



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Ubiquitous convalescent plasma: An artificial universal plasma for COVID-19 patients

Mahla Sattarzadeh Bardsiri^{a,b,c}, Seyedeh Mehrnaz Kouhbananinejad^{b,c}, Reza Vahidi^d, Saeed Soleimany^e, Masoud Moghadari^f, Ali Derakhshani^{b,d}, Bahareh Kashani^g, Alireza Farsinejad^{b,c,*}

^a Student Research Committee, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran

^b Cell Therapy and Regenerative Medicine Comprehensive Center, Kerman University of Medical Sciences, Kerman, Iran

^c Department of Hematology and Laboratory Sciences, Faculty of Allied Medical Sciences, Kerman University of Medical Sciences, Kerman, Iran

^d Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran

^e Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

^f Department of Traditional Medicine, Faculty of Traditional Medicine, Kerman University of Medical Sciences, Kerman, Iran

^g Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:

Convalescent plasma
COVID-19
Adsorption procedure
SARS-CoV-2
Antibody titer

ABSTRACT

Objectives and background: In December 2019, the first case of COVID-19 was reported in Wuhan, China. Its causative virus, is a novel strain of RNA viruses with high mortality rate. There is no definitive treatment, but among available approaches the use of recovered patients' plasma containing specific antibodies can enhance the immune response against coronavirus. However, the dearth of eligible donors and also ABO incompatibility in plasma transfusion, have limited this therapeutic method. Therefore, it is highly desirable to introduce a simple procedure that allows efficient reduction or even removal of natural ABO antibodies. Accordingly, we aimed to evaluate a RBC-mediated adsorption technique that reduces the titer of the mentioned antibodies in plasma.

Methods/materials: This experimental study was conducted in Kerman University of Medical Sciences, Kerman, Iran. The pre- and post-incubation antibody titers of 168 plasma samples were determined. For incubation, each plasma sample was exposed (60 min) to different percentages of RBCs at room temperature or 4 °C.

Results: The results evidenced that both the concentration of RBCs and temperature had significant decreasing effects on antibody titer ($P < 0.001$) and all concentrations significantly reduced titer. Compared to RT, 4 °C further reduced the antibody titer. Overall, the best incubation condition for reducing antibody titer in all blood groups was 4 °C and 2% RBCs concentration.

Conclusion: The presented adsorption procedure is able to produce universal plasma (we call it Ubiquitous Convalescent Plasma) with a non-immunogenic level of ABO mismatch antibodies which can be used for COVID-19 patients with any type of blood group with desirable simplicity, feasibility, and efficacy.

1. Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in late December 2019 in Wuhan, China and rapidly spread worldwide [1,2]. Until December 2020, the COVID-19 pandemic infected 82 million people and caused nearly 1.79 million deaths [3]. The exponentially increasing rate of cases infected with COVID-19 has prompted researchers to seek effective and definitive therapeutic

options. Besides pharmacotherapy [4,5], other remedies such as convalescent plasma (CP) therapy, vaccination, and stem cell therapy are under investigation in several clinical trials [1,6]. Nowadays, CP therapy is considered a safe, effective, and FDA-approved immunotherapy approach [7] which is utilized for different diseases such as SARS, Middle East respiratory syndrome coronavirus, Ebola, Zika, and pandemic influenza A [1,8,9]. In this therapeutic option, recovered patients' plasma containing pathogen-specific antibodies are used to provide immediate immunity [1,5,8]. Although this approach can be

* Corresponding author at: Cell Therapy and Regenerative Medicine Comprehensive Center, Kerman University of Medical Sciences, Kerman, Iran.

E-mail address: afarsinejad@kmu.ac.ir (A. Farsinejad).

<https://doi.org/10.1016/j.transci.2021.103188>

Received 8 March 2021; Received in revised form 5 June 2021; Accepted 8 June 2021

Available online 10 June 2021

1473-0502/© 2021 Elsevier Ltd. All rights reserved.

effective in the COVID-19 patients [10], it has some limitations including the dearth of eligible recovered patients and ABO-incompatibility. Except for individuals with AB blood group (universal plasma donors) [11], others have cold reactive natural antibodies directed against red blood cells (RBCs), high concentration ($\geq 1:64$) of which leads to various complications and symptoms [12]. Despite the mentioned practical advantage of the AB group, low prevalence limits its widespread application for CP therapy; the frequency of blood groups is race/ethnicity-dependent, but overall, their prevalence in white populations is as follows: O > A > B > AB (45 %, 40 %, 11 %, and 4%, respectively) [13]. Therefore, it is critical to find methods that allow transfusion of ABO-mismatched plasma. Accordingly, for the first time, we decided to optimize an adsorption method to produce a universal plasma that can be transfused into patients with any blood group. By obviating the deficiency of matched donors, this process allows the widespread use of CP therapy in the treatment of various diseases such as COVID-19.

2. Materials and methods

2.1. Blood collection

The current study was approved by the ethical review committee affiliated with Kerman University of Medical Sciences, Kerman, Iran. Before blood collection, informed consent was obtained from 168 healthy donors (56 samples from each A, B, and O blood group; 7 samples for each incubation condition). In a sterile condition, whole blood was collected into bags containing an FDA-approved anticoagulant preservative solution (Citrate-Phosphate-Dextrose-Adenine). The ABO group, Rh type, and anti-viral antibodies (anti-hepatitis C virus and anti-human immunodeficiency virus 1/2 antibodies) were determined for each donor. Using differential centrifugation, plasma was separated from whole blood and platelets, then stored at -20°C .

2.1.1. Antibody titration

Based on the type of plasma and packed cells, for initial titration, the samples were divided into four groups: 1) Plasma A tittered with B packed cells (A–B), 2) Plasma B tittered with A packed cells (B–A), 3) Plasma O tittered with A packed cells (O–A), and 4) Plasma O tittered with B packed cells (O–B). Next, antibody titration was performed as follows: Ten test tubes were assigned for serial dilution and 500 μL of saline was transferred to tubes 2–10. After the addition of 500 μL plasma to tubes 1 and 2, tube 2 was mixed thoroughly and 500 μL of its content was transferred into the next tube. This process was repeated for the next tubes and eventually, 500 μL of contents of tube 10 was removed. Therefore, tubes 1–10 contained undiluted plasma and dilutions of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, and 1/512 of plasma, respectively. Finally, 10 μL of related packed RBCs was transferred into each tube (final concentration = 2%) and after centrifugation ($1000\times g/30\text{ s}$), agglutination was examined macroscopically. The last serum dilution with visible agglutination was defined as the antibody titer [14].

2.2. Adsorption procedure

According to the packed red cell concentrations (0.5, 1, 2, and 4%) and incubation temperature [room temperature (RT) and 4°C], the samples were divided into 32 groups: 1) Plasma A incubated with B packed cells (0.5 %) at RT, 2) Plasma A incubated with B packed cells (1%) at RT 3) Plasma A incubated with B packed cells (2%) at RT, 4) Plasma A incubated with B packed cells (4%) at RT, 5) Plasma A incubated with B packed cells (0.5 %) at 4°C , 6) Plasma A incubated with B packed cells (1%) at 4°C , 7) Plasma A incubated with B packed cells (2%) at 4°C , 8) Plasma A incubated with B packed cells (4%) at 4°C , and as the same way, 8 groups for plasma B (incubated with A packed cells), 8 groups for plasma O (incubated with A packed cells), and 8

groups for plasma O (incubated with B packed cells). To perform adsorption procedure, in each group, 5 mL of plasma was mixed with 25, 50, 100, or 200 μL of related packed red cells (final concentrations of packed cells = 0.5, 1, 2, and 4%) and incubated for 60 min at RT or 4°C . The mixture was shaken during the incubation phase periodically and then centrifuged at $1000\times g$ for 5 min to form tightly-packed cells. Finally, the supernatant was harvested for the secondary antibody titration according to section 2.2. procedure.

2.3. Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics 23 software. Reciprocal antibody titers were transformed into $\log_2^{(x+1)}$ prior to analysis [15,16]. The comparisons of antibody titer reduction in different temperatures and RBC concentrations were performed by two-way analysis of covariance (ANCOVA). The initial titer was used as the concomitant covariate in ANCOVA analysis for adjustment. The initial assumptions of ANCOVA analysis (normality and homogeneity of regression slopes) were also checked. The level of significance was considered $P < 0.05$. Results were presented as mean \pm standard error (SE).

3. Results

Overall, the concentration of RBC and incubation temperature had significant decreasing effects on antibody titer ($P < 0.001$). However, the interaction between the concentration of RBCs and temperature was not statistically significant (A-B: $P = 0.242$, B-A: $P = 0.891$, O-A: $P = 0.198$, and O-B: $P = 0.374$). The Bonferroni pairwise comparison test between different concentrations of RBCs showed that all concentrations significantly reduced antibody titer ($P < 0.05$). Concerning temperature, this comparison test evidenced that 4°C had a significantly higher decreasing effect on antibody titer compared to RT (A–B, O–A, and O–B: $P < 0.001$ and B–A: $P < 0.01$). Furthermore, incubation at 4°C had a higher decreasing effect on antibody titer in all concentrations of RBCs. According to Fig. 1, the antibody titer alterations had a linear relationship with RBCs concentrations. The lowest concentration of RBCs that declined the antibody titer to an acceptable level ($< 1:64$) was 2 percent.

4. Discussion

There is no specific approved protocol for the treatment of COVID-19, but some antiviral drugs and adjunctive therapies (use of blood products and immunomodulators) can be applied for the alleviation of coronavirus inflammatory effects [17,18]. For instance, stem cells with immunomodulatory properties as well as CP can be added to the treatment protocols of COVID-19 [19]. Donated plasma from recovered patients has been investigated in the treatment of many infectious diseases such as H_1N_1 influenza, SARS, and Ebola [20]. This blood product is cost-effective and provides passive immunization by virus-specific antibodies, limits virus replication, and accelerates immune response to viral infection [21,22]. Recently, this modality has been noticed as promising supportive treatment for COVID-19 patients and its proper usage at an early stage of the disease can reduce hospitalization [23]. In this regard, Duan et al. treated 10 COVID-19 patients with 200 mL CP within 4 h (antibody titer $> 1:640$); the results evidenced improvement of clinical symptoms, enhancement of oxyhemoglobin, increase/maintenance of the pathogen-neutralizing antibody titer, and lack of adverse effects [1]. Furthermore, in a clinical trial carried out by Chen and colleagues, transfusion of CP in 10 COVID-19 patients led to the improvement of respiratory problems, lymphocyte increase, and inflammation suppression [10]. Another case study by Shen et al. demonstrated that CP transfusion (400 mL, antibody titer $> 1:1000$) in 5 patients with severe clinical symptoms increased the pao₂/fio₂ ratio and neutralizing antibody titer from 40–60 to 80–320 [2]. In addition,

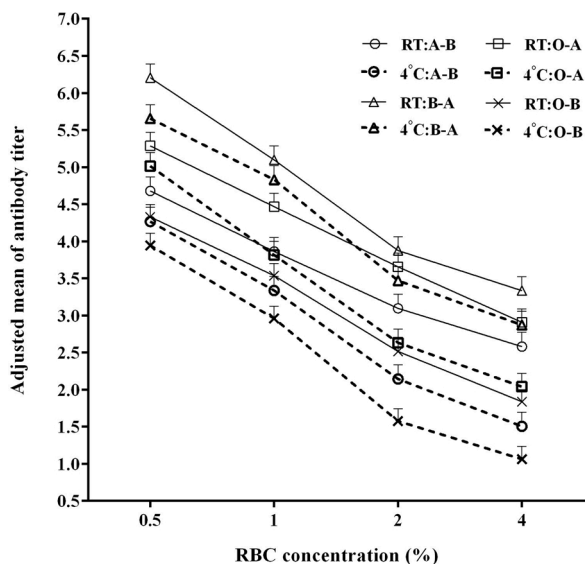


Fig. 1. The effect of temperature and RBC concentration on antibody titer in different blood groups. As can be seen, antibody titer was declined in both temperatures in all RBC concentrations. However, 4 °C had a significantly higher decreasing effect on antibody titer compared to RT. The linear form of the graph indicates that the antibody titer decreases with increasing RBC concentrations. The lowest concentration of RBCs that declines the antibody titer to an acceptable level (<1:64) is 2%. Reciprocal antibody titers were transformed into $\log_2(x+1)$. **RT:A-B:** Plasma A incubated with B packed cells at RT; **4°C:A-B:** Plasma A incubated with B packed cells at 4 °C; **RT:B-A:** Plasma B incubated with A packed cells at RT; **4°C:B-A:** Plasma B incubated with A packed cells at 4 °C; **RT:O-A:** Plasma O incubated with A packed cells at RT; **4°C:O-A:** Plasma O incubated with A packed cells at 4 °C; **RT:O-B:** Plasma O incubated with B packed cells at RT; **4°C:O-B:** Plasma O incubated with B packed cells at 4 °C. Statistical significance was measured by two-way ANCOVA. Data were presented as adjusted mean for initial antibody titer (mean \pm standard error).

available findings from other experiments revealed that CP therapy along with recommended drug protocols could be considered as a safe and effective method for disease improvement and mortality reduction [24,25]. However, ABO-matched donor availability from COVID-19-recovered patients is a challenging issue since incompatible plasma transfusion is associated with some antibody-related adverse complications such as acute hemolytic transfusion reactions (AHTRs), allergic reactions, transfusion-related acute lung injury (TRALI), and febrile non-hemolytic transfusion reactions (FNHTRs) [26]. These problematic antibodies can be removed by attaching to specific antigens of RBCs through the adsorption process. For instance, Das and colleagues performed polyethylene glycol (PEG) and low ionic strength saline (LISS)-papain adsorption methods in warm autoimmune hemolytic anemia patients to remove the corresponding autoantibodies. They reported that the PEG method is a more efficient autoadsorption technique for complete autoantibody elimination [27]. Concerning COVID-19, theoretically, by reducing the titer of ABO-antibodies the final plasma will contain a non-immunogenic level of these antibodies (<1:64) while preserving the coronavirus-specific antibodies.

One of the effective factors in the adsorption procedure is the antibody, which its reaction with antigen depends on different elements such as temperature, antigen concentration, plasma/cell ratio, pH, and incubation time [28]. ABO natural antibodies (cold agglutinins, CA) are mainly IgM and the optimum temperature for their reaction is 0–4 °C. The temperature range of cold agglutinin reaction with antigen is known as thermal amplitude and determines the pathogenicity of CA. Because these antibodies can also react at >4 °C, two temperatures (RT and 4 °C) were selected in the present study [29]. Furthermore, since the use of a high concentration of antigen for antibody neutralization may cause

plasma contamination during the adsorption process, different RBC concentrations (0.5, 1, 2, and 4%) were investigated to obtain the lowest concentration that provides an acceptable reduction in the antibody titer. According to our results, 4 °C had a significantly higher decreasing effect on antibody titer compared to RT. The linear form of the graph in Fig. 1 indicates that the antibody titer decreases with increasing RBCs concentration. The lowest concentration of RBCs which declined the antibody titer to an acceptable level (<1:64) was 2 percent, and although concentrations >2% caused more titer diminution, these reductions may be associated with potential RBCs-related contamination of the final plasma. Therefore, we selected 2% as an optimum RBC concentration. Overall, the best incubation condition for reducing antibody titer in all blood groups was 4 °C and 2% RBCs concentration.

Despite the promising results of the present study, the effectiveness of this product in clinical trials has not been evaluated. In addition, ensuring the accuracy of the results requires further studies with larger sample sizes.

In conclusion, our modified adsorption procedure is able to produce universal plasma (we call it Ubiquitous Convalescent Plasma) with a non-immunogenic level of ABO mismatch antibodies which can be used for different infectious diseases such as COVID-19 patients with any type of blood group with desirable simplicity, feasibility, and efficacy.

CRedit authorship contribution statement

Mahla Sattarzadeh Bardsiri: Investigation, Formal analysis, Writing- Original draft, preparation, Visualization. **Seyedeh Mehrnaz Kouhbananejad:** Investigation, Formal analysis, Visualization, Review & Editing. **Reza Vahidi:** Review & Editing. **Saeed Soleimany:** Methodology. **Masoud Moghadari:** Resources. **Ali Derakhshani:** Resources. **Bahareh Kashani:** Editing. **Alireza Farsinejad:** Conceptualization, Supervision, Methodology, Validation, Writing- Reviewing and Editing.

Funding

We would like to be grateful to Student Research Committee, Faculty of Allied Medicine of Kerman University of Medical Sciences for supporting this study by grant No. 400000151.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

A.F proposed the original concept and designed the experiment and supervised all aspects of the work. M.SB and SM.K, participated in investigations, data acquisition, Formal analysis, and writing- original draft. S.S participated in practical work. M.SB, SM.K, and R.V contributed to the data analysis, reviewing & editing. M.M and A.D contributed to the references collection. B.K participated in manuscript editing.

References

- [1] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci U S A* 2020;117:9490–6.
- [2] Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA* 2020;323:1582–9.
- [3] World Health Organization. Coronavirus disease (COVID-19) pandemic. Available at: 2020. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>.
- [4] Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Investig* 2020;130:2757–65.
- [5] Tiberghien P, de Lamballerie X, Morel P, Gallian P, Lacombe K, Yazdanpanah Y. Collecting and evaluating convalescent plasma for COVID-19 treatment: why and how? *Vox Sang* 2020;115:488–94.
- [6] Metcalfe SM. Mesenchymal stem cells and management of COVID-19 pneumonia. *Med Drug Discov* 2020;5:100019.

- [7] FDA issues emergency use authorization for convalescent plasma as potential promising COVID-19 treatment, another achievement in administration's fight against pandemic. Press Announcements; 2020. Available at: <https://www.fda.gov/news-events/press-announcements/fda-issues-emergency-use-authorization-convalescent-plasma-potential-promising-covid-19-treatment>.
- [8] Liu G, Li S. Convalescent plasma: a valid option in the treatment of COVID-19. *Insights Clin Cell Immunol* 2020;4:001–2.
- [9] Wong HK, Lee CK. Pivotal role of convalescent plasma in managing emerging infectious diseases. *Vox Sang* 2020;115:545–7.
- [10] Chen L, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. *Lancet Infect Dis* 2020;20:398–400.
- [11] Balvers K, Saleh S, Zeerleder SS, Klinkspoor JH, Goslings JC, Juffermans NP. Are there any alternatives for transfusion of AB plasma as universal donor in an emergency release setting? *Transfusion* 2016;56:1469–74.
- [12] Methods section 4, cold agglutinin titer. In: Brecher ME, editor. AABB technical manual. 15th ed. Maryland: AABB press publisher; 2005. p. 778–9.
- [13] Paridar M, Shoushtari MM, Kiani B, Nori B, Shahjahani M, Khosravi A, et al. Distribution of ABO blood groups and rhesus factor in a Large Scale Study of different cities and ethnicities in Khuzestan province, Iran. *Egypt J Med Hum Genet* 2016;17:105–9.
- [14] Antibody detection, antibody identification, and serologic compatibility testing. In: Brecher ME, editor. AABB technical manual. 15th ed. Maryland: AABB press publisher; 2005. p. 768–9.
- [15] Biller-Takahashi JD, Montassier HJ, Takahashi LS, Urbinati EC. Proposed method for agglutinating antibody titer analysis and its use as indicator of acquired immunity in pacu, *Piaractus mesopotamicus*. *Braz J Biol* 2014;74:238–42.
- [16] Bailone RL, Martins ML, Mourão JL, Vieira FN, Pedrotti FS, Nunes GC, et al. Hematology and agglutination titer after polyvalent immunization and subsequent challenge with *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Arch Med Vet* 2010;42:221–7.
- [17] Rezabakhsh A, Ala A, Khodaei SH. Novel coronavirus (COVID-19): a new emerging pandemic threat. *JRCM* 2020;8:5.
- [18] Zhong J, Tang J, Ye C, Dong L. The immunology of COVID-19: is immune modulation an option for treatment? *Lancet Rheumatol* 2020.
- [19] Golchin A, Seyedjafari E, Ardehshirylajimi A. Mesenchymal stem cell therapy for COVID-19: present or future. *Stem Cell Rev Rep* 2020:1–7.
- [20] Liu ST, Lin HM, Baine I, Wajsborg A, Gumprecht JP, Rahman F, et al. Convalescent plasma treatment of severe COVID-19: a propensity score-matched control study. *Nat Med* 2020;26:1708–13.
- [21] Casadevall A, Pirofski LA. The convalescent sera option for containing COVID-19. *J Clin Invest* 2020;130:1545–8.
- [22] Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, et al. Neutralizing antibodies responses to SARS-CoV-2 in COVID-19 inpatients and convalescent patients. *Clin Infect Dis* 2020.
- [23] Cheraghali AM, Abolghasemi H, Eshghi P. Management of COVID-19 virus infection by convalescent plasma. *Iran J Allergy Asthma Immunol* 2020.
- [24] Rajendran K, Narayanasamy K, Rangarajan J, Rathinam J, Natarajan M, Ramachandran A. Convalescent plasma transfusion for the treatment of COVID-19: systematic review. *J Med Virol* 2020.
- [25] Ahn JY, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, et al. Use of convalescent plasma therapy in two COVID-19 patients with acute respiratory distress syndrome in Korea. *J Korean Med Sci* 2020:35.
- [26] Food and Drug Administration. EUA of COVID-19 convalescent plasma for the treatment of COVID-19 in hospitalized patients: fact sheet for health care providers. Available at: 2020. <https://www.fda.gov/media/141478/download>.
- [27] Das SS, Chaudhary R. Utility of adsorption techniques in serological evaluation of warm autoimmune haemolytic anaemia. *Blood Transfus* 2009;7:300.
- [28] Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. *Blood Transfus* 2007;5:227.
- [29] Berentsen S. New insights in the pathogenesis and therapy of cold agglutinin-mediated autoimmune hemolytic anemia. *Front Immunol* 2020;11.