Addition of haptoglobin to RBCs storage, a new strategy to improve quality of stored RBCs and transfusion

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ABSTRACT

Transfusion of red blood cells (RBCs) is an effective therapy in surgery and critical care. Comparing to fresh RBCs, the therapeutic effect of stored RBCs is greatly limited because of its loss of NO during storage, which leads to vasodilatation dysfunction upon transfusion. So far, there is no effective solution to this problem. Here, we summarize the protective effects of Haptoglobin (Hp) in RBCs storage and transfusion, by using data extracted from literature review. Because Free Hemoglobin (FHb) is the major factor responsible for rapid NO loss during storage, addition of FHb-sequestering protein Haptoglobin will prevent the loss of NO and improve the quality of stored RBCs.

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Background

Transfusion of red blood cells (RBCs) is a life-saving therapy in treating anemia, trauma and surgical patients with massive blood loss. Over the last 30 years, great care has been taken on the varied transfusion-related complications. Due to the strict screening during blood collection, risk of transfusion-related virus infection has decreased significantly. On the other hand, clinical studies suggest that RBCs stored for long periods (often described as >14–21 days) may mediate adverse effects in the recipient, leading to morbidity and mortality [1,2]. This is due to the fact that the biochemical and mechanical properties of RBCs deteriorate progressively during storage, so-called storage lesions. When stored in preservative solutions, the metabolism of RBCs can be maintained stable at 4 °C for as long as 42 days; however, properties such as its flexibility [3], adhesivity [4] and oxygen affinity [5] change continuously with time prolonging. Besides, the consequences of RBC storage, such as impaired blood flow, impaired O2 delivery to tissues, hemolysis, and imbalance in nitric oxide (NO) homeostasis, are well-documented [6]. Among them, the NO homeostasis is more visible recently and the INOBA (insufficient NO bioavailability) is considered the main cause of vascular effects resulting from RBC storage lesion by Robact [7]. His studies of this hypothesis have observed older RBC units could block NO-mediated vasodilation in rat aortic ring models [7].

NO, the key vasodilator, is generally synthesized by nitric oxide synthase (NOS) using L-arginine as substrate in endothelium. It matches the blood flow and oxygen delivery to local hypoxemic tissue, thus plays a very important role in microcirculatory function. NO binds to hemoglobin (Hb) on cysteine-93 to form S-nitroso hemoglobin (SNO-Hb) [8] which is the main mode to store and transport NO in blood. When reaching the hypoxia tissue, SNO-Hb is bioactivated and releases NO to induce vasodilation [9]. Study in rat brain has also found that extracellular SNO-Hb can couple local blood flow to tissue oxygen tension by constricting or dilating blood vessels [10].

However, the SNO-Hb in banked blood was declining during storage. In the ex vivo blood the concentration of SNO-Hb declines rapidly. Reynolds et al. reported that after 1 day the SNO-Hb levels had declined by 70%, and at 1 week the decline was 83% of the initial levels in stored swine blood [11]. Similar results were also seen in stored human whole blood [11]. Bennett-Guerrero et al. found that SNO-Hb were depressed in processed samples at 3 h (1.2 × 10^-4 at 3 h vs. 6.5 × 10^-4 (fresh) mol SNO/mol Hb tetramer) and remained low over the 42-day period [12]. Such rapidly declining of SNO-Hb levels will result in reduced vasodilation, an important ending point in critical care. The results rooting from in vivo experiments, which showed strong correlation between vasodilation of banked RBCs and the amounts of SNO-Hb, and a greater blood flow during infusing SNO-replete blood than SNO-depleted blood in canine coronary ischemic model, suggests that SNO-Hb deficiency during storage impairs the vasodilatory response of banked blood, especially to hypoxia [11]. Therefore, finding ways to preventing the rapid NO decline in banked blood may ameliorate the storage lesion and improve transfusion efficacy.

In blood, free hemoglobin (FHB) is considered a chief criminal to oxidize NO at a high rate into nitrate (NO3) and methemoglobin (MetHb) [13], which resulting in vasodilative disorders. Recent research indicates that the NO consumption in intact RBCs is much slower than that in FHB solution with an equivalent Hb.
concentration [14]. With the RBCs storage time, the concentration of FHb increases as RBCs break down [12]. The review has listed the significantly increased plasma Hb during processing and storage in many researcher [15]. And it is reported that the hemolysis appears donor independent [16]. When used for transfusion, the bolus Hb would scavenge NO and inhibit the binding between hemoglobin within erythrocyte and NO. Composed with fresh RBCs, RBCs stored for 3–14 days produced a significant shift in the dose–response curve, with a reproducible 50% greater inhibition of relaxation in aortic ring assays in vitro. The inhibitory activity further increased with extended storage: RBCs stored for 28–42 days almost completely eliminated ACh-stimulated NO-mediated vasodilation [7]. Until now, actual measurement of the NO consumption in vivo after storage RBCs transfusion is also lacking, but we can get information via the studies in other diseases. Jeffers et al. [17] computed that levels of plasma Hb as low as 1 μM can limit nitric oxide bioavailability significantly in hemolytic anemia. Pohl et al. [18] showed reduced NO responsiveness in isolated perfused rabbit hearts infused with as little as 6 μM Hb. Another study also confined that the forearm blood flow, responses to infusions of nitroprusside (an NO donor), were more than 80% reduced at plasma heme concentrations ≥6 μM in sickle-cell disease [19]. Although the preserving method is continuously improving, the hemolysis during storage can not be prevented. It was shown that FHb in stored blood increases steadily in the medium reaching approximately 20 μM at the end of the 6-weeks storage in CP2D (citrate–phosphate–dextrose–dextrose) anticoagulant solution [12]. Importantly, washing the stored RBCs did not prevent the adverse effects, as the aged cells continued to hemolyze intravascularly after transfusion [20]. It is reported that despite washing, extracellular Hb concentrations remained high (up to 0.7 g/L in a given blood bag, equally to 22 μM according to the MW (32 kDa) of FHb) during preparative blood salvage, even though this value is lower than most concentrations reported in the literature [21]. Besides the effect on NO consumption, the FHb is associated with the generation of hydroxyl radical and lipid peroxidation. When FHb is filtered through the glomerular barrier and reabsorbed by proximal tubular cells, acute renal failure may occur. Taking together, removing FHb from stored blood will significantly reduce NO consumption, thus serve as a novel solution to improve the quality of stored RBCs, promote the oxygen supply and lighten the oxidative stress induced by FHb.

The hypothesis

Haptoglobin (Hp), an acute phase reaction protein, is well known for its ability in binding and transporting FHb. We hypothesize that addition of Hp to the RBC storage suspension should sequester FHb and prevent its damage on stored RBCs.

Evaluation of the hypothesis

Haptoglobin was first described in 1940 for its Hb binding properties [22]. It is a plasma a2-glycoprotein consisting of an alpha and a beta polypeptide chain [23]. There are two classes of alleles for Hp (1 and 2) existing in human. After being synthesized in hepatocyte and released into the blood, Hp binds rapidly and irreversibly to the alpha chain of the oxygenated, free Hb [24]. This binding is one of the strongest known non-covalent interactions in biology with a very high affinity of approximately 10−15 mol/L [25]. The Hp–Hb complex, whose half-life is 10–30 min [23], binds to the CD163 receptor which located on the cell surface of monocytes and macrophages, after which the Hp–Hb–CD163 complex is rapidly cleared by the reticuloendothelial system in the liver. The Hp 1–1–Hb complex is endocytosed and cleaned much more rapidly than the Hp 2–2–Hb complex [26]. Therefore, the major function of the Hp–Hb complex is the hepatic clearance and degradation of FHb [24]. Previous report confirms that Hp concentration correlated negatively with time in storage blood which resulting in increased FHb [27]. During surgery for major trauma, serum Hp concentration decreased after blood transfusion of ≥1000 ml of whole blood with mean storage time of 12.2 days [28]. It suggests important physiological functions of Hp during blood storage and transfusion. In clinic, the determination of Hp concentration in serum or plasma is used for the detection of hemolysis since the Hp–Hb complexes are quickly removed from the circulation after hemolysis [29].

How does the Hp–Hb complex inhibit the rapid consumption of NO by scavenging FHb? With abundant references, we explain the phenomenon as follows. (1) FHb loses its oxidizing ability after irreversibly binding to haptoglobin [30]. The oxidizing ability is the key of the NO inactivation when being exposed to FHb. It is the main factor for the NO protecting. (2) The Hp–Hb complex may be effective on reducing nitrite to NO. The Hb in the Hp–Hb complex is R conformation [31] and has higher reducibility [32,33]. It is a different pathway to supply the NO in stored RBCs. (3) The Hp–Hb complex keeps the integrated structure of Hb [34]. The normal structure of Hb is convenient to play its' role on forming SNO-Hb [35] and transporting O2 to hypoxia tissue [36]. But once released from erythrocyte, FHb is readily oxidized to produce MetHb and loses the O2 and NO binding ability. The Hp–Hb complex can inhibit the oxidation and keep the physiological function of Hb. Moreover, the Hp–Hb complex could not be excreted in kidney via its high molecular weight. It reduces the risk of renal oxidative stress damage induced by FHb [37].

Although the mechanism is unknown, it is widely observed that Hp could raise the bioavailability of NO. The above might be the reasonable explanations supported by previous literatures. Some researchers reported that Hp did not change the consumption of NO in vitro by using computer simulation [38]. At the same time, the reporters admitted that they stood on the viewpoint that Hp could improve the bioavailability of NO, and thus could not explain their computer simulation results. We speculate that the simplicity of the simulation in vitro and the insensitivity of testing method generate the inaccurate consequence.

The hypothesis that FHb can scaveng NO and reduce its bioavailability is further supported by studies in many other diseases. The transgenic mice with the Hp 1–1 genotype had markedly less vasospasm as compared to Hp 2–2 mice after the induction of subarachnoid hemorrhage (SAH) [39]. Hp affects nitric oxide bioavailability in preeclampsia [40]. On the above basis, the exogenetic Hp is used in animal experience to determine its effect in vivo. Boretti et al. [41] showed that both the induced expression of Hp via glucocorticoid pathway and the exogenous injection of Hp could remove FHb, neutralize the vasculotoxic oxidative stress and protect against systemic hypertension in canine and guinea pig models. Furthermore, the recent study showed transfusion with old blood (stored 28 days) led to adverse effects, such as intravascular hemolysis, acute hypertension, vascular injury, and kidney dysfunction associated with pathophysiology driven by FHb, all of which could be dramatically attenuated when Hp (750 mg) was administered at the time of transfusion in guinea pigs [42]. This study proves the effect of Hp on FHb suppression in vivo during old blood transfusion for the first time and encourages us to explore its application in clinical transfusion extensively.

The characters of Hp also bring it to become the proper assistant of transfusion. In any individual the Hp level remains fairly constant and it is 0.3–3 mg/ml in normal human body [43]. Due to the 1:2 binding between Hp and Hb dimers, the normal concentration of Hp could combine with micromole levels of FHb theoretically. Actually, research about sickle cell disease with chronic...
hemolysis found that patients had approximately 4 µM FHb in plasma and undetectable haptoglobin [19]. Research indicates that the concentration of FHb gets to 20 µM in the stored blood for 6 weeks [12] and even reaches to 22 µM or even more in body after the blood transfusion [20,21]. Actually, decreased concentration of Hp has been observed in patients transfused with massive prolonged stored RBCs [28]. Apart from this, Hp is not recycled. When it is completely saturated with FHb, Hp levels can take 5–7 days to recover, because synthesis is not increased by low Hp levels [44]. It is possible that the FHb after transfusion is so excessive that the NO-mediated vasodilation is inhibited. In such cases, once the FHb in stored blood is scavenged by added Hp, the RBCs will reach the tissue more easily and lead to better effect of transfusion.

Consequences of the hypothesis and discussion

The rapid decomposition of NO in stored RBCs has caused important untoward reactions upon transfusion. It both limits the period and quality of stored RBCs and weakens the effect of transfusion. Several retrospective studies have shown increased mortality, higher risk of infections, and multiorgan failure in patients transfused with older blood [45–48]. To resolve the problem of NO rapid consumption in stored RBC suspension, researchers raised the proposal of supplementing the NO anew by adding NO or NO donor. Reynolds et al. [11] suggest that SNO-Hb repletion via exploring the ramifications and lipid peroxidation, subsequently results in systemic activation and inflammation in subarachnoid hemorrhage (SAH) [39]. And the Hp infusion in vivo did not show adverse effect on recipients’ condition overall [56].

(1) How to determine the standard concentration of Hp?

Hp is an native protein in human blood and stored RBCs [27]. From the current view, to abolish the adverse effect of FHb in RBCs storage and transfusion, the concentration of additive Hp requires about 10 µM at least (according to the 20 µM FHb [12] after 6 weeks storage and 1 Hp:2 Hb dimers combination). Until now, there are not sufficient data in the literature about the harm of high concentrations of Hp in vivo. But animal experiments which administrate Hp at dose of 201.23 mg/dl (about 5.03–25.15 µM) in dogs [41] and 750 mg in pigs [42] did not show the adverse effect. To assure the adequate and nontoxic dose of Hp in RBCs preservation, the precise concentration should be determined via further experiment. Moreover, the detailed effect of Hp on specific patients, such as newborns, little kids and patients with kidney or liver failure, need to be determine, too.

(3) What is the effect of higher concentration of Hp–Hb complexes in RBCs in vivo?

Hp–Hb complex is alleged to increase White Blood Cells activation and the inflammatory response in vivo due to degradation in monocytes and macrophages. Actually, this scavenging system elicits an anti-inflammatory response. Firstly, the interaction between Hp and Hb attenuates Hb-mediated oxidative organ damage [52]. Secondly, as mentioned above, Hp highly expedites Hb clearance, leading to production of anti-inflammatory metabolites [53]. Thirdly, researches have shown the anti-inflammatory effect of Hp–Hb complex [54–56]. It has been suggested that intracellular signaling cascades activated by Hp–Hb binding to cell-surface CD163 may contribute even further to an anti-inflammatory response [54] and elicit secretion of IL-10 [54,55]. The transgenic mice with the Hp 1–1 genotype had markedly decreased inflammation in subarachnoid hemorrhage (SAH) [39]. And the Hp infusion in vivo did not show adverse effect on recipients’ condition overall [56].

(4) others

In clinic, the stored RBCs is washed by saline before transfusion, which is considered removing FHb effectively. But we learn that despite washing, extracellular Hb concentrations remained high (up to 0.7 g/L in a given blood bag) [21]. The phenomenon prompt the research to explore new strategy to address the question.

In summary, Hp is considered as both the protector of stored RBCs and the adjuvant of transfusion. It is logical to believe that therapeutics increasing Hp level might help to limit toxic effects in pathological conditions associated with transfusion. James et al. [57] have realized the necessity of augmenting the capacity of the intrathecal Hp scavenging system to enhance FHb efflux in subarachnoid hemorrhage. Future research should be performed to explore the safeness and proper dosage of Hp addition in RBCs storage and transfusion therapy.

Conclusion

Data from literature review supports our hypothesis that Hp could inhibit rapid consumption of NO by sequestering FHb. Addition of Hp to the RBC storage suspension is a promising strategy to improve quality of blood storage, thus potentially lead to better transfusion result and higher success rate in emergency treatment. These data pave ground for the next step in laboratory investigations and clinical studies.

Authorship statement

(1) The paper is not under consideration elsewhere; (2) none of the paper’s contents have been previously published; (3) all
authors have read and approved the manuscript; and (4) There are no relationships with industry and no competing financial interests exist.

Conflicts of interest
None declared.

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