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Citral and Mechanical Perturbation Improve Viability of RBCs in Blood Banks

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Abstract

Objective: To explore the effect of citral preservation solution and physical disturbance before infusion on reducing blood stock damage. Methods: The red blood cell suspension with citral was disturbed after 7 days of storage, then the NO level were detected by nitrite kit. The physiological indexes were detected by routine blood test. The particle size of supernatant was measured by Malvern nano laser particle size analyzer. Results: Adding citral to the preservation solution and physical perturbation before infusion could increase the NO production of erythrocytes, reduce hemolysis and maintain the physiological indexes of the RBCs. Conclusion: We proposed for the first time that adding citral before RBC storage or mild physical perturbation at the later stage of stock can improve the rheological and physiological indexes of RBCs in blood bank.

Keywords

Blood bank, Citral, Mechanical perturbation, NO, Routine blood test.

1. Introduction

Blood transfusion is considered to be one of the most effective treatments to save lives. But during blood storage, red blood cells (RBC) undergo a series of biochemical changes that affect cell morphology and physiology. These changes are collectively referred to as it is "storage damage" of red blood cells[1], and the damage is more significant with the increase of stock time. However, it has potential harm to the health of patients. Therefore, it is of great physiological significance to reduce inventory damage before infusion [2].

A series of studies in recent years have shown that nitric oxide (NO) is closely related to the physiological activity of stored RBCs. NO is not only related to the deformability of RBCs[3; 4], but also can be used as a vasodilator molecule to reduce the adhesion between erythrocyte and vascular endothelium, and also has an antithrombotic effect that reduces platelet activation[5]. However, when red blood cells are stored at low temperature, the rapid decrease of NO and its derivatives in red blood cells may be the initiation factor of storage damage. Therefore, reducing NO consumption in RBC is of great significance for reducing storage damage and thus improving the prognosis of transfused patients[6]. Since shear stress can regulate the nitric oxide synthase activity[7; 8; 9; 10], In order to

ISSN: 2414-1895 DOI: 10.6919/ICJE.202004_6(4).0018

provide a simple method for the activation of stock cells, We use oscillation to investigate the effect of physical perturbation on the NO of red blood cells in stock.

Citral is the main component of the essential oil of Litsea cubeba[11], a traditional Chinese medicine. It has many biological activities such as anti-bacteria, anti-fungus, insecticidal and anti-inflammatory[12; 13], no acute and cumulative toxicity to animals and human [14], and it has been approved as a food additive by the Ministry of health of China[13]. As a natural plant component, it has been applied in many clinical treatments, mainly with antithrombus, treatment of cardiovascular disease, anticancer activity[15] and antioxidant effect[16; 17]. The application of citral in stored blood has not been reported. This paper is the first time to explore the effect of citral additive on the physiological indexes of stored red blood cells, and aims to provide natural anti-stock damage components for blood preservation solutions to maintain the red blood cells' biochemical activity.

2. Materials and Methods

2.1 Sample and materials

Red blood cell suspension (From the Blood Transfusion Department of the First Affiliated Hospital of Jinan University). Malvern Nano Laser Particle Size Analyzer (Zetasizer Nano ZS); High-speed refrigerated centrifuge (Allegra 64R); Full-automatic blood analyzer (Sysmex XS 800i); Bench-top thermostat (QYC 200); Citral (Sigma); EDTA anticoagulation Tube (SanLi)

2.2 Physical perturbation induces RBC-NO production

The red blood cell suspension was dispensed into 2 mL EDTA tubes (SanLi) , a total of 18 tubes, of which 9 tubes were added with citral (Sigma) according to the volume ratio of blood sample to each group of drugs: 9: 1, labeled as the Citral group; the remaining 9 tubes were labeled as CK Group; mark and wrap it with tin foil, then put it in a refrigerator at 4 °C for 7 days. After 7 days of stroage, suspended RBCs were subjected to mechanical perturbed with a shaker (QYC 200) at 200 rpm for 0,1 and 5 min. NO production was measured by Nitrite Kit (Shanghai Enzyme) at 495/515 nm.

2.3 Blood test by Full-automatic blood analyzer (Sysmex XS 800i)

A suspension of RBCs after 7 days stroage were sent to the Overseas Chinese Affiliated Hospital of Jinan University for blood tests.

2.4 Supernatant particle size measurement

After shaking treatment, 2 mL of blood was centrifuged by high-speed refrigerated centrifuge (Allegra 64R) in a 5 mL centrifuge tube, and the supernatant was taken to test particle size measurement by Malvern Nano Laser Particle Size Analyzer (Zetasizer Nano ZS).

ISSN: 2414-1895 DOI: 10.6919/ICJE.202004_6(4).0018

2.5 Statistical analysis

All experiments were performed in triplicate (n=3) unless otherwise specified. Data were analysed with GraphPad Prism 7 and SPSS 16.0. and "x ± s" is used for measurement data. T-test was used when two conditions were compared and one-way Anova used for multiple comparisons. Values of $p < 0.001$ were selected as showing a statistically significant difference.

3. Results

3.1 Citral and physical perturbation improves RBCs-NO level

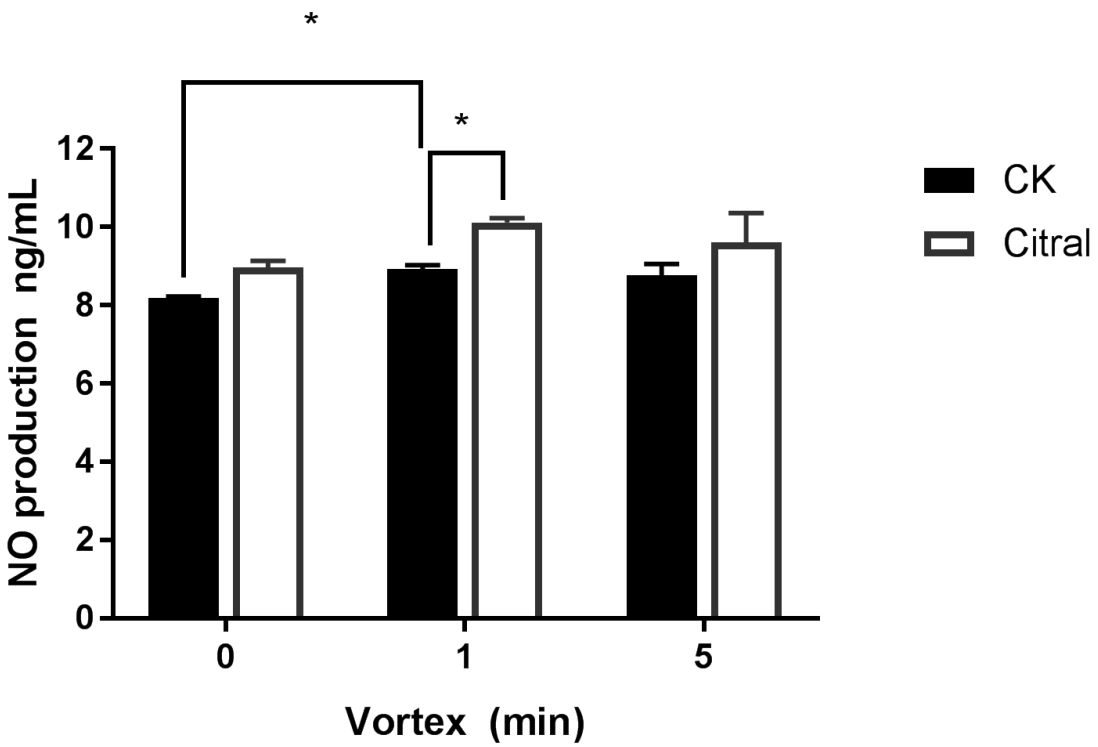

Figure 1. Physical perturbation induces RBC-NO production.

RBCs were incubated with citral at 4 ℃ for 7 days and subjected to mechanical perturbed with a shaker (QYC 200) at 200 rpm for 1 and 5 min (n =3； *p<0.001), versus control RBC (static), CK means group without citral.

Citral improves RBCs-NO level. As can be seen from the figure above, when the natural plant component citral is added before blood preservation, compared with the non-medicated group, citral can increase the NO of red blood cells in the storage, which may be that citral can reduce the activity attenuation of NO in low-temperature storage . Therefore, the addition of citral in the preservation solution is conducive to maintaining the deformability of the infused cells, and it is beneficial to dilate the blood vessels and prevent blockage after infusion.

Physical perturbation induces RBCs-NO production. When the red blood cells were in stock for 7 days, a physical disturbance was given. It can be clearly seen that the mechanical disturbance can promote the release of NO, but the amount of NO released and the physical disturbance time do not have a positive correlation. This shows that when the red blood cells are mechanically disturbed, they will respond to shear stress and then trigger the NO synthesis pathway. This provides us with a new and simple way to reduce inventory damage before infusion.

In order to prove that physical perturbation and citral did not negatively affect other physiological indexes of red blood cells, we also performed blood test and supernatant particle size tests on the treated red blood cells.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202004_6(4).0018

3.2 Citral and physical disturbance improve physiological index of blood bank

Table 1. Blood test parameters of physically disturbed RBC stocks after 7 days

	CK	CK-1min	CK-5min	Reference range
RBC	6.33±0.31	4.51±0.14	5.255±0.04	3.8-5.8
HGB	159.5±9.19	122±1.41	131.5±2.12	110-170
HCT	48±2.40	37.65±0.64	40.3±0.28	35-51
MCV	75.85±0.07	83.5±1.27	76.7±0	80-100
MCH	25.15±0.21	27.05±0.49	25.05±0.21	27-35
MCHC	332±2.83	324±1.41	326±2.83	320-360
RDW-SD	59.65±0.92	53.45±3.04	58.8±0.42	RDW-SD
RDW-CV	22.85±0.35	17.8±1.27	22.1±0	11.5-15.0

Physical disturbance improve physiological index **of blood bank.** From the reference range, whether the red blood cells are shaken or not, their HGB, HCT and MCHC are within the normal range, while the average red blood cell volume (MCV) and the average hemoglobin (MCH) only return to normal range after 1 minute of disturbance, indicating that mechanical disturbance can reverse the erythrocytes with stock damage. The MCV refers to the average volume of a single red blood cell in the human body, which is closely related to the deformability of the cell. If the cell deformability is damaged, its clinical infusion effect will have potential hidden dangers. Red blood cell distribution width (RDW) is a parameter that reflects the heterogeneity of red blood cell volume[18], and is usually expressed by the coefficient of variation of red blood cell volume. The RDW reflects the consistency of the size and shape of red blood cells in the blood sample. When the width is larger, means the shape and size of red blood cells are more different. Otherwise, indicating that the shape and size of the red blood cells in the sample are consistent. RDW is usually expressed in two forms: RDW-CV and RDW-SD. Where SD is the absolute value and CV is the relative value of SD relative to the measured value[19]. As can be seen from the table above, when the blood in the storage for 7 days is physically disturbed, the RDW is significantly reduced, indicating that the disturbance is conducive to the uniform and consistent red blood cell morphology.

Table 2. Blood test parameters after 7 days of citral in red blood cell suspension

	Citral	Citral -1min	Citral -5min	Reference range
RBC	4.835±0.32	5.23±0.04	5.45±0	3.8-5.8
HGB	124.5±2.12	130.5±0.71	136.5±0.71	110-170
HCT	37.95±0.57	39.9±0.28	41.55±0.07	35-51
MCV	78.55±4.88	76.25±0.07	76.2±0.14	80-100
MCH	25.75±1.48	24.95±0.07	25.05±0.07	27-35
MCHC	328±0.71	327±0	328.5±2.12	320-360
RDW-SD	58.15±9.62	57.7±0	57.9±0.85	
RDW-CV	21.05±4.24	21.95±0.21	22.1±0.28	11.5-15.0

Citral can improve physiological index of blood bank. The table above is the result of perturbation after 7 days of erythrocyte suspension and citral storage. According to the value of the reference range,

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it can be seen that after adding citral, the RBC, HGB, HCT, MCV and MCHC are within the normal reference range, which are two more normal indicators than group without citral. It can be concluded that citral does not cause abnormal changes in the conventional indicators of red blood cells after it acts on the stock suspension, but helps maintain the normal physiological state of red blood cells.

3.3 Citral can improve physiological index of blood bank

Table 3. PdI value of the RBC supernatant after 7 days of storage

	PdI		PdI		PdI
CK	0.75±0.06	CK -1 min	0.68±0.04	CK -5 min	0.66±0.004
Citral	0.74±0.06	Citral-1 min	0.65±0.005	Citral-5 min	0.58±0.02

PdI (Polydispersity Index) is a polydispersity index, which is usually used to describe the molecular weight distribution of a polymer, indicating the degree of uniformity of the particle size distribution. For a polymer, its molecular weight is usually non-uniform, and it is essentially a mixture. The larger the PdI, the wider the molecular weight distribution range; the smaller the PdI, the more uniform the molecular weight distribution.

Citral and physical perturbation reduce PdI index of blood bank. From the PdI results, it can be seen that with the physical disturbance, both the CK group and the citral group showed a decrease. From this, it can be seen that the disturbance is beneficial to maintain the stability of the cells and improve the homogeneity of the particle size distribution of the supernatant. Compared with the addition of the citral component before the stock, the PdI of the supernatant decreased, and the PdI of the citral group that disturbed the same time was also lower than the blank group, indicating that the molecular weight distribution of the particles in the supernatant was more concentrated after the citral effect. It suggests that citral can reduce the difference and abnormal change of particle size in RBC suspension and improve the particle homogeneity in the supernatant.

Table 4. Peak particle size distribution of erythrocyte suspension in stock for 7 days

	CK		Citral	
	Size(d.nm)	%Intensity	Size(d.nm)	%Intensity
Peak1	15.9	7.4	21.5	7.6
Peak2	156.0	70.0	157.5	66.7
Peak3	2133.0	22.6	1984.0	25.7

From the measurement results of the particle size distribution of the supernatant of RBCs, it can be seen that the particles with a size ranging from 100 to 300 nm account for the largest proportion, which are hemolyzed aggregated products of RBCs, and have the potential risk to cause exogenous particulate blood vessel deposition in the recipient, which is the main indicator of the particle size distribution of the supernatant; particles with a size of a few to tens of nanometers are tetramers or polymers of hemoglobin and with a particle size of several thousand microns are RBC membrane vesicles or fragments.

Citral protect the integrity of RBCs. As can be seen from the above table, three peaks in the particle size of the supernatant appeared in the CK group and citral group, and the peaks occupying the largest share were Peak 2. The percentage of Peak 2 in the citral group was smaller than that in the CK group , Suggesting that citral can reduce the aggregation of RBC hemolysate; and Peak 3, which characterizes the size of membrane vesicles, is obviously smaller than the CK group, suggesting that citral can protect the integrity of RBCs and reduce the production of membrane vesicles.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202004_6(4).0018

4. Conclusion

The citral can not only reduce the NO prodution of the red blood cells in the storage and maintain the deformability of the red blood cells, but also can improve the uniformity of the particle size of the supernatant of the RBC. The integrity of RBC, reduction of erythrocyte membrane vesicle formation, and decrease of hemolysate aggregation, which have important physiological effects on blood transfusion into recipients. It can reduce its risk of depositing and forming thrombus in blood vessels, and improve the safety and efficiency of blood transfusion.

Physical perturbation induces RBCs-NO production after storage, which is beneficial to maintain the deformability of RBCs. The deformability of RBCs is very important for the optimal rheology and gas exchange function in the process of capillary flow, which is helpful for blood transfusion to play its physiological role.

In summary, adding a appropriate citral concentration before RBC storage or mild physical perturbation at the later stage of RBC storage can improve the rheological and physiological indexes of RBC in blood bank.

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