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ABSTRACT: Blood disinfection is currently being evaluated as a means of reducing or eliminating the risks of disease transmission associated with blood transfusion. For the past 10 years our laboratory has used baboons to evaluate the effects of various disinfection treatments on autologous red blood cell and platelet viability and function in vitro and in vivo. We have used radioactive (51Cr) methods to evaluate the posttransfusion survival and lifespan of disinfected red blood cells. The in vivo recovery and lifespan of platelets were assessed using either radioactive 51Cr or 111Indium. The function of the platelets was evaluated by their ability to correct an aspirin-induced thrombocytopathy. We observed that in some instances in which the disinfected autologous red blood cells showed no alterations in vitro measurement, recovery in vivo and lifespan were decreased. In view of these findings, we believe that it is prudent to continue studies in the baboon before attempting studies in normal volunteers.

INTRODUCTION: For the past 20 years, our laboratory has used the baboon (Papio sp.) to evaluate the effects of methods to preserve red blood cells and platelets. We have

been using the baboon in studies to evaluate whether blood disinfection adversely affects red blood cells. When in vitro measurements indicate that a disinfection treatment does not significantly damage the baboon red blood cells, in vivo studies are performed to assess the effect of disinfection of autologous baboon red blood cells on 24-hour posttransfusion survival and lifespan.

Disinfected platelets also can be assessed in the baboon. The posttransfusion survival, lifespan and function of disinfected platelets can be studied in baboons subjected to an aspirin-induced thrombocytopathy, an approach that has been used in studies at the Naval Blood Research Laboratory for more than 20 years in normal volunteers and for more than 10 years in baboons.[1-3]

Our laboratory also has used the baboon to study the effects of hypothermia on hemostasis before we conducted the experiments in normal volunteers and patients.[4-10] We have found that data obtained from baboon studies are similar to those seen in studies of normal volunteers and believe that human studies can be reduced by using the baboon in specific areas of research. The following experiments were performed to evaluate the effects of various disinfection treatments on the survival and function of red blood cells and platelets.

METHODS

A. Disinfected Red Blood Cells:

1. In Vitro Measurements: To determine whether red blood cells are damaged by a particular disinfection treatment, the following measurements were made: hematocrit, hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration measured using an electronic particle counter; plasma hemoglobin measured using a dual-beam spectrophotometer;[11] intracellular and extracellular sodium and potassium measured using a flame photometer;[12] and adenosine triphosphate (ATP) measured using a fluorometer.[13] The function of the red blood cells was evaluated by their ability to transport oxygen as assessed by measurement of 2,3 diphosphoglycerate (DPG)[14] and red blood cell p50,[15] the partial pressure at which 50% of the hemoglobin is saturated with oxygen.

2. In Vivo Measurements: The 24-hour posttransfusion survival and lifespan were measured in baboons and normal volunteers using a double-label isotope procedure. The baboon's blood volume was measured using 125-Iodinated human serum albumin, and the 24-hour posttransfusion survival and lifespan of the disinfected autologous red blood cells were measured using 51-Cr disodium chromate.[16-19]

3. Disinfection Treatments: The following disinfection treatments were evaluated:

a. **Benzoporphyrin derivative (BPD) and photoactivation:**--Blood was collected into the CPD-ADSOL blood collection system, and red blood cells were prepared with a final hematocrit of 55 V%. BPD was added to the red blood cells to achieve a final concentration of 0.5 ug/ml; the red blood cells were stored at room temperature for 60 minutes and then washed 3 times with phosphate buffered saline (PBS). The red blood cells were then exposed to photoactivation to achieve a total fluence of 6.5 J/cm2, washed once with a solution referred to as American Red Cross Solution 8 (ARC8), resuspended in the ARC8 solution to achieve a final hematocrit of 55 V%, and stored at 4 C for 24 hours prior to in vitro and in vivo evaluation.

a1. **Control:** Blood was collected into the CPD-ADSOL blood collection system but the BPD solution was not added and photoactivation was not employed. Storage and washing procedures were the same as those for the treated red blood cells.

b. **Panavirocide**--Two different formulations of Panavirocide, a solution containing glutaraldehyde and detergents, were evaluated. Solutions 1 and 2 contained the same amount of glutaraldehyde; however, solution 2 contained significantly less detergent.

Blood collected into CPD anticoagulant was treated by adding Panavirocide solution to achieve a final concentration of 10% Panavirocide solution in blood. The Panavirocide was allowed to remain with the blood for 60

minutes at room temperature, and the blood was washed with PBS.

b1. **Control:** Blood collected into CPD anticoagulant was treated as described above except PBS solution was added to the blood in place of the Panavirocide solution.

c. **Aluminum phthalocyanines and photoactivation**[20-22]--Eighty-five (85) ml of baboon blood were collected into CPD anticoagulant. The blood was centrifuged to prepare a red blood cell concentrate, and the red blood cells were treated with 10 uM aluminum phthalocyanine and 44 J/cm2 visible light. The treated red blood cells were washed with PBS prior to evaluation.

c1. **Control:** Baboon blood was collected into CPD anticoagulant and red blood cells prepared as above. The red blood cells were not treated with phthalocyanine or light, but were washed as above.

d. **Formaldehyde**[23-25]--Seventy-five (75) ml of blood were collected into 10.5 ml of CPD anticoagulant in a 300 ml polyvinylchloride (PVC) transfer pack. The blood was placed on an Eberbach shaker set at low speed (180 lateral oscillations per minute) and the formaldehyde solution was added to the blood over a 1-2 minute period to achieve a final concentration of 250 ppm. The formaldehyde-treated blood was stored at 4 C for 24 hours and an aliquot was removed for evaluation.

d1) **Control:** Instead of the formaldehyde solution, a similar volume of sodium chloride solution was added to the CPD-collected blood and the treated blood was stored at 4 C for 24 hours.

e. **Sodium chlorite**[26]--Blood collected into citrate-phosphate-dextrose (CPD) anticoagulant was treated with sodium chlorite solution. Sodium chlorite activated with a CPD solution was added to whole blood to achieve a final concentration of 0.75 to 15 mM. The blood was not washed following disinfection.

e1. **Control:** Sodium chlorite was added to a sodium chloride solution to prepare a "non-activated" form of the disinfectant. The non-activated sodium chloride disinfectant was added to the whole blood to achieve the same final concentration as the activated sodium chlorite disinfectant.

Disinfectant Effectiveness and Viability

We did not evaluate the disinfectant treatments for virucidal and/or bacterial killing. The doses and protocols required for disinfection with each method were provided either by the company or by persons supplying the disinfectant. Neither did we evaluate the toxicity of the disinfectant treatments.

B. Disinfected Platelets:

1. In Vitro Measurements: Both baboon and human platelets have been assessed in vitro by their ability to aggregate to arachidonic acid (AA) and adenosine diphosphate (ADP), ability to produce thromboxane A2 during aggregation to AA and ADP, response to hypotonic stress, and measurements of pH, pO2 and pCO2.[27]

2. In Vivo Measurements: Platelet survival in vivo has been measured in both humans and baboons using the double-label isotope procedure used to measure red blood cell survival in vivo.[28,29] Blood volume was measured using either 51-Cr labeled autologous fresh red blood cells or 125-Iodinated human serum albumin; platelet survival was measured using either 51-Cr labeled or 111-Indium- oxine labeled autologous platelets. Posttransfusion survival and lifespan have been measured in baboon platelets preserved using both liquid and frozen procedures.[29]

The ability of preserved platelets to function in vivo has been assessed by producing an aspirin-induced thrombocytopathy in the baboon. The administration of 325 mg of aspirin into the baboon produced a prolonged bleeding time measured using a standard template (Simplate) method. Blood collected from the bleeding time site (referred to as "shed blood") had a significantly reduced thromboxane A2 level. The ability of the preserved or treated platelets to increase the shed blood thromboxane A2 level and reduce the

extended bleeding time is the measure of their function in vivo.

Statistical Analyses: Non-paired and paired t-tests were used to test the significance of the effects of disinfection; a p value of 0.05 was considered significant.

RESULTS

A. Red Blood Cells

1. Comparison of Human and Baboon Blood Prior to and Following Disinfection

Tables 1 and 2 report the in vitro effects of disinfecting human and baboon blood using two of the disinfection methods described above (Panavirocide and BPD/Photoactivation). These data demonstrate that both disinfection methods resulted in a significant reduction in intracellular potassium in both baboon and human blood.

When human blood was treated with Panavirocide Solution, Formulation 1, the intracellular potassium level decreased significantly from 7.1 to 0.7 mEq/10^{12} RBC, the plasma hemoglobin increased from 15 mg/dl to 308 mg/dl, and the percent hemolysis increased from 0.08 to 0.60%. Baboon blood treated with Panavirocide Solution, Formulation 1, showed results similar to those observed with human blood with the exception that following treatment, the increase in plasma hemoglobin was not as great in baboon blood as that seen with human blood. Treatment of baboon blood with Panavirocide Solution, Formulation 2, resulted in increased plasma hemoglobin and intracellular potassium levels (Table 1).

Treatment of human and baboon blood with benzoporphyrin derivative and photoactivation resulted in a decrease in

intracellular potassium and increases in plasma hemoglobin and percent hemolysis. Red blood cell ATP was slightly increased in both the human and baboon blood following treatment. This increase was due to the post-treatment storage of the red blood cells in the ARC8 resuspension media and was not a result of the BPD/photoactivation treatment. Red blood cell 2,3 DPG and P50 were unaffected by the treatment (TABLE 2)

2. **Disinfection Treatments**

Tables 3 and 4 report the 24-hour posttransfusion survival and lifespan of baboon red blood cells after treatment with the five disinfection treatments described above. Baboon red blood cells treated with Panavirocide solution, Formulation 1, had a 24-hour posttransfusion survival value of 0% and those treated with BPD and photoactivation had a value of 22%. These values are significantly ($p<0.001$) lower than the 85-90% 24-hour posttransfusion survival values seen in the control studies.

In the study using the non-activated sodium chlorite, which was performed as a control for the activated sodium chlorite studies, there was actually more damage to the red blood cells at the 15 mM concentration. The 24-hour posttransfusion survival value was 75% for the red blood cells in the control studies and 87% for the red blood cells treated with activated sodium chlorite.

These data demonstrate significant reductions in the 24 hour posttransfusion survival of the baboon red blood cells treated with Panavirocide solution #1, BPD/photoactivation and 15 mM of non-activated sodium chlorite. Treatment with Panavirocide solution #2, 250 ppm formaldehyde, phthalocyanines and photoactivation, or activated sodium chlorite produced no significant effect on the 24-hour posttransfusion survival values.

Table 4 reports that red blood cells treated with Panavirocide solution #2, BPD and photoactivation or 15 mM of non-activated sodium chlorite had a significantly shorter lifespan than did the control values.

B. Platelets

Ingestion of 325 mg of aspirin to produce an aspirin-induced thrombocytopathy in baboons increases bleeding time from 3 to 5 minutes. Associated with the increased bleeding time is a decrease in shed blood thromboxane A2 from about 1500 pg/0.1 ml to 200 pg/0.1 ml. Results in baboon studies have been found to be similar to those obtained in studies in normal volunteers[2,3] and patients[10] treated with aspirin. Baboons transfused 1 unit of platelets stored at 22 C for 18 hours or 2 units of previously frozen platelets exhibit a significant reduction in bleeding time and an increase in shed blood thromboxane A2. No such decrease in bleeding time or increase in shed blood thromboxane A2 level was seen in baboons transfused 2 units of platelets stored at 22 C

for 5 days (unpublished data). In vivo recovery and lifespan values following the transfusion of fresh or preserved platelets were similar in baboons and normal volunteers.

DISCUSSION

Baboon red blood cells exposed to different disinfectant treatments responded in a manner similar to that observed of human red blood cells. Red blood cell damage related to the disinfection treatment was reflected in a decreased intracellular potassium level and reduced 24-hour posttransfusion survival value. Intracellular potassium levels correlated with 24-hour posttransfusion survivals: when these levels were decreased, as with the Panavirocide solution Formulation 1 and BPD/photoactivation treatment, the 24-hour posttransfusion survival was reduced. However, red blood cells with reduced lifespan values did not necessarily have reduced 24-hour posttransfusion survival values, as in the case of the blood treated with Panavirocide Solution Formulation 2. This indicates that the damage to the red blood cells, assessed by the 24-hour value, was not caused by the treatment, but rather that the treatment resulted in a change in red blood cell antigenicity, assessed by the lifespan T 1/2 value

Other investigators have reported a correlation between red blood cell ATP and 24-hour posttransfusion survival. However, in our study, we observed a significant reduction in intracellular potassium for red blood cells treated with BPD and photoactivation associated with an increase in red blood cell ATP. The significant reduction in the 24-hour posttransfusion survival of these red blood cells suggests that the intracellular potassium level may be a more

important in vitro predictor of the 24-hour posttransfusion survival than the red blood cell ATP level.

These data demonstrate that the baboon can be used to study the in vitro and in vivo effects of a variety of treatments, including red blood cell and platelet disinfection.

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REFERENCES

1. Handin, R.I. and C.R. Valeri (1971) Hemostatic effectiveness of platelets stored at 22 C. NEJM 285, 538-543.

2. Valeri, C.R. (1974) Hemostatic effectiveness of liquid-preserved and previously frozen platelets. NEJM 290, 353-358.

3. Valeri, C.R. (1976) Circulation and hemostatic effectiveness of platelets stored at 4 C or 22 C: Studies in aspirin-treated normal volunteers. Transfusion 16, 20-23.

4. Valeri, C.R., H. Feingold, G.P. Cassidy, G. Ragno, S.F. Khuri and M.D. Altschule (1987) Hypothermia induced-reversible platelet dysfunction. Ann Surg 205, 175-181.

5. Valeri, C.R., H. MacGregor, F. Pompei and S.F. Khuri (1991) Acquired abnormalities of platelet function. N Engl J Med 324, 1670.

6. Valeri, C.R., H. MacGregor, G. Cassidy, R. Tinney and F. Pompei (1995) Effects of temperature on bleeding time and clotting time in normal male and female volunteers. Crit Care Med 23, 698-704.

7. Michelson, A.D., H. MacGregor, M.R. Barnard, A.S. Kestin, M.J Rohrer and C.R. Valeri (1994). Reversible inhibition of platelet activation by hypothermia in vivo and in vitro. J Thromb Haemost 5, 633-640.

8. Khuri, S.F., A.D. Michelson and C. R. Valeri (1994) The effect of cardiopulmonary bypass on hemostasis and coagulation. In Thrombosis and Hemorrhage (Edited by J. Loscalzo and A.I. Schafer), pp 1051-1073, Blackwell Scientific, Cambridge.

9. Khuri, S.F., J.A. Wolfe, M. Josa, T. C. Axford, I. Szymanski, S.N. Assousa, G. Ragno, M. Patel, A. Silverman, M. Park and C.R. Valeri (1992) Hematologic changes during and after cardiopulmonary bypass and their relationship to bleeding time and non-surgical blood loss. J Thorac Cardiovasc Surg 104, 94-107.

10. Valeri, C.R., K.R. Khabbaz, S.F. Khuri, C. Marquardt, G. Ragno, H. Feingold, A.D. Gray and T. Axford (1992) Effect of skin temperature on platelet function in patients undergoing extracorporeal bypass. J Thorac Cardiovasc Surg 104, 108-116.

11. Blakney, G.B. and A.J. Dinwoodie (1975) A spectrophotometric scanning technique for the rapid determination of plasma hemoglobin. Clin Biochem 8, 96-102.

12. Runck, A., C.R. Valeri and W. Sampson (1968) Comparison of effects of ionic and non-ionic solutions on volume and intracellular potassium of frozen and non-frozen human red cells. Transfusion 8, 9-18.

13. Lamprecht, W. and I. Trautschold (1963) Determination with hexokinase and glucose-6-phosphate dehydrogenase. In Methods of Enzymatic Analysis (Edited by H.U. Bergmeyer) pp. 543-558, New York: Academic Press.

14. Keitt, A.S. (1966) Pyruvate kinase deficiency and related disorders of red cell glycolysis. Am J Med 41, 762-785.

15. Asakura, T. and M.P. Reilly (1984) Methods for the measurement of oxygen equilibrium curves of red cell suspensions and hemoglobin solutions. In Oxygen Transport in Red Blood Cells (Edited by C. Nicolau), pp 57-75, Proc. of the 12th Aharon Katzir Katchlasky Conference, Tours, France, 4-7 April.

16. Valeri, C.R., J.R. Lindberg, T.J. Contreras, L.E. Pivacek, R.M. Austin, D.A. Valeri, A. Gray and C.P. Emerson (1981) Measurement of red blood cell volume, plasma volume, and total blood volume in baboons. Am J Vet Res 42, 1025-1029.

17. Valeri, C.R., J.R. Lindberg, T.J. Contreras, L.E. Pivacek, R.M. Austin, D.A. Valeri, A. Gray and C.P. Emerson (1981) Liquid preservation of baboon red blood cells in acid-citrate-dextrose or citrate-phosphate-dextrose anticoagulant: Effects of washing liquid stored red blood cells. Am J Vet Res 42, 1011-1013.

18. Valeri, C.R., J.R. Lindberg, T.J. Contreras, G.B. Lowrie, L.E. Pivacek, A. Gray, D.A. Valeri and C.P. Emerson (1981) Freeze-preserved baboon red blood cells: Effect of biochemical modification and perfusion in vitro. Am J Vet Res 42, 1590-1594.

19. Valeri, C.R. (1971) Viability and function of preserved red cells. NEJM 284, 81-88.

20. Horowitz, B., S. Rywkin, H. Margolis-Nunno, B. Williams, N. Geacintov, A.M. Prince, D. Pascual, G. Ragno, C.R. Valeri and T. Huima-Byron (1992) Inactivation of viruses in red cell and platelet concentrates with aluminum phthalocyanine (AlPc) sulfonates. Blood Cells 18, 141-150.

21. Margolis-Nunno, H., A. Bartley, E. Ben-Hur, T. Huima-Byron, H. MacGregor, R. Robinson, C.R. Valeri and B. Horowitz (1993) Quencher mediated protection of platelet integrity during psoralen photoinactivation of viruses in platelet concentrates. Blood 82(10), 401a.

22. Rywkin, S., E. Ben-Hur, D. Pascual, A.M. Prince, M. Reid, M. Kenney, N.L. Oleinick, J.E. van Lier and B. Horowitz (1993) New photosensitizers to inactivate viruses in red blood cells. Blood 82(10), 205a.

23. Council on Scientific Affairs (1989) Council report: Formaldehyde. JAMA 261, 1183-1187.

24. Louderback, A.L. (1989) Method of inactivating HTLV-III virus in blood. Patent #3,833,165. 23 May.

25. Louderback, A. (1992) Sterilization of red blood cells. AABB Annual Meeting, San Francisco, CA November.

26. Sarin, P.S., D.I. Scheer and R.D. Kross (1985) Inactivation of human T- cell lymphotropic retrovirus (HTLV-III) by LDtm. N Engl J Med 313, 1416.

27. Feingold, H.M., L.E. Pivacek, A.J. Melaragno and C.R. Valeri (1986) Coagulation assays and platelet aggregation patterns in human, baboon and canine blood. Am J Vet Res 47, 2197-2199.

28. Valeri, C.R., H. Feingold and L.D. Marchionni (1974) The relation between response to hypotonic stress and the 51Cr recovery in vivo of preserved platelets. Transfusion 14, 331-337.

29. Melaragno, A.J., W. Abdu, R. Katchis, A. Doty and C.R. Valeri (1981) Liquid and freeze preservation of baboon platelets. Cryobiology 18, 445-452.

TABLE 1

RED BLOOD CELL DISINFECTION USING PANAVIROCIDE SOLUTION

	RED CELL RECOVERY (%)	INTRA K^+ ($mEq/10^{12}$ RBC)	PLASMA HGB (mg/dl)	HEMOLYSIS (%)
Human Blood				
Pre-Treatment				
X:	---	7.1	15	0.08
SD:		1.1	10	0.03
N:		3	3	2
Post-Treatment Formulation 1				
X:	98	0.7	308	0.60
SD:	1	0.2	237	0.30
N:	2	3	3	2
p value:		<.001	NS	NS
Baboon Blood				
Pre-Treatment				
X:	--	7.4	13	0.07
SD:		0.6	9	0.04
N:		4	4	4
Post-Treatment Formulation 1				
X:	98	1.6	98	0.31
SD:	3	0.6	54	0.04
N:	3	4	4	4
p value:		<.001	<.05	<.001
Post-Treatment Formulation 2				
X:	--	8.6	315	---
SD:		0.4	30	
N:		7	7	
p value:		<.01	<.001	

TABLE 2

RED BLOOD CELL DISINFECTION TREATMENT USING BENZOPORPHYRIN DERIVATIVE (BPD) AND PHOTOACTIVATION

	RED CELL RECOVERY (%)	INTRA K+ (mEq/10^{12} RBC)	PLASMA HGB (mg/dl)	HEMOLYSIS (%)	ATP (uM/gHb)	2,3DPG	P50 (mmHg)
Human Blood							
Pre-Treatment							
X:	--	8.7	39	0.11	3.7	11.0	32.7
SD:		0.7	6	0.01	0.2	1.6	3.0
N:		3	3	3	3	3	3
Post-Treatment with 0.5 ug/ml BPD							
X:	96.1	4.0	131	0.33	5.7	11.0	31.9
SD:	0.8	0.9	47	0.12	0.3	1.2	0.7
N:	3	5	5	5	3	3	3
p value:		<.001	<.05	<.05	<.001	NS	NS
Baboon Blood							
Pre-Treatment							
X:	--	8.7	31	0.08	2.9	9.1	32.2
SD:		0.8	6	0.01	0.6	1.8	0.0
N:		3	3	3	3	3	3
Post-Treatment with 0.5 ug/ml BPD							
X:	95.8	3.7	167	0.41	5.0	9.4	32.3
SD:	4.1	1.3	91	0.19	0.4	0.7	1.0
N:	3	5	5	5	3	3	2
p value:		<.001	<.05	<.05	<.001	NS	NS

TABLE 3

EFFECT OF DISINFECTION TREATMENT ON IN VIVO 24-HOUR POSTTRANSUFSION SURVIVAL OF RED BLOOD CELLS IN BABOONS

	BPD/Photo-activation	Panavirocide Soln #1	Panavirocide Soln #2	Formaldehyde (250 ppm)	Phthalocyanine/ photoactivation	Sodium Chlorite 0.75 mM	Sodium Chlorite 15 mM
TREATED							
MEAN:	22	0	89	90	92	87	87
SD:	10	0	5	5	--	3	2
N:	2	3	7	8	2	2	2
CONTROL							
MEAN:	89	88	--	88	93	87	75
SD:	2	3		5	--	2	5
N:	3	3		4	2	2	8
Non-paired t-test p value:	<.001	<.001	NS	NS	NS	NS	<.01

TABLE 4

EFFECT OF DISINFECTION TREATMENT ON IN VIVO RED BLOOD CELL LIFESPAN IN BABOONS

	BPD/Photo-activation	Panavirocide Soln #1	Panavirocide Soln #2	Formaldehyde (250 ppm)	Phthalocyanine/ photoactivation	Sodium Chlorite 0.75 mM	Sodium Chlorite 15 mM
TREATED							
MEAN:	7.7	---	11.6	14.9	13.2	14.0	13.0
SD:	1.7		1.7	3.2	---	1.0	1.0
N:	2		7	8	2	2	2
CONTROL							
MEAN:	13.5	15.2	---	13.6	14.1	13.0	10.0
SD:	2.5	2.3		0.9	--	1.0	1.0
N:	3	3		4	2	2	8
Non-Paired t-test p value:	<.001		<.001	NS	NS	NS	<.01