proBNP > 2000 pg/ml, left ventricular ejection fraction (LVEF) < 0.35.
- 10 patients with acute myocardial infarction tip 1. The admission criteria: precordial pain > 20 minutes, acute changing in the ST-T wave forms, elevated levels of cTnI > 99th percentile of URL (according to the universal definition of acute

Table1. Baseline Characteristics of the Study Patients

	Ischemic cardiomyopathy	**Acute myocardial infarction**	***p***
Gender: m (%)	17/23 (73.91%)	6/10 (60%)	NS
Age, y	57.26 ± 4.29	64.2 ± 4.56	0.03
Diabetes mellitus	6/23 (26.09%)	3/10 (30%)	NS
Hypertension :systolic blood pressure > 140 and/or diastolic blood pressure > 90 mmHg	6/23 (26.09%)	6/10 (60%)	NS
Total cholesterol > 180 mg/dl	16/23 (69.5%)	6/10 (60%)	NS
Triglicerides > 150 mg/dl	6/23 (26.09%)	4/10 (40%)	NS
Smoking: curent, previous	10/23 (43.4%)	4/10 (40%)	NS
LVEF	0.25 ± 0.1	0.4 ± 0.03	< 0.0001

Table 2. Serum levels of TRAIL, OPG and RANKL

	TRAIL (mean ± SEM) pg/ml	**OPG (mean ± SEM) pg/ml**	**RANKL (mean ± SEM) pg/ml**
Controls (n =11)	103.86 ± 7.98	60.18 ± 5.06	2.04 ± 0.31
AMI with heart failure (n = 10) *Statistical significance*	91.62 ± 24.6 *vs controls, NS*	129.8 ± 20.2 *vs controls, p < 0.0008*	5.06 ± 2.57 *vs controls, NS*
Cardiomyopathy with heart failure (n = 23) *Statistical significance*	82.6 ± 7.53 *vs controls, p < 0.05* *vs AMI, NS*	137.74 ± 19.81 *vs controls, p < 0.006* *vs AMI, NS*	19.78 ± 7.95 *vs controls, NS* *vs AMI, NS*

NS = not significant

myocardial infarction), in the Killip II class.

- 11 controls (healthy volunteers)

The exclusion criteria were: chronic inflammatory diseases, cancer, bone disease, acute coronary syndrome in the previous 6 months.

Methods:

The parameters were analyzed from venous blood samples obtained in the morning of the day of the admission in hospital. OPG, RANKL and TRAIL were measured with colorimetric sandwich ELISA (R&D System), sensitivity: for OPG: 4.5 pg/ml, range: 31.2 - 2000pg/ml; for RANKL: < 5pg/ml, range 31.2 - 2000pg/ml; for TRAIL < 7.87 pg/ml, range 15.6 - 1000pg/ml.

Statistical analyses

Data were calculated as mean ± SEM. Comparisons between groups were performed with Student's t test and Fisher test. Differences were considered statistically significant when $p < 0.05$. Correlations were tested by Spearman's correlation coefficient.

Results

The LVEF was higher (0.4 ± 0.03) in patients with Killip II class acute myocardial infarction as compared with the patients with ischemic cardiomyopathy and chronic heart failure (0.25 ± 0.1; NYHA III and IV) ($p < 0.0001$) (*Table 1*). No correlation between LVEF and TRAIL, OPG and RANKL serum levels were found.

As compared to controls: serum levels of TRAIL are significantly decreased only in the group with cardiomyopathy (82.6 ± 7.53 vs. 103.86 ± 7.98; $p < 0.05$), serum levels of OPG are increased in acute myocardial infarction

Table 3. The OPG/TRAIL, RANKL/TRAIL, OPG/RANKL ratio

	OPG/ TRAIL	RANKL/ TRAIL	OPG / RANKL
Controls (n =11)	0.6 ± 0.08	0.02 ± 0.004	33.55 ± 4.11
AMI with heart failure (n = 10)	1.97 ± 0.36	0.13 ± 0.08	88.29 ± 45.03
Statistical significance	*vs controls, p < 0.0004*	*vs controls, NS*	*vs controls, NS*
Cardiomyopathy with heart failure (n = 23)	4.25 ± 2.05	0.58 ± 0.32	37.27 ± 9.28
Statistical significance	*vs controls, NS* *vs AMI, NS*	*vs controls, NS* *vs AMI, NS*	*vs controls, NS* *vs AMI, NS*

NS = not significant

(129.8 ± 20.2 vs. 60.18 ± 5.06; p < 0.0008) and in ischemic cardiomyopathy (137.74 ± 19.81 vs. 60.18 ± 5.06; p < 0.006) (*Table 2*), the OPG/TRAIL ratio is significantly increased only in patients with acute myocardial infarction (1.97 ± 0.36 vs. 0.6 ± 0.08; p < 0.0004) (*Table 3*). Positive correlations between serum levels of OPG and the patients' age were found (in ischemic cardiomyopathy: r = 0.44; p<0.05; in AMI: r = 0.68; p<0.05) (*Figure 1*; *Figure 2)*.

In patients with cardiomyopathy and NT proBNP > 2000pg/ml negative correlations between serum levels of TRAIL and OPG (r = - 0.574, p < 0.01) (*Figure 3*) and serum levels of TRAIL and RANKL (r = - 0.458; p < 0.05) (*Figure 4*) were found.

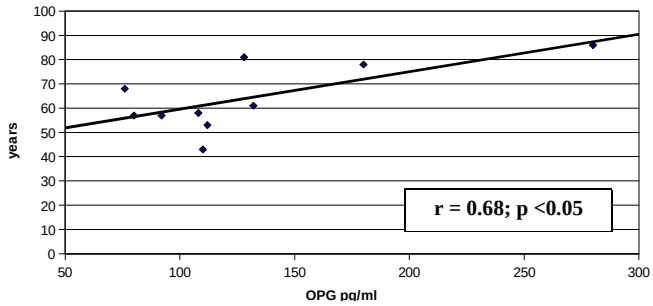

Figure 1. Correlation between serum levels of OPG and the age of patients with AMI

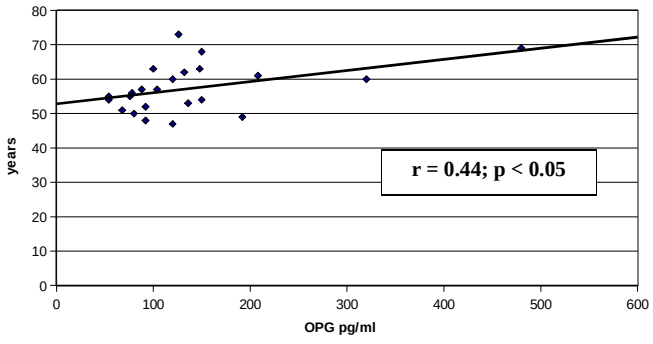

Figure 2. Correlation between serum levels of OPG and the age of the patients with ischemic cardiomyopathy

Discusions

Heart failure is a syndrome resulting from any structural or functional cardiac disorder that impairs the ability of the heart to support the physiological circulation. Despite advances in the understanding and treatment of heart failure, it has a poor prognosis. For this reason there is an increasing interest in the development of new biomarkers.

TRAIL particularly plays an important role in development of plaque rupture, acute myocardial infarction and heart failure (13). TRAIL is member of the TNF superfamily ligands and is expressed as a type II membrane protein. TRAIL has been shown to bind any one of five cognate receptors (TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4 and osteoprotegerin). TRAIL-R1 and TRAIL-R2 are commonly referred to as the death receptors for their well characterized activity of transducing apoptotic signals. TRAIL –R1 and TRAIL-R2 as well as other members of the TNF receptor family showing intra-cytoplasmatic death domains are also involved in non-apoptotic func-

tion. TRAIL-R3 was originally proposed as acting mainly as an antagonizing decoy receptor for the domain containing TRAIL receptors. TRAIL-R4 acts as regulatory rather than decoy receptor that inhibits apoptosis signaling by TRAIL. Circulating TRAIL levels that are clearly detected in the plasma of normal individuals may be involved in regulation of endothelial cell function (14).

It was shown that TRAIL has an anti-inflammatory role on human endothelial cells. Recombinant TRAIL activates the ERK/MAPK and the AKT/eNOS intracellular pathways when added in endothelial cell cultures. The activation of these pathways by TRAIL was associated with the induction of endothelial cell proliferation and release of nitric oxide with its vaso-relaxation and anti-inflammatory effects (14).

The activity best characterized for OPG is the inhibition of the receptor activator of the NF-kB ligand (RANKL). OPG can also interact with TRAIL showing a comparable affinity to that of transmembrane TRAIL-R3 and TRAIL-R4. OPG has been shown to act in a paracrine and autocrine manner by binding TRAIL and promoting survival cells (14).

It has been proposed that OPG induces endothelial cell survival by blocking the interaction of TRAIL endogenously produced by endothelial cells and TRAIL-R1 or TRAIL-R2 on serum and extracellular matrix-starved endothelial cells. OPG may exert direct biological activities independent of neutralizing effects toward TRAIL or RANKL. Recombinant OPG inhibits the proliferation of vascular smooth muscle cells while promoting monocyte locomotion in vitro. TRAIL does not affect the migration of monocytes but counteracts their adhesion to endothelial cells, showing anti-inflammatory potential in vitro.

The ability of recombinant OPG to enhance the recruitment and infiltration of monocyte/macrophages suggests that abnormal elevation of serum OPG may be involved in endothelial cells dysfunction (15). Monocytes are able to stimulate vascular smooth cells mineralization, a key feature of atherosclerosis, once they have migrated to the subendothelium region (16).

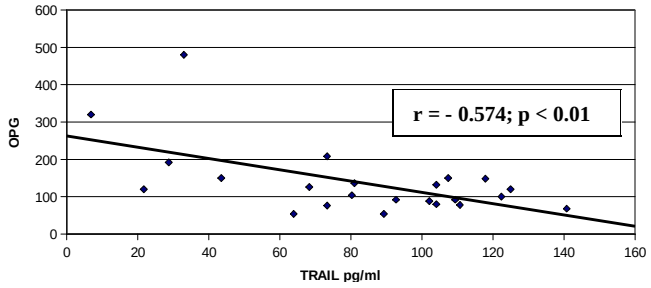

Figure 3. Correlation between serum levels of TRAIL and OPG in patients with cardiomyopathy and NT-proBNP > 2000 pg/ml

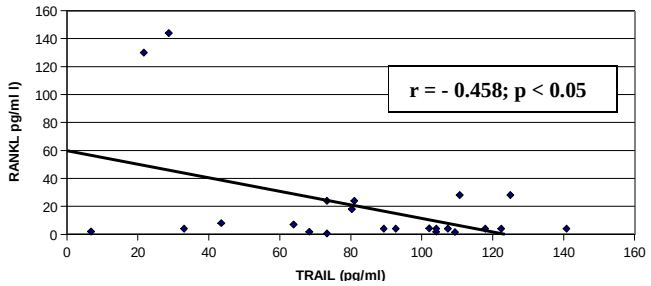

Figure 4. Correlation between serum levels of TRAIL and RANKL in patients with cardiomyopathy and NT-proBNP > 2000 pg/ml

OPG is produced in many tissues including lung, cardiovascular system, kidney, intestine, and bone (3). OPG is produced in the normal artery wall and in cultured arterial cells such as coronary smooth muscle cells and endothelial cells. RANKL and RANK are not expressed in normal artery wall but they are expressed in calcified arteries of OPG deficient mice, and they co-localize with osteoclast-like cells.

Increased serum levels of OPG and RANKL were found in patients with severe atherosclerotic lesions, acute myocardial infarction and heart failure. Increased serum levels of OPG and RANKL were significantly correlated with functional, hemodynamic and neurohormonal parameters for disease severity. Enhanced systemic expression of RANKL was accompanied by in-

creased expression of the RANK in cardiomyocytes. Enhanced systemic and myocardial expression of the OPG/RANKL/RANK system in heart failure suggests a possible role for known mediators of bone homeostasis in the pathogenesis of heart failure (3,17).

Patients with CAD have higher OPG serum levels than healthy and the relative risk of cardiovascular mortality is increased by 3- to 4-fold in patients with high serum levels (5,18). OPG has been reported to predict survival in patients with heart failure after acute myocardial infarction, to predict hospitalization and mortality in patients with acute coronary syndrome (19, 20).

In a previous study (21), unlike in other studies, we did not find a significant decrease of the TRAIL serum levels in acute myocardial infarction. The high OPG levels and the correlation between serum OPG or the OPG/RANKL ratio and the patients' age corresponded to the data in literature.

In this study we set out to find whether in the same etiological context, ischemic cardiomyopathy and acute myocardial infarction, the patterns of TRAIL, OPG and RANKL serum levels are different. The left ventricular function is the most severely affected in the patients with cardiomyopathy and chronic heart failure. No correlation between serum levels of TRAIL, OPG and RANKL and the LVEF were found.

Serum TRAIL levels were decreased in both patient groups, reaching statistical significance in comparison to controls only in the patients with ischemic cardiomyopathy and chronic failure with no differences between ischemic cardiomyopathy and AMI. Serum OPG levels were significantly increased in both groups as compared to controls, with no differences between the two study groups. In both group we found positive correlations between serum levels of OPG and patients' age. The increase of the OPG/TRAIL ratio is due to a more marked increase of the OPG than the TRAIL decrease. In ischemic cardiomyopathy with heart failure increased OPG was accompanied by the increase of RANKL, thus the OPG/RANKL ratio had similar average values to the control group. The OPG/RANKL ratio was higher, though not statistically significant, in acute myocardial infarction.

In the same etiological context, Killip II AMI and cardiomyopathy with chronic heart failure, there are different patterns of the serum TRAIL, OPG and RANKL. Regardless of the patient group, TRAIL levels decreased, while OPG and RANKL increased. In chronic heart failure, with a significantly lower LVEF, the decrease of TRAIL and increase of OPG are more marked. The pattern of the OPG/RANKL ratio in the patients with ischemic heart disease reflects in fact that OPG provides only a partial protection against the deleterious effects of RANKL.

Although OPG is a known inhibitor of RANKL, its biological effect may depend on the molar ratio between RANKL and OPG (3). Under high OPG/RANKL ratios OPG attenuates RANKL-mediated effects; in low OPG/RANKL ratios it has been found to enhance RANKL-mediated effects on MMP levels in vascular smooth muscle cells. A reliable biomarker in CVD is not necessarily an important mediator in the disease but rather a stable marker of up-stream pathways that are involved in the pathogenesis of CVD. Thus, because OPG circulates at much higher levels than RANKL, the role of OPG as a marker in CVD may not be related to its role as a mediator but reflects its role as a stable marker of activity in the RANKL/OPG/RANK axis (22).

Conclusion

In patients with heart failure, in the same etiological context - ischemic cardiomyopathy and acute myocardial infarction - the patterns of TRAIL, OPG and RANKL serum levels are different. No correlations between serum levels of TRAIL, OPG and RANKL or with the LVEF were found.

This study cannot identify the origin of the increase of OPG or TRAIL. It is unclear which of the two markers has a pathogenetic or a compensatory role. The limitations of this study are due to the small number of patients included.

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