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Research Article

Desferal treatment and serum oxidant/ antioxidant balance in experimentally induced chronic venous insufficiency

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Abstract. Background and aim: Iron ions have proinflammatory properties, and redox-active Fe can contribute to lipid peroxidation, to the activation of endothelial cells and the generation of reactive oxygen species. The increase of iron ion concentration stimulates the formation of free radicals and plays an important role in the pathogenesis of chronic venous insufficiency (CVI). We aimed to study, in an experimental CVI model, the serum changes in the oxidant/antioxidant (O/AO) balance and the effect of Desferal administration on the O/AO balance. Material and methods: White Wistar rats were assigned to two groups: group I – with CVI, control group, (n=20 animals/group), group II – with CVI, treated with Desferal (n=20 animals/group). CVI was induced by the ligation of the common femoral vein in the right lower limb. Desferal was administered postoperatively, by intramuscular injection in the contralateral lower limb. The serum O/AO balance was determined from blood collected from the retro-orbital sinus. The indicators of oxidative stress (OS) were: malondialdehyde (MDA), protein carbonyls (PC), oxidized glutathione (GSSG); the indicators of antioxidant defense (AO) were: reduced glutathione (GSH), ceruloplasmin (CP). Results: Experimentally induced CVI caused the following significant changes in the serum: MDA values significantly decreased after Desferal administration, while in the control group, MDA values continued to increase; after desferal administration serum PC values were significantly reduced compared to the control group; the group treated with Desferal had a significant increase of GSH values at T1, T2, T4 ccompared to control group. Conclusions: Deferoxamine administration causes an increase of serum AO on account of GSH. Deferoxamine administration in animals with experimentally induced CVI causes a decrease of serum oxidative stress on account of MDA compared to untreated animals. Our data, experimentally tested in rats, have clinical relevance, recommending the use of Desferal in the treatment of CVI.

Key Words: Desferal, chronic venous insufficiency, oxidative stress

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Introduction

An important role in the development of varicose ulcer skin changes in chronic venous insufficiency is attributed to erythrocyte extravasation (Wenner et al 1980). This occurs as a result of increased pressure in the venules, which forces erythrocytes to migrate along the capillary wall in the dermis (Gloviczki et al 2009). Starting from these considerations, we assumed that the administration of iron chelators such as Desferal might improve venous circulation by removing iron and reducing oxidative stress induced by the presence of iron through the Fenton reaction.

Oxidative stress plays an important role in the pathogenesis of chronic venous insufficiency (CVI), which is demonstrated by the increase of serum malondialdehyde (MDA) concentration in CVI. Even in the early stages of CVI, an increase in the formation of free oxygen radicals on account of the increase of serum iron concentration has been observed (Budzyn et al 2011). Iron ions also have proinflammatory properties (Wenk et al 2001; Zamboni et al 2003); there is a link between venous leg ulcer and genetic abnormalities involved in iron metabolism (Zamboni et al 2003; Zamboni et al 2006).

Redox-active iron may contribute to lipid peroxidation, to the activation of endothelial cells and the generation of ROS (particularly the hydroxyl radical, via the Haber-Weiss reaction) (Duffy et al 2001).

The presence of high iron levels in the skin of patients with CVI, observed for the first time by Myers in 1965, is currently explained by the extravasation of erythrocytes and their dysfunction, which results in the degradation of hemoglobin (Caggiati et al 2010). Erythrocyte extravasation with local iron accumulation can generate ROS and initiate inflammatory reactions (Krzyściak et al 2012). In the evolution of CVI, an increase of hemosiderin levels resulting from iron metabolism can be seen. At the same time, hemosiderin in physiological concentrations protects against ROS action (Krzyściak et al 2011).

Desferal (DFO) is a trihydroxamic acid of vegetal origin, belonging to the class of siderophores (iron chelator), with low molecular weight, which solubilizes and transports Fe3+ in aqueous environment. It is the only Fe chelator accepted as a drug used in the treatment of some diseases (transfusion-induced hemosiderosis, idiopathic hemochromatosis, Fe overload associated with porphyria cutanea tarda, treatment of acute Fe poisoning, treatment of chronic aluminum overload, in patients with end-stage renal failure, atherosclerosis, rheumatoid arthritis, neurodegenerative diseases).

It has a protective antioxidant effect, binding free plasma or cellular Fe2+ and forming the ferrioxamine complex, and to a small extent, ferritin and hemosiderin Fe, but it can also have a prooxidant effect, through the formation of OH• by the Fe2+ autoxidation reaction.

We aimed to study on an experimental CVI model the serum changes in the O/AO balance and the effect of the administration of Desferal, an iron chelator, on the O/AO balance.

Materials and methods

The research was performed with the approval of the Bioethics Commission of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, according to the international animal protection norms in force.

The experiment was carried out on white male Wistar rats of the Rattus norvegicus variety, aged 16 weeks, with a mean weight of 200 grams, from the Biobase of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca. The research was conducted in the Experimental Laboratory and the Laboratory for the Study of Oxidative Stress of the Physiology Department of UMPh, between February 2014 and April 2014.

Groups

The animals were assigned to two groups:

- group I – with CVI, control group (n=20 animals/group)
- group II – with CVI, treated with Desferal (n=20 animals/group)

CVI was induced by the ligation of the common femoral vein in the right lower limb, according to the method of Pascarella et al (2005).

Desferal, Novartis Pharma – Gmbh, was administered postoperatively, by intramuscular injection of 200 mg/kg body weight/day substance in the contralateral lower limb.

The animals of groups I and II were divided into 4 subgroups (n=5 animals/group), depending on the time of sacrifice: T1 - at 3 days, T2 – at 7 days, T3 – at 14 days, T4 – at 21 days. We chose these time points for sacrificing the animals taking into consideration iron metabolism.

Biochemical methods

The serum oxidant/antioxidant (O/AO) balance was determined from blood collected from the retro-orbital sinus, at the previously mentioned time points.

The indicators of OS were: malondialdehyde (MDA), serum values in nmol/ml – determined according to the Conti method (1991); protein carbonyls (PC), serum values in nmol/ml – determined using the Reznick and Packer method (1994); oxidized glutathione (GSSG), serum values in nmol/ml – determined by the Vats method (2008).

The indicators of AO defense were: reduced glutathione (GSH), serum values in nmol/ml – determined using the Hu method (1994); serum ceruloplasmin (CP), values expressed in mg ceruloplasmin/100 ml serum – determined by the method of Ravin, Manta et al (1976).

Statistical processing was carried out with the StatsDirect v.2.7.2 software and the OpenEpi v.3.01 application. The tabular representation of the results was performed using the Excel application (Microsoft Office 2007). For the statistical analysis of the ranks, the non-parametric Mann-Whitney (U) test was used for two unpaired samples, and for the analysis of three or more samples, the non-parametric Kruskal-Wallis test was used. The $\chi 2$ test was also employed for the statistical processing of some data. The significance threshold for the tests used was $\alpha = 0.05$ (5%), $\alpha = 0.01$ (1%) or $\alpha = 0.001$, thus: $0.01 < p < 0.05$ – statistically significant difference; $0.001 < p < 0.01$ – very statistically significant difference; $p < 0.001$ – highly statistically significant difference; $p > 0.05$ – statistically insignificant difference.

Results

Analysis of OS

MDA

Depending on the studied moments, we found the highest MDA values at T1 in the group with Desferal treatment compared to the group without Desferal treatment. MDA values significantly decreased after Desferal administration, while in the control group, MDA values continued to increase. At T3 and T4, MDA values slightly increased in both groups (Table 1).

PC

Protein carbonyl (PC) values showed the same trend. After Desferal administration to animals with experimentally induced chronic venous insufficiency, serum PC values were significantly reduced compared to the control group. The greatest reductions were seen at T2, like in the case of MDA (Table 2).

GSSG

In the control group, there were significant increases between the studied moments. In the group treated with Desferal, the highest increase was found at T4 (Table 3)

Analysis of AO defense

Table 1. Comparative analysis of MDA values in the studied groups and statistical significance

Group	Moment	Median	Minimum	Maximum	Statistical significance (p) between moments			
I	T1	2.055	1.170	2.370	T1-T2	0.0007	T2-T4	0.8199
	T2	2.930	1.570	3.600	T1-T3	0.0048	T3-T4	0.7291
	T3	2.645	2.360	3.530	T1-T4	0.0104		
	T4	2.811	1.812	3.933	T2-T3	0.8199		
II	T1	3.010	1.410	5.000	T1-T2	0.0371	T2-T4	0.0059
	T2	1.800	1.010	3.710	T1-T3	0.6708	T3-T4	0.8096
	T3	2.803	2.127	3.438	T1-T4	0.5129		
	T4	2.737	1.854	3.748	T2-T3	0.0488		
Statistical significance (p) between groups	T1	T2	T3	T4				
	0.0337	0.0288	<0.0001	0.0007				

Table 2. Comparative analysis of PC values in the studied groups and statistical significance

Group	Moment	Median	Minimum	Maximum	Statistical significance (p) between moments			
I	T1	2.479	2.060	2.955	T1-T2	0.0331	T2-T4	<0.0001
	T2	2.847	2.209	3.817	T1-T3	0.1395	T3-T4	<0.0001
	T3	2.270	1.856	2.710	T1-T4	<0.0001		
	T4	1.300	0.948	1.610	T2-T3	0.0019		
II	T1	2.034	1.733	2.259	T1-T2	0.0777	T2-T4	0.0300
	T2	1.865	1.647	2.090	T1-T3	0.0645	T3-T4	0.3750
	T3	1.736	0.872	3.303	T1-T4	0.0016		
	T4	1.555	0.945	2.117	T2-T3	0.4922		
Statistical significance (p) between groups	T1	T2	T3	T4				
	0.0009	<0.0001	0.0029	0.0962				

Table 3. Comparative analysis of GSSG values in the studied groups and statistical significance

Group	Moment	Median	Minimum	Maximum	Statistical significance (p) between moments			
I	T1	1.400	0.780	1.980	T1-T2	0.1836	T2-T4	0.2270
	T2	1.665	1.380	1.840	T1-T3	0.1101	T3-T4	0.3294
	T3	1.625	1.380	1.960	T1-T4	0.0328		
	T4	1.720	1.240	2.280	T2-T3	0.6266		
II	T1	1.840	1.420	2.000	T1-T2	0.0149	T2-T4	0.0013
	T2	1.635	1.480	1.740	T1-T3	0.0371	T3-T4	0.3750
	T3	2.148	1.680	3.800	T1-T4	0.2015		
	T4	2.045	1.460	2.380	T2-T3	0.0039		
Statistical significance (p) between groups	T1	T2	T3	T4				
	0.011	0.9420	0.0007	0.1824				

GSH

In the control group, there was a significant increase of GSH values at T3 compared to T1. The group treated with Desferal had a significant increase of GSH values at T1, T2, T4 ccompared to control group (Table 4).

CP

In the group treated with Desferal, a significant increase of CP values was found at T2 compared to T1, which was followed by a significant reduction of values during the rest of the studied period (Table 5).

Discussion

Chronic venous insufficiency is one of the important causes of morbidity in the adult population. In the pathogenesis of diseases associated with vascular injuries, reactive oxygen species play an important role in the acceleration of endothelial destruction, resulting in the loss of endothelial integrity (Bishop et al 1985; Michalska et al 2010). It is highly possible that these changes in the vein wall structure seen in chronic venous insufficiency are caused by an overproduction of reactive oxygen species (ROS). ROS induce lipid, protein and DNA oxidation, determining the development of oxidative stress (Bandyopadhyay et al 1999; Budzyn et al 2011). Many studies show that ROS play a critical role in the development and progression of chronic venous insufficiency (Glowinski et al 2002; Kozka et al 2009; Karatepe et al 2010). Among the mechanisms involved in the production of oxidative stress in chronic venous insufficiency, an important role is played by iron overload. As early as 1988, Ackerman showed the presence of an increased iron concentration in the skin of patients with varicose ulcer and its role in the production of reactive oxygen species (Akerman et al 1988). Although an important element involved in many vital processes, iron accumulated in large amounts is the source, via the Fenton reaction, of a highly reactive free radical, the hydroxyl radical (OH+) (Budzyn et al 2011).

Under the conditions of venous stasis associated with chronic venous insufficiency, venous hypertension results in the extravasation of erythrocytes through the pores of endothelial cells (Wenner et al 1980), their destruction with the release of iron from hemoglobin, of ferritin and hemosiderin (Thomas et al 1985; Biemond et al 1988; Wenk et al 2001).

Similar studies evidencing a close relationship between iron and venous insufficiency have been conducted by Zamboni, who shows that a mutation in the HFE gene in patients with hereditary hemochromatosis increases the risk of ulcer in chronic venous insufficiency (Zamboni et al 2006; Budzyn et al 2011). In this study, we tested the hypothesis according to which an iron chelator can reduce oxidative stress present in this disorder and thus, might be used as an adjuvant therapy in this disease. Our study focused on the identification of the antioxidant scavenger potential of an iron chelator, deferoxamine (Desferal), dynamically monitoring the evolution of oxidative stress in animals with experimentally induced chronic venous insufficiency. Lipid peroxidation plays an important role in the pathogenesis of oxidative destruction in chronic venous insufficiency, the overproduction of reactive oxygen species being an important

Table 4. Comparative analysis of GSH values in the studied groups and statistical significance

Group	Moment	Median	Minimum	Maximum	Statistical significance (p) between moments			
I	T1	3.540	2.450	5.250	T1-T2	0.0090	T2-T4	0.1370
	T2	4.865	2.450	7.300	T1-T3	0.0060	T3-T4	0.0485
	T3	6.570	3.950	13.900	T1-T4	0.4289		
	T4	4.168	0.225	7.830	T2-T3	0.0453		
II	T1	12.777	3.925	35.000	T1-T2	0.0195	T2-T4	<0.0001
	T2	4.019	3.125	4.750	T1-T3	0.0840	T3-T4	0.3365
	T3	7.296	6.015	9.455	T1-T4	0.1055		
	T4	7.835	6.195	9.375	T2-T3	<0.0001		
Statistical significance (p) between groups	T1	T2	T3	T4				
	0.0003	0.0490	0.7313	0.0008				

Table 5. Comparative analysis of CP values in the studied groups and statistical significance

Group	Moment	Median	Minimum	Maximum	Statistical significance (p) between moments			
I	T1	71.167	44.293	93.958	T1-T2	0.1410	T2-T4	<0.0001
	T2	63.972	47.731	74.226	T1-T3	0.0398	T3-T4	<0.0001
	T3	60.976	49.893	72.870	T1-T4	<0.0001		
	T4	32.890	28.175	39.069	T2-T3	0.3999		
II	T1	58.704	46.489	86.616	T1-T2	0.0098	T2-T4	0.0020
	T2	83.972	49.665	108.246	T1-T3	0.0039	T3-T4	0.2324
	T3	44.560	38.133	50.969	T1-T4	0.0488		
	T4	50.335	33.915	78.663	T2-T3	<0.0001		
Statistical significance (p) between groups	T1	T2	T3	T4				
	0.0630	0.0022	<0.0001	0.0003				

factor at the origin of endothelial destruction (Kozka et al 2009; Budzyn et al 2011). Lipid peroxidation is dependent on the autoxidation of Fe2+ to Fe3+, and the binding of iron by deferoxamine can inhibit the formation of the hydroxyl radical and reduce oxidative stress (Miller et al 1992).

In our study, we determined malondialdehyde (MDA) and demonstrated a statistically significant increase of its plasma concentration as a marker of lipid peroxidation in the group with experimental chronic venous insufficiency. After Desferal administration, MDA levels decreased in the study group at all studied time points, which can be explained by the known action of deferoxamine to bind Fe3+ released from extravasated erythrocytes and thus reduce lipid peroxidation (Miller et al 1992). The fact that the rate of Fe3+ binding by deferoxamine is higher at a more acid pH (Miller et al 1992) can explain the presence of the highest oxidative stress reduction value through the reduction of the MDA value at time T2, when serum pH decreased because of venous stasis consecutive to venous insufficiency. Our results are in accordance with the studies of Budzyn (2011), showing an increase of serum MDA in patients with chronic venous insufficiency associated with increased serum iron levels. The high MDA levels can be significantly reduced in the presence of dissolved deferoxamine or deferoxamine-coupled cellulose, in dermal fibroblasts subjected to an atmosphere of free radicals produced via the Fenton reaction (Wenk et al., 2001). These authors anticipate a protective effect of deferoxamine against iron-mediated oxidative stress.

Kozka (2009), who studied oxidative stress in patients with chronic venous insufficiency associated with obesity, also showed a significant increase of MDA values. Studying the in vitro effect of deferoxamine on dermal fibroblasts subjected to high iron concentrations, Wenk demonstrated its role in reducing oxidative stress by decreasing MDA levels (2001). In 2013, Condezo-Hoyos et al, studying oxidative stress in early chronic venous insufficiency, showed an increase of lipid peroxidation. High plasma MDA levels were also described in in vitro studies on varicose vein fragments, compared to healthy tissue (Krzysciak et al. 2011). However, Yasim et al. showed an insignificant difference of MDA values between the study group and the control group of patients with chronic venous insufficiency, at an early stage (2008).

The oxidative changes of structural enzymes and proteins play an important role in the etiology and even the progression of various disorders (Dalle-Donne et al 2003). The determination of carbonyl proteins as oxidative stress markers can provide information facilitating diagnosis as early as the presymptomatic stage (Dalle-Donne et al 2003). In our study, significantly lower PC values were found after Desferal administration compared to initial values and to the control group. The reduction was recorded at T2, in parallel to the time of reduction of MDA values, which suggests not only lipid but also protein peroxidation as part of oxidative stress in chronic venous insufficiency, as well as the effect of deferoxamine in both metabolic lines. Our results are in accordance with those of other studies (Esterbauer et al 1991; Dalle-Donne et al 2003; Vaida-Voevod 2013), which found increased PC values in disorders evolving with oxidative stress, without being dynamically studied. However, Condezo-Hoyos studying oxidative stress parameter changes in the early

stages of chronic venous insufficiency, show that lipid peroxidation precedes protein peroxidation in venous pathology (2013). This increase of oxidative stress markers (MDA, PC) shows the magnitude of oxidative stress, which exceeds the capacity of antioxidants to neutralize them.

In the evolution of oxidative stress, an important role is played by antioxidant systems, which counteract/attempt to counteract the action of reactive oxygen species. If in the evolution of GSSG values, differences are weakly/not statistically significant, reduced glutathione (GSH) exhibits a marked reduction at T2 in the study group.

Ceruloplasmin is a plasma α2-glycoprotein that binds and transports 7 copper atoms. Among its many functions, the most important is that of ferroxidase, converting Fe2+ to Fe3+ (Gutteridge 1980; Ganini et al 2012).

However, ceruloplasmin is also an important plasma enzymatic antioxidant. It protects against free oxygen radicals through its ferroxidase function itself. Thus, by converting Fe2+ to Fe3+, it inhibits the Fenton and Haber-Weiss reactions (Ramakrishnan et al 2007) and prevents the use of Fe2+ for the formation of the hydroxyl radical (Gutteridge 1980; Chapman et al 2013).

In our study, we obtained a decrease of plasma CP levels in the control group associated with a MDA increase at the same time of the experiment (T2), which were maintained low throughout the study. This can be explained by the antioxidant effect of CP and its consumption in the effort to neutralize free radicals, which exceeds its hepatic resynthesis capacity.

Similar results were obtained by Selvi (2007), who studied iron and ceruloplasmin levels in patients with chronic venous insufficiency and Eales disease. High ceruloplasmin levels are associated with many pathological conditions such as: cardiovascular diseases (Fox et al 2000; Engström et al 2002; Giurgea et al 2005), viral infections (Novicova et al 2011), cancer (Wang et al 2002) and others, where ceruloplasmin seems to have both an antioxidant role and an important anti-inflammatory role. Our study confirms the results of these studies. We obtained an increase of plasma CP levels at time T2 of the experiment in the study group. This effect can be explained by the acute phase protein role of ceruloplasmin (Iskra et al 1999; Gruys et al 2005), a concomitant inflammatory syndrome being possibly associated. The increase in the concentration of CP, which is a regulator of oxidative stress response, concomitantly with the decrease of GSH, can be interpreted in the same way, as an inflammatory syndrome associated with oxidative stress.

Recent studies show that the plasma iron-reducing activity of iron chelators is related to ceruloplasmin concentrations: thus, the magnitude of the reduction of iron concentration in the cerebrospinal fluid of patients with Parkinson's disease increases as ceruloplasmin concentrations increase (Grolez et al 2015).

Conclusion

Deferoxamine administration causes an increase of serum AO on account of GSH. Deferoxamine administration in animals with experimentally induced CVI causes a decrease of serum OS on account of MDA compared to untreated animals. Our data, experimentally tested in rats, have clinical relevance, recommending the use of Desferal in the treatment of CVI.

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