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Influence of the Administration of a TNF-α Inhibitor on the Oxidant/Antioxidant Balance in Chronic Venous Insufficiency

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Abstract
Purpose: many studies in experimental models have evidenced the presence of inflammation in venous vascular dysfunction and the implication of TNF-α in vascular pathology, through its prooxidant and proinflammatory effect. Starting from these studies, we experimentally investigated the effect of treatment with a TNF-α inhibitor, etanercept, on the oxidant/antioxidant balance in rats, with the partial obstruction of the common femoral vein. *Methods*: the researches were carried out in 7 groups of animals (n=10 rats/group), 6 groups undergoing surgery and a control group. In 3 groups, the ligation of the common femoral vein was performed (groups 1, 2, 3), 3 groups underwent the ligation of the common femoral vein and received etanercept (groups 4, 5, 6), in a dose of 1 mg/kg, one dose per week, according to data found in literature. The serum indicators of the O/AO balance were determined: indicators for oxidative stress (malondialdehyde (MDA), protein carbonyls (PC)), indicators for non-enzymatic AO defense (thiol groups (SH), hydrogen donors (HD), glutathione (GSH)) at weeks 1, 2 and three. *Results and Conclusions*: chronic venous insufficiency with and without anti-TNF-α treatment induced changes in the oxidant/antioxidant balance compared to the control group; after the administration of the anti-TNF-α preparation, oxidative stress was maintained on account of MDA that increased significantly, and decreased significantly on account of PC compared to the untreated groups; after the administration of the anti-TNF-α preparation, at two and three weeks there was an insignificant increase in antioxidant defense on account of HD and GSH compared to the untreated groups; the administered anti-TNF-α preparation had late and insignificant effects in chronic venous insufficiency.

Keywords: Oxidative stress; Chronic venous insufficiency; Antioxidants.

Introduction

TNF-α is a major cytokine with a polypeptide structure, produced by endothelial cells, tumor cells, vascular smooth muscle fibers, mast cells, NK cells, T and B cells and, predominantly, by activated macrophages. It has multiple roles: proinflammatory, angiogenetic, proapoptotic, prooxidant, proatherogenic [1].

Many studies in experimental models have evidenced the presence of inflammation in venous vascular dysfunction and the implication of TNF-α in vascular pathology [2-4], through its

prooxidant and proinflammatory effect [1,5]. On the other hand, reactive oxygen and nitrogen species are involved in the regulation of immune response [6]. Starting from these studies, we experimentally investigated the effect of treatment with a TNF-α inhibitor, etanercept, on the oxidant/antioxidant balance in rats, with the partial obstruction of the common femoral vein.

Etanercept is the soluble dimeric form of the TNF-α receptor. The anti-inflammatory effects of etanercept are due to its ability to bind TNF, preventing in this way the interaction of TNF with cell surface receptors. Etanercept can modulate biological responses induced and mediated by TNF-α, including the expression of adhesion molecules responsible for the migration of leukocytes, serum cytokine levels and matrix metalloproteins [7].

Objectives

Serum changes in the oxidant/antioxidant balance were dynamically studied in animals in which venous insufficiency was induced by the partial ligation of the common femoral vein, with and without anti-TNF-α treatment protection.

Material and Method

Groups

The researches were carried out in 7 groups of animals (n=10 rats/group), 6 groups undergoing surgery and a control group.

In 3 groups, the ligation of the common femoral vein was performed (groups 1, 2, 3), 3 groups underwent the ligation of the common femoral vein and received etanercept (groups 4, 5, 6), in a dose of 1 mg/kg, one dose per week, according to data found in literature [5].

Groups were defined as follows:

- G 0 – control group
- G 1 – CFV ligation, harvesting at one week
- G 2 – CFV ligation, harvesting at two weeks
- G 3 – CFV ligation, harvesting at three weeks
- G 4 – CFV ligation, harvesting at one week, etanercept administration
- G 5 – CFV ligation, harvesting at two weeks, etanercept administration
- G 6 – CFV ligation, harvesting at three weeks, etanercept administration

Male Wistar rats with a weight between 150 and 250 g, from the UMPh biobase, were used for the experimental model.

Under general anesthesia, an incision was performed on the anterior side of the thigh, the common femoral vascular nervous bundle was identified, the common femoral vein was isolated and partially ligated, using a guide (prolene thread 4-0).

Biochemical Methods

The serum indicators of the oxidant/antioxidant balance were measured in the Laboratory for the Study of Oxidative Stress of the Department of Physiology of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca.

The serum indicators of the O/AO balance were determined:

- Indicators for oxidative stress: malondialdehyde (MDA) [8]; protein carbonyls (PC) [9]
- Indicators for non-enzymatic AO defense- thiol groups (SH) [10]: hydrogen donors (HD) [11], glutathione (GSH) [10]

The time moments for harvesting and sacrificing were: T1 – week 1 (group 1 and group 4), T2 – week 2 (group 2 and group 5), T3 – week 3 (group 3 and group 6).

Statistical Methods

The data resulting from the study were processed using the SPSS medical statistics program (version 13.0) and Microsoft EXCEL. Normality of data was investigated using the Kolmogorov-Smirnov test; depending on the result of this test, we applied Student's t test and ANOVA variance analysis with post-hoc multiple comparison analysis (Scheffe test) for normally distributed variables, or the Kruskall-Wallis and U Mann-Whitney tests for non-normally distributed variables. We interpreted the two-tailed p value, using a significance threshold alpha at 0.05.

Results

Descriptive and analytical results regarding the investigated serum indicators of the O/AO balance are presented in Tables 1-5.

Table 1. Descriptive and analytical results regarding MDA levels

Group A-Group B	Group A-Group B						
	Mean	StDev	95%CI	Mean	StDev	95%CI	P
G 0 - G 1	1.26	0.31	(1.038; 1.48)	1.15	0.11	(1.07; 1.22)	0.912
G 0 - G 2				1.23	0.85	(0.62; 1.84)	0.406
G 0 - G 3				2.52	0.43	(2.218; 2.8	0.000
G 0 - G 4				2.06	0.25	(1.80; 2.32)	0.003
G 0 - G 5				2.06	0.25	(1.80; 2.32)	0.995
G 0 - G 6				2.63	0.79	(1.82; 3.49)	0.002
G 1 - G 2	1.15	0.11	(1.07; 1.22)	1.23	0.85	(0.62; 1.84)	0.001
G 1 - G 3				2.52	0.43	(2.218; 2.8	0.000
G 1 - G 4				2.06	0.25	(1.80; 2.32)	0.001
G 1 - G 5				2.06	0.25	(1.80; 2.32)	0.001
G 1 - G 6				2.63	0.79	(1.82; 3.49)	0.001
G 2 - G 3	1.23	0.85	(0.62; 1.84)	2.52	0.43	(2.218; 2.8)	0.002
G 2 - G 4				2.06	0.25	(1.80; 2.32)	0.013
G 2 - G 5				2.06	0.25	(1.80; 2.32)	0.009
G 2 - G 6				2.63	0.79	(1.82; 3.49)	0.007
G 3 - G 4	2.52	0.43	(2.218; 2.8319)	2.29	0.4	(1.86; 2.73)	0.310
G 3 - G 5				2.06	0.25	(1.80; 2.32)	0.03
G 3 - G 6				2.63	0.79	(1.82; 3.49)	0.673
G 4 - G 5	2.29	0.4	(1.86; 2.73)	2.06	0.25	(1.80; 2.32)	0.256
G 4 - G 6				2.63	0.79	(1.82; 3.49)	0.347
G 5 - G 6	2.06	0.25	(1.80; 2.32)	2.63	0.79	(1.82; 3.49)	0.109

StDev = standard deviation;G 0 = control group; G 1 = CFV ligation, harvesting at one week; G 2 = CFV ligation, harvesting at two weeks; G 3 = CFV ligation, harvesting at three weeks; G 4 = CFV ligation, harvesting at one week, etanercept administration; G 5 = CFV ligation, harvesting at two weeks, etanercept administration; G 6 = CFV ligation, harvesting at three weeks, etanercept

Table 2. Descriptive and analytical results regarding PC levels

Group A-Group B	Group A-Group B						
	Mean	StDev	95%CI	Mean	StDev	95%CI	p
G 0 - G 1	0.74	0.06	(0.69; 0.78)	1.97	0.44	(1.65; 2.28)	**0.000**
G 0 - G 2				2.25	0.46	(1.92; 2.58)	**0.000**
G 0 - G 3				1.38	0.19	(1.25; 1.51)	**0.000**
G 0 - G 4				1.04	1.147	(0.89; 1.2)	**0.002**
G 0 - G 5				1.02	0.26	(0.74; 1.3)	**0.003**
G 0 - G 6				1.127	0.26	(0.85; 1.40)	**0.001**
G 1 - G 2	1.97	0.44	(1.65; 2.28)	2.25	0.46	(1.92; 2.58)	1
G 1 - G 3				1.38	0.19	(1.25; 1.51)	**0.000**
G 1 - G 4				1.04	1.147	(0.89; 1.2)	**0.01**
G 1 - G 5				1.02	0.26	(0.74; 1.3)	0.002
G 1 - G 6				1.127	0.26	(0.85; 1.40)	**0.002**
G 2 - G 3	2.25	0.46	(1.92; 2.58)	1.38	0.19	(1.25; 1.51)	**0.000**
G 2 - G 4				1.04	1.147	(0.89; 1.2)	**0.03**
G 2 - G 5				1.02	0.26	(0.74; 1.3)	**0.002**
G 2 - G 6				1.127	0.26	(0.85; 1.40)	**0.002**
G 3 - G 4	1.38	0.19	(1.25; 1.51)	1.04	1.147	(0.89; 1.2)	**0.993**
G 3 - G 5				1.02	0.26	(0.74; 1.3)	**0.013**
G 3 - G 6				1.127	0.26	(0.85; 1.40)	**0.065**
G 4 - G 5	1.04	1.147	(0.89; 1.2)	1.02	0.26	(0.74; 1.3)	**0.423**
G 4 - G 6				1.127	0.26	(0.85; 1.40)	**0.873**
G 5 - G 6	1.02	0.26	(0.74; 1.3)	1.127	0.26	(0.85; 1.40)	**0.337**

StDev = standard deviation; G 0 = control group; G 1 = CFV ligation, harvesting at one week; G 2 = CFV ligation, harvesting at two weeks; G 3 = CFV ligation, harvesting at three weeks; G 4 = CFV ligation, harvesting at one week, etanercept administration; G 5 = CFV ligation, harvesting at two weeks, etanercept administration; G 6 = CFV ligation, harvesting at three weeks, etanercept

Table 3. Descriptive and analytical results regarding HD levels

Group A-Group B	Group A-Group B						
	Mean	StDev	95%CI	Mean	StDev	95%CI	p
G 0 - G 1	37.17	5.6	(33.156; 41.184)	28.91	2.56	(26.61; 30.27)	**0.002**
G 0 - G 2				20.465	6.42	(15.87; 25.057)	**0.000**
G 0 - G 3				23.48	3.89	(20.697; 26.27)	**0.001**
G 0 - G 4				23.43	5.7	(17.44; 29.41)	**0.002**
G 0 - G 5				26.05	2.79	(23.124; 28.98)	**0.02**
G 0 - G 6				27.168	7.74	(19.045; 35.291)	**0.04**
G 1 - G 2	28.91	2.56	(26.61; 30.27)	20.465	6.42	(15.87; 25.057)	0.004
G 1 - G 3				23.48	3.89	(20.697; 26.27)	**0.001**
G 1 - G 4				23.43	5.7	(17.44; 29.41)	**0.013**
G 1 - G 5				26.05	2.79	(23.124; 28.98)	0.08
G 1 - G 6				27.168	7.74	(19.045; 35.291)	**0.83**
G 2 - G 3	20.46	6.42	(15.87; 25.057)	23.48	3.89	(20.697; 26.27)	**0.257**
G 2 - G 4				23.43	5.7	(17.44; 29.41)	**0.23**
G 2 - G 5				26.05	2.79	(23.124; 28.98)	**0.05**
G 2 - G 6				27.168	7.74	(19.045; 35.291)	**0.398**
G 3 - G 4	23.48	3.89	(20.697; 26.27)	23.43	5.7	(17.44; 29.41)	**0.83**
G 3 - G 5				26.05	2.79	(23.124; 28.98)	**0.23**
G 3 - G 6				27.168	7.74	(19.045; 35.291)	**0.13**
G 4 - G 5	23.43	5.7	(17.44; 29.41)	26.05	2.79	(23.124; 28.98)	**0.34**
G 4 - G 6				27.168	7.74	(19.045; 35.291)	**0.26**
G 5 - G 6	26.05	2.79	(23.124; 28.98)	27.168	7.74	(19.045; 35.291)	**1**

StDev = standard deviation; G 0 = control group; G 1 = CFV ligation, harvesting at one week; G 2 = CFV ligation, harvesting at two weeks; G 3 = CFV ligation, harvesting at three weeks; G 4 = CFV ligation, harvesting at one week, etanercept administration; G 5 = CFV ligation, harvesting at two weeks, etanercept administration; G 6 = CFV ligation, harvesting at three weeks, etanercept

Table 4. Descriptive and analytical results regarding SH levels

Group A-Group B	Group A-Group B						
	Mean	StDev	95%CI	Mean	StDev	95%CI	p
G 0 - G 1	0.192	0.053	(0.15; 0.23)	0.14	0.02	(0.12; 0.15)	**0.004**
G 0 - G 2				0.133	0.03	(0.11; 0.15)	**0.002**
G 0 - G 3				0.13	0.027	(0.108; 0.15)	**0.01**
G 0 - G 4				0.106	0.034	(0.07; 0.14)	**0.003**
G 0 - G 5				0.30	0.41	(-0.13; 0.73)	**0.103**
G 0 - G 6				0.11	0.3	(0.08; 0.14)	**0.007**
G 1 - G 2	0.14	0.02	(0.12; 0.15)	0.133	0.03	(0.11; 0.15)	0.65
G 1 - G 3				0.133	0.027	(0.108; 0.15)	**0.13**
G 1 - G 4				0.106	0.034	(0.07; 0.14)	**0.04**
G 1 - G 5				0.30	0.41	(-0.13; 0.73)	0.33
G 1 - G 6				0.11	0.3	(0.08; 0.14)	**0.05**
G 2 - G 3	0.133	0.03	(0.11; 0.15)	0.13	0.027	(0.108; 0.15)	**0.364**
G 2 - G 4				0.106	0.034	(0.07; 0.14)	**0.08**
G 2 - G 5				0.30	0.41	(-0.13; 0.73)	**0.588**
G 2 - G 6				0.11	0.3	(0.08; 0.14)	**0.083**
G 3 - G 4	0.127	0.027	(0.108; 0.15)	0.106	0.034	(0.07; 0.14)	**0.386**
G 3 - G 5				0.30	0.41	(-0.13; 0.73)	**0.193**
G 3 - G 6				0.11	0.3	(0.08; 0.14)	**0.808**
G 4 - G 5	0.106	0.034	(0.07; 0.14)	0.30	0.41	(-0.13; 0.73)	**0.07**
G 4 - G 6				0.11	0.3	(0.08; 0.14)	**1**
G 5 - G 6	0.30	0.41	(-0.13; 0.73)	0.11	0.3	(0.08; 0.14)	**0.07**

StDev = standard deviation; G 0 = control group; G 1 = CFV ligation, harvesting at one week; G 2 = CFV ligation, harvesting at two weeks; G 3 = CFV ligation, harvesting at three weeks; G 4 = CFV ligation, harvesting at one week, etanercept administration; G 5 = CFV ligation, harvesting at two weeks, etanercept administration; G 6 = CFV ligation, harvesting at three weeks, etanercept

Table 5. Descriptive and analytical results regarding glutathione levels

Group A-Group B	Group A-Group B						
	Mean	StDev	95%CI	Mean	StDev	95%CI	p
G 0 - G 1	2.087	1.29	(1.947; 3.79)	18.89	8.19	(14.41; 26.14)	**0.000**
G 0 - G 2				15.15	1.27	(14.23; 16.06)	**0.000**
G 0 - G 3				12.24	1.71	(11.02; 13.46)	**0.000**
G 0 - G 4				18.49	5.69	(12.51; 24.46)	**0.005**
G 0 - G 5				17.63	6.72	(10.55; 24.66)	**0.001**
G 0 - G 6				13.65	2.15	(11.41; 15.92)	**0.001**
G 1 - G 2	18.89	8.19	(14.41; 26.14)	15.15	1.27	(14.23; 16.06)	0.65
G 1 - G 3				12.24	1.71	(11.02; 13.46)	**0.000**
G 1 - G 4				18.49	5.69	(12.51; 24.46)	**0.914**
G 1 - G 5				17.63	6.72	(10.55; 24.66)	0.083
G 1 - G 6				13.65	2.15	(11.41; 15.92)	**0.003**
G 2 - G 3	15.14	1.27	(14.23; 16.06)	12.24	1.71	(11.02; 13.46)	**0.000**
G 2 - G 4				18.49	5.69	(12.51; 24.46)	**0.104**
G 2 - G 5				17.63	6.72	(10.55; 24.66)	**1**
G 2 - G 6				13.65	2.15	(11.41; 15.92)	**0.386**
G 3 - G 4	12.24	1.71	(11.02; 13.46)	18.49	5.69	(12.51; 24.46)	**0.005**
G 3 - G 5				17.608	6.72	(10.55; 24.66)	**0.005**
G 3 - G 6				13.65	2.15	(11.41; 15.92)	**0.159**
G 4 - G 5	18.49	5.69	(12.51; 24.46)	17.63	6.72	(10.55; 24.66)	**0.522**
G 4 - G 6				13.65	2.15	(11.41; 15.92)	**0.05**
G 5 - G 6	17.63	6.72	(10.55; 24.66)	13.65	2.15	(11.41; 15.92)	**0.262**

StDev = standard deviation; G 0 = control group; G 1 = CFV ligation, harvesting at one week; G 2 = CFV ligation, harvesting at two weeks; G 3 = CFV ligation, harvesting at three weeks; G 4 = CFV ligation, harvesting at one week, etanercept administration; G 5 = CFV ligation, harvesting at two weeks, etanercept administration; G 6 = CFV ligation, harvesting at three weeks, etanercept

Discussion

Literature data support the fact that chronic etanercept treatment improves endothelial function and diminishes the activity and expression of NAD(P)H oxidase in aged arteries [5].

Other studies have shown that TNF-α inhibition does not affect acetylcholine mediated responses or the production of reactive oxygen species in young animals [12].

These literature data suggest a relationship between aging, oxidative stress and endothelial dysfunction [12-14].

Other authors have evidenced the fact that anti-TNF-α treatment with infliximab improves endothelial dysfunction in human subjects with vascular inflammation characterized by high TNF-α levels [15].

Inflammatory factors such as TNF-α play an important role in the pathogenesis of vascular disorders [2].

In our study, comparative analysis with the control group (L0) showed a significant increase in oxidative stress on account of PC in all groups, a significant decrease in antioxidant defense on account of HD and SH in all groups, and a significant increase in antioxidant defense on account of GSH.

Following the comparative analysis between the groups with CVI without anti-TNF-α treatment (G1, G2, G3) and with anti-TNF-α treatment (G4, G5, G6), our results showed a significant increase in oxidative stress on account of MDA in groups 4 and 5 and a significant decrease in oxidative stress on account of PC in groups 4, 5, 6.

Changes in antioxidant defense were insignificant for SH and GSH and significant for HD in groups 4 and 6.

Antioxidant defense decreased insignificantly for SH and GSH and significantly for HD in group 4.

Antioxidant defense increased insignificantly for SH and GSH and significantly for HD in group 5.

The results of the dynamic comparative analysis between the untreated groups revealed the following significant changes for the untreated groups compared to group 1: an increase in oxidative stress on account of MDA in G2 and G3, a decrease in oxidative stress on account on PC in G2 and G3, a decrease in antioxidant defense on account of HD in G2 and G3, a decrease in antioxidant defense on account of GSH in G3.

The dynamic comparative analysis between the treated groups indicated for the treated groups the following insignificant changes compared to G4: a decrease in MDA, PC and GSH for G5, an increase in MDA, PC, HD and SH for G6.

Conclusions

1. Chronic venous insufficiency with and without anti-TNF-α treatment induced changes in the oxidant/antioxidant balance of our experimental rat group compared to the control group.
2. After the administration of the anti-TNF-α preparation, oxidative stress was maintained on account of MDA that increased significantly, and decreased significantly on account of PC compared to the untreated rat groups.
3. After the administration of the anti-TNF-α preparation, at two and three weeks there was an insignificant increase in antioxidant defense on account of HD and GSH compared to the untreated groups.
4. The administered anti-TNF-α preparation had late and insignificant effects in chronic venous insufficiency induced in our experimental rat model.

Ethical Issues

All experiments were performed respecting international rules concerning experiments on animals.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. Biochem Pharmacol 2009;78(6):539-52.
2. Blake GJ, Ridker PM. Tumour necrosis factor-α, inflammatory biomarkers, and atherogenesis. European Heart Journal 2002;23:345-347.
3. Pascarella L, Schmid-Schönbein GW, Bergan J. An animal model of venous hypertension: the role of inflammation in venous valve failure. J Vasc Surg 2005;41:303-311.
4. Takase S, Pascarella L, Lerond L, Bergan JJ, Schmid-Schönbein GW. Venous hypertension, inflammation and valve remodeling. Eur J Vasc Endovasc Surg 2004;28:484-493.
5. Csiszar A, Labinskyy N, Smith K, Rivera A, Orosz Z, Ungvari Z. Vasculoprotective Effects of Anti-Tumor Necrosis Factor-α Treatment in Aging. Am J Pathol 2007;170(1):388-698.
6. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology 2007;39:44-84.
7. Di Paola R, Mazzon E, Muià C, Crisafulli C, Terrana D, Greco S, Britti D, Santori D, Oteri G, Cordasco G, Cuzzocrea S. Effects of etanercept, a tumour necrosis factor-α antagonist, in an experimental model of periodontitis in rats. Br J Pharmacol 2007;150(3):286-297.
8. Conti M, Morand PC, Levillain P, Lemonnier A. Improved fluorimetric determination of malondialdehyde. Clin Chem 1991;37:1273-1275.
9. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In: Methods in enzymology 1994;233:357-363.
10. Hu ML. Measurement of protein thiol groups and glutathione in plasma. In: Methods in Enzymology vol. 233, Academic Press, Inc. 1994, pp. 380-384.
11. Janazsewska A, Bartosz G. Assay of antioxidant capacity: comparison of four methods as applied to human blood plasma. Scand J Lab Invest 2002;62:231-236.
12. Ungvari Z, Csiszar A, Edwards JG, Kaminski PM, Wolin MS, Kaley G, Koller A. Increased superoxide production in coronary arteries in hyperhomocysteinemia: role of tumor necrosis factor-alpha, NAD(P)H oxidase, and inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 2003;23:418–424.
13. Frey RS, Rahman A, Kefer JC, Minshall RD, Malik AB. PKCzeta regulates TNF-alpha-induced activation of NADPH oxidase in endothelial cells. Circ Res 2002;90:1012-1019.
14. De Keulenaer GW, Alexander RW, Ushio-Fukai M, Ishizaka N, Griendling KK. Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. Biochem J 1998;329:653-657.
15. Booth AD, Jayne DR, Kharbanda RK, McEniery CM, Mackenzie IS, Brown J, Wilkinson IB. Infliximab improves endothelial dysfunction in systemic vasculitis: a model of vascular inflammation. Circulation 2004;109:1718-1723.