Effects of nitric oxide donor S-nitrosoglutathione on apoptosis of apheresis platelets

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\begin{abstract}
\textbf{Objectives:} The aim of this study is to investigate the effects of a Nitric oxide (NO) donor, S-nitrosoglutathione (GSNO), on apoptosis and the improvement of preservation quality in apheresis platelets.

\textbf{Methods:} A GSNO solution - to make the final GSNO concentration of 100 \textmu M was added into fresh apheresis platelets, and the parameters associated with platelet morphology, metabolism, and apoptosis were dynamically monitored for seven days.

\textbf{Results:} The results showed that the NO level was remarkably higher during the whole storage stage after GSNO injection. The number of depolarized platelets and platelets with phosphatidylserine valgu were significantly reduced in the GSNO group compared to that of the control group at some time point. The expression of Bcl-xL mRNA on day 5 of storage in the experimental group was significantly higher than that of the control group, but the expression of Bcl-\texttimes L protein was not significantly higher than that in the control group. In addition, Bak and Bax mRNA expression levels in the experimental group were significantly lower than those in the control group, but Bak and Bax protein expression levels were not statistically different. Meanwhile, caspase-3 activity was significantly inhibited.

\textbf{Discussion and conclusion:} These data suggest that the addition of a certain dose of GSNO as a NO donor during platelet storage could inhibit platelet apoptosis and reduce platelet storage lesion (PSL) to a certain extent.
\end{abstract}

\section*{Introduction}

The quality of preserved platelets prior to infusion is particularly important in order to achieve better results from platelet transfusion therapy.

The \textit{in vitro} quality of platelet preservation is affected by a variety of factors, including the processes of platelet collection and preparation, material and ability of platelet storage bags, and storage conditions. More importantly, platelets, unfortunately, undergo numerous physiological, biochemical, and morphological changes that alter their functional integrity during storage, all of which lead to platelet storage lesion (PSL) \cite{1,2}. Not only does PSL seriously affect the quality of the platelets and greatly shorten the shelf life, but it also affects the platelets’ survival and hemostasis functions in patients who received platelet transfusions \cite{3}. A large number of studies have shown that platelet activation and apoptosis are important causes of PSL \cite{4}; however, the mechanism of PSL is not yet fully understood. Therefore, to maximize the platelet function closest to the physiological state of the body, it is critical to find a way to inhibit platelet activation and apoptosis \textit{in vitro} and thereby reduce PSL.

Many studies have shown that adding some antiplatelet agents or drugs during platelet preparation and preservation can partially inhibit platelet activation and improve platelet quality \cite{5}. Nitric oxide (NO), an important messenger and effector molecule, is a small molecule of gas with multiple biological activities in the body. Studies have shown that \cite{6} NO has important protective effects on erythrocyte morphology and function and membrane viscoelasticity. NO is also an antiplatelet agent that could inhibit platelet aggregation and activation \textit{in vitro} and \textit{in vivo} \cite{7–9}, which provides a novel way to improve the quality of platelet preservation \textit{in vitro}. Using NO gas aqueous solution or an NO donor as a source of NO can inhibit platelet aggregation and activation and improve platelet quality to a certain extent \cite{10}. However, it has not yet been reported whether NO has an effect on platelet apoptosis during platelet storage. In this study, S-nitrosoglutathione (GSNO) was used as a NO donor to monitor apheresis platelet apoptosis indexes under simulated...
platelet storage conditions. The aim of this study was to investigate whether it is possible to improve platelet function by adding NO to preserved platelets, and in particular, if it is possible to regulate apoptosis, thereby reducing PSL and improving platelet quality during platelet storage.

**Material and methods**

**Samples**

Apheresis platelets were collected from 30 healthy blood donors in Ningbo Central Blood Station, Zhejiang Province, China. None of the donors took aspirin or other drugs that would affect platelet function for two weeks prior to the donation. Written-informed consent was obtained from all of the donors, and this study was approved by the local ethical committee.

Each sample included 150 ml platelet concentrate (PC) with more than $1 \times 10^9$ PLT ml$^{-1}$. On the day of the PC collection (day 0), each PC in a bag was equally divided into two parts using a connecting device instrument (TSCD-II, Terumo Sterile Tubing Welder, TerumoBCT, Japan) and a digital balance (Sartorius, Germany). GSNO powder (Sigma-Aldrich, Germany) was dissolved in phosphate buffer saline (PBS) with a store concentration of 1 mM and filtered using 0.22 μm filters (Millipore, USA). The final concentration of 100 μM was chosen based on a pilot study. GSNO solution was added to one group of bags, which made up the GSNO group, with a calculated volume of sterile normal saline was added to the other group of bags to make up the control group. Both groups of PCs were kept in a platelet incubator at 22 ± 2°C and agitated constantly for seven days. All of the parameters were tested on days 1, 3, 5, and 7 of storage.

**NO assay**

The NO levels in the PCs were measured using an NO assay kit (Jiancheng, Nanjing, China). Following the manufacturer’s protocol, the reduction of nitrate to nitrite was done after 15 min at 37°C. By centrifuging the mixture for 10 min at 4000 rpm, the supernatant was obtained and used for the assessment of nitrite levels, which reflected the free NO content. The results were provided as μmol/L.

**Platelet morphology visualized by scanning electron microscope**

After platelet sampling, the samples were centrifuged at 3000 rpm for 5 min, the supernatant solution was discarded, and the samples were fixed in 2.5% glutaraldehyde overnight. They were then dehydrated with 30–100% ethanol step by step, suspended in anhydrous tetrahydrofuran, and finally, they were freeze-dried for 8 h. A small amount of dry powder was taken with a toothpick and fixed directly on the sample stage using conductive tape. The morphology of the platelets was observed by Hitachi S-4800 scanning electron microscope.

**RNA isolation**

The total platelet RNA was extracted using a total RNA isolation kit (Beijing CoWin Bioscience Co, Ltd., Beijing, China) according to the manufacturer’s protocol. The RNA concentration was estimated with a Nanodrop spectrophotometer (ND1000; Saveen & Werner, Linhamn, Sweden). The sample was kept at −70°C for further analysis.

**Quantitative real-time polymerase chain reaction**

A qRT-PCR assay was performed to measure the mRNA expression of Bak, Bax, and Bcl-xL using the UltraSYBR One Step RT-qPCR Kit (Beijing CoWin Bioscience Co, Ltd.). Results were normalized to the expression of 5s rRNA. The PCR cycling conditions were as follows: 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min for 40 cycles. The qRT-PCR data were normalized using the 2$-^{ΔΔCt}$ method. Melting curve analysis was utilized to test the specificity and quality of the qRT-PCR amplification. The data were processed using StepOneTM software v2.2.2 (Applied Biosystems, USA). The primers were synthesized using the sequences listed in Table 1.

**Flow cytometry**

To assess $ΔΨ_m$, depolarization using flow cytometry, a Mitochondrial Membrane Potential Detection JC-1 kit (BD Biosciences, San Diego, CA, USA) was used according to the manufacturer’s instruction. $ΔΨ_m$ depolarization was quantified as the percentage of depolarized cells. For analysis of PS externalization, flow cytometry was employed using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer’s recommendations. Fluorescence intensity was analyzed on the Cytomics FC

| Gene     | Primer (5′–3′)                |
|----------|------------------------------|
| 5s rRNA-F| TACGCCCATACCCACCTGGAA        |
| 5s rRNA-R| TAACAGGGGCGAGCCCTGCT         |
| Bak-F    | TGAGTACTTCAACCCAGATTCA       |
| Bak-R    | AGTCGGCCATGCCTGGTAGAC        |
| Bax-F    | GAGCGGGCGATGTAGGA            |
| Bax-R    | TGGATGAAAACCGCTGAGCAA       |
| Bcl-xL-F | TTACCTGAGAGGACACCTA          |
| Bcl-xL-R | ATTCCGCAGTAAAGATGGA          |
500 (Beckman Coulter, Fullerton, CA, USA). Data on 50,000 platelets per sample were collected and analyzed. Each experiment was performed at least three times.

Western blotting
Platelet proteins were separated on 12% Bis-Tris NuPage gels (Invitrogen, Basel, Switzerland) under reducing conditions, electroblotted onto PVDF membranes (Bio-Rad, Hercules, CA, USA) and immunoblotted with polyclonal antibodies against Bcl-xL, Bak, Bax, and β-actin (Abcam, Cambridge, MA, USA). Then, the membranes were incubated with horseradish peroxidase-conjugated IgG (Abgene Inc., Epsom, UK) and detected with enhanced chemiluminescence. Integrated density values were calculated using Quantity One (Bio-Rad).

Statistical methods
Data were presented as mean ± standard deviation. GraphPad Prism 6 statistical software (GraphPad Software, La Jolla, CA, USA) was used for the analysis. The statistical difference between the GSNO and control groups was assessed with the student’s t test. Given the non-Gaussian distribution of the results, non-parametric Kruskal–Wallis ANOVA was applied to compare the data from groups. A p-value of less than .05 was considered to be statistically significant.

Results

NO measurement
We examined the NO levels in the PCs, which indicated the availability of the NO donor by an NO assay kit. The levels of NO in two groups are presented in Figure 1. In the control group, the concentration of NO increased slightly over time, while the concentration of NO in the GSNO group first increased sharply, than decreased slightly after reaching a peak on day 3. The levels of NO in the GSNO group were markedly higher than those in the control group during the whole storage stage.

Platelet morphology
Scanning electron microscopy revealed the platelet morphology in Figure 2. The platelet morphology in the control and GSNO groups were both normal on days 1 and 3 of the storage. In the control group, a portion of the platelets exhibited an irregular shape on days 5 and 7 of storage, which was transitioned from a bimodal to a spherical shape; meanwhile, a large number of platelets aggregated spontaneously. However, most of the platelets were still intact on days 5 and 7 in GSNO group, but some of the platelets appeared the spinous pseudopodia and weak aggregation. No apoptotic bodies were found in both groups.

GSNO inhibits apoptosis in platelets
To investigate whether the addition of GSNO could inhibit platelet apoptosis, we examined apoptosis markers, such as PS exposure, and ΔΨm depolarization in platelets by flow cytometry. The proportion of platelets with surface-exposed PS in the GSNO group, was no significant difference compared with the control group, as assessed by flow cytometry Annexin V-staining (P > .05) (Figure 3A). Additionally, the mitochondrial depolarized platelets from the GSNO group tended to be lower compared to the control group at days 5 and 7 (P = .001 and P = .002) (Figure 3B).

The mRNA expression level of Bcl-xL/Bax in the platelets from the GSNO group and control group was found with a significant difference at days 1, 3, and 7 (P = .001, P = .023, and P = .014) (Figure 4(A)). While the mRNA expression level of Bcl-xL/Bak in the platelets from two groups also had a statistical difference at days 1, 5, and 7 (P = .002, P = .007, and P = .004) (Figure 4(B)).

As seen in Figure 5(A), the protein expression level of Bcl-xL/Bax was higher in the platelets from the GSNO group than in the controls, but the change was not significant (Figure 5(B)) (P > .05). And so was Bcl-xL/Bak (Figure 5(C)).

Discussion
The in vivo life span of platelets is short, and the regulatory mechanism is not yet clear. Numerous studies have shown that apoptosis plays an important role in the activation and senescence of platelets [11]. Platelet apoptosis is an important indicator of platelet quality and function, but it is also an important reason for PSL [11]. Animal experiments have shown that platelet apoptosis results in aging and a decrease in the viability of platelets in the body after transfusion [12].
Studies have shown that NO can improve platelet function in vitro and in vivo [10,13,14]. The antiplatelet effect of NO is characterized by a prolonged bleeding time, inhibition of platelet adhesion, and aggregation, reduced platelet activation, decrease of degranulation, and the release of active substances [15]. The effect of

Figure 2. Morphology photos of GSNO-treated PCs (GSNO) and PCs without GSNO (Control) at different days of storage which was caught by scanning electron microscope, the magnification is 5000×.

Figure 3. Flow cytometry charts of GSNO-treated PCs (GSNO) and PCs without GSNO (Control) at different days of storage. (A) PS exposure in platelets using the FITC Annexin V Apoptosis Detection kit to marker the apoptotic cell, gate B shows the percentage of apoptotic platelets; (B) ΔΨm, depolarization in platelets using a Mitochondrial Membrane Potential Detection JC-1 kit, gate K4 shows the percentage of depolarized platelets.
NO may vary depending on the types of cells and the types of isoenzymes. Endothelial nitric oxide could prevent platelet adhesion to the vessel wall and thereby inhibit platelet aggregation [16,17]. Platelet-derived NO limits the activation of adjacent platelets in order to avoid the formation of thrombus [13].

Since the platelet in vitro experiences a loss of endothelial-derived NO, the amount of NO it produces cannot inhibit its activation [18]. In this study, we added GSNO as the NO donor to stored platelets to explore whether it can inhibit platelet apoptosis and reduce PSL, thereby improving the quality of platelet preservation. Our preliminary experimental data showed that 100 mM GSNO achieved the best platelet preservation effect, so we used 100 mM GSNO to carry out our study in the subsequent experiments. The results suggesting that the platelets themselves also produced a certain amount of NO to inhibit platelet activation in vitro. However, the NO level produced by the platelet itself is not enough to inhibit the activation. Meanwhile, the platelet consumption of NO is greater than the GSNO release NO rate from the day 3.

Studies have shown that platelets, despite their lack of nuclei and nuclear DNA, are rich in mitochondrial and mitochondrial mRNA, which can carry out protein synthesis and energy metabolism [19,20]. Platelets contain mitochondria, which are not only a control center for life activities, but they are also a control center for cell metabolism and energy supply or a control center for apoptosis. The apoptotic pathway of platelets is similar to that of nucleated cells, except that there is no nucleus. Our research suggests that GSNO helps keep platelets from undergoing apoptosis and from activating during platelet storage from scanning electron microscopy results, phosphatidylserine valgus tests and mitochondrial membrane potential tests. Studies by Verhoeven [21] and Leytin [22] suggest that prolonged storage of platelets affected platelet function, leading to a decline in platelet quality. Studies by Senser et al. [23] showed that L-arginine and GSNO-derived NO can inhibit the adenosine diphosphate (ADP)-induced decline in platelet mitochondrial membrane potential, indicating that NO can inhibit mitochondrial membrane potential depolarization and improve platelet mitochondrial function.

The Bcl-2 family of proteins is a group of key regulators of the endogenous mitochondrial apoptosis pathway and they are divided into two types: pro-apoptotic proteins (Bak, Bax, etc.) and anti-apoptotic proteins (Bcl-2, Bcl-xl, etc.). The two types of proteins bind and inhibit each other to maintain a relatively balanced state and regulate cell apoptosis. The platelet apoptosis depends on the balance of pro-apoptotic factors and anti-apoptotic factors in the Bcl-2 family [24]. The caspase family of proteins plays essential roles in the initiation, execution, and regulation of apoptosis. Caspase-3, a key factor in apoptosis execution, degrades apoptotic substrate, ultimately causing apoptosis.

In conclusion, our results suggest that not only can 100 mM GSNO as the NO donor inhibit platelet apoptosis, reduce PSL, and maintain the quality of stored platelets.

Studies on GSNO are mostly focused on platelet activation and adhesion and other functions. It has been suggested that GSNO can reduce the prevalence of cerebral embolism in patients with carotid endarterectomy who received aspirin and heparin therapy, and it can also reduce platelet adhesion during bypass grafting [25]. Senser et al. [23] found that 100 mM GSNO significantly reduced ADP-induced platelet activation and aggregation. Other studies have suggested that the addition of GSNO to frozen platelets could inhibit platelet activation and maintain their aggregation activity and platelet function, indicating that GSNO may be used as a protectant for platelet cryopreservation [26,27].

In this study, we explored GSNO as a NO source that may have an effect on platelet apoptosis during platelet preservation at normal temperature.
The results showed that 100 mM GSNO could inhibit the apoptosis of platelets to a certain extent. Chatto-padhyay et al. [28] found that the precursor of GSNO, L-arginine, could reduce apoptosis during ischemia-reperfusion in rats, and an additional study showed that L-arginine could inhibit rat mesangial cells and neutrophils apoptosis in vitro [29,30]. Senser et al. [23] demonstrated that GSNO also could reduce platelet apoptosis in the study of platelet agonist ADP-induced platelet activation. Platelet apoptosis and activation are two different processes; however, apoptosis was associated with a high level of platelet activation, and there was a certain degree of overlap in the process [11]. This is consistent with our results, and further suggests that GSNO can inhibit platelet apoptosis, reduce PSL, and improve the quality of platelets during preservation.

Relevant studies suggest that an increase in glycolysis during platelet storage leads to a decrease in pH, which may activate the cell apoptotic pathway and thus being cleared [31]. Our previous study showed that NO may maintain the platelet pH value in the normal range by increasing fatty acid oxidation, reducing glycolysis, and accumulating lactic acid [32]. Nicolay et al. [33] reported that an NO generator, which produces NO, could significantly reduce calcium overload, glycogen depletion, and high osmotic stress-induced red blood cell shrink age and apoptosis. Similarly, the rate of erythrocyte clearance in the circulatory system was significantly faster in the endothelium-derived nitric oxide synthase (eNOS) gene knockout mice than that of wild-type mice, indicating that NO is involved in maintaining the physiological state and survival of red blood cells and reducing red blood cell apoptosis. The mechanism is that NO increases the platelet cGMP concentration and the inhibition of Ca$^{2+}$ influx, which is caused by erythrocyte aging and apoptosis. Therefore, a reduction in intracellular Ca$^{2+}$ concentration will likely prevent a series of age-related changes [34]. Similarly, as a nucleus-free platelet, we speculate that the inhibition of platelet apoptosis of GSNO may also be through the cGMP-mediated pathway to inhibit Ca$^{2+}$ influx to have an anti-platelet apoptosis effect. The underlying mechanism needs to be further studied.

The relationship between an NO donor and platelet storage quality is not only still at the experimental research stage, but there is a lack of related animal experiments and clinical trials to verify its function and safety in vivo. This study explored the relationship between the inhibition of platelet apoptosis of NO and platelet storage quality, which provides new theoretical ideas for NO donors to improve the quality of platelet preservation.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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References

[1] Seghatchian J, Kraladisiri P. The platelet storage lesion. Clin Lab Med. 1997;11(2):130.

[2] Thon JN, Schubert P, Devine DV. Platelet storage lesion: a new understanding from a proteomic perspective. Transfus Med Rev. 2008;22(4):268–279.

[3] Zhang JG, Carter CJ, Culibrk B, et al. Buffy-coat platelet variables and metabolism during storage in additive solutions or plasma. Transfusion. 2008;48(5):847–856.

[4] Schubert P, Devine DV. Towards targeting platelet storage lesion-related signaling pathways. Blood Transfus. 2010; Suppl. 8, 3(4):s69–s72.

[5] Wood EM, Colton E, Yongovian RA, et al. Prevention of monocyte adhesion and inflammatory cytokine production during blood platelet storage: an in vitro model with implications for transfusion practice. J Biomed Mater Res. 2000;51(2):147.

[6] Bennettguerrero E, Veldman TH, Doctor A, et al. Prevention of cytokine and metabolic variables and metabolism during storage in additive solutions during platelet storage: an in vitro model with implications for transfusion practice. J Biomed Mater Res. 2000;51(2):147.

[7] Radomski MW, Zakar T, Salas E. Nitric oxide in platelets. Meth Enzymol. 1996;269(1):88–107.

[8] Wong K, Li X. Nitric oxide infusion alleviates cellular activation during blood platelet storage: an in vitro model with implications for transfusion practice. J Biomed Mater Res. 2000;51(2):147.

[9] Major TC, Brant DO, Reynolds MM, et al. The attenuation of platelet and monocyte activation in a rabbit model of extracorporeal circulation by a nitric oxide releasing polymer. Biomaterials. 2010;31(10):2736–2745.

[10] Marjanovic JA, Stojanovic A, Brovkovych VM, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci. 2007;104(43):17063–17068.

[11] Wood EM, Colton E, Yongovian RA, et al. Prevention of monocyte adhesion and inflammatory cytokine production during blood platelet storage: an in vitro model with implications for transfusion practice. J Biomed Mater Res. 2000;51(2):147.

[12] Marjanovic JA, Stojanovic A, Brovkovych VM, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci. 2007;104(43):17063–17068.

[13] Wood EM, Colton E, Yongovian RA, et al. Prevention of monocyte adhesion and inflammatory cytokine production during blood platelet storage: an in vitro model with implications for transfusion practice. J Biomed Mater Res. 2000;51(2):147.

[14] Oberprieler NG, et al. cGMP-independent inhibition of platelet adhesion to collagen through cGMP-dependent and independent mechanisms: the potential role for S-nitrosoylation. Platelets. 2009;20(7):478–486.

[15] Leytin V. Apoptosis in the anucleate platelet. Blood Rev. 2012;26(2):51–63.

[16] Mason KD, Carpinelli MR, Fletcher JE, et al. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128(6):1173–1186.

[17] Verhoeven AJ, Verhaar Robin, Gouwerok Eric GW, et al. The mitochondrial membrane potential in human platelets: a sensitive parameter for platelet quality. Transfusion. 2005;45(1):82–89.

[18] Leytin V, Allen DJ, Mutlu A, et al. Platelet activation and apoptosis are different phenomena: evidence from the sequential dynamics and the magnitude of responses during platelet storage. Br J Haematol. 2008;142(3):494–497.

[19] Sener A, Egemen G, Cevik O, et al. In vitro effects of nitric oxide donors on apoptosis and oxidative/nitrative protein modifications in ADP-activated platelets. Hum Exp Toxicol. 2013;32(3):225–235.

[20] Dowling MR, Josefsson EC, Henley JF, et al. Platelet senescence is regulated by an internal timer, not damage inflicted by hits. Blood. 2010;116(10):1776.

[21] Miller MR, Megson IL. Recent developments in nitric oxide donor drugs. Br J Pharmacol. 2007;151(3):305–321.

[22] Wu Tao, Zhang Changhong, Wang Zantao, et al. Inhibitory role of S-nitrosoglutathione in the aggregation of frozen platelets, and its effect on the expression of membrane glycoproteins. Exp Ther Med. 2013;6(3):831.

[23] Lee JH, Kim JT, Cho YG. Effect of nitric oxide on the cryopreservation of platelets. Korean J Lab Med. 2008;28(2):136.

[24] Chattopadhyay P, Shukla G, Wahi AK. Protective effect of L-arginine against necrosis and apoptosis induced by experimental ischemic and reperfusion in rat liver. Saudi J Gastroenterol. 2009;15(3):156.

[25] Trachtman H, Futterweit S, Mermelstein A, et al. Anti-apoptotic effect of L-arginine in cultured rat mesangial cells. Int J Mol Med. 2000;6(4):485–489.

[26] Stépovaya Y, Zhavoronok TV, Starikov YV, et al. Regulatory role of nitric oxide in neutrophil apoptosis. Bull Exp Biol Med. 2008;146(6):737–740.

[27] Overberger NG, et al. cGMP-independent inhibition of integrin αIIbβ3-mediated platelet adhesion and outside-in signalling by nitric oxide. FEBS Lett. 2007;581(7):1529–1534.

[28] Gkaliagkousi E, Ritter J, Ferro A. Platelet-derived nitric oxide signaling and regulation. Circ Res. 2007;101(7):654–662.

[29] Oberprieler NG, et al. cGMP-independent inhibition of integrin αIIbβ3-mediated platelet adhesion and outside-in signalling by nitric oxide. FEBS Lett. 2007;581(7):1529–1534.

[30] Overberger NG, et al. cGMP-independent inhibition of integrin αIIbβ3-mediated platelet adhesion and outside-in signalling by nitric oxide. FEBS Lett. 2007;581(7):1529–1534.

[31] Sandgren P, Stejpanovic A. High-yield platelet units revealed immediate pH decline and delayed mitochondrial dysfunction during storage in 100% plasma as compared with storage in SSP+. Vox Sang. 2012;103(1):55–63.

[32] Yu L, Yu S, He Y, et al. Effect of nitric oxide donor on metabolism of apheresis platelets. Indian J Hematol Blood Transfus. 2017;2(2):1–7.

[33] Nicolay JP, et al. Inhibition of suicidal erythrocyte death by nitric oxide. Pflügers Archiv – Eur J Physiol. 2008;456(2):293–305.

[34] Lang F, Lang KS, Lang PA, et al. Mechanisms and significance of cryopreservation. Antioxid Redox Signal. 2006;8(7–8):1183–1192.