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DEPARTMENT OF PATHOLOGY
LOS ANGELES COUNTY HARBOR-UCLA MEDICAL CENTER
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TORRANCE, CALIFORNIA 90509

January 23, 1990

Captain Anthony Melaragno
Department of the Navy
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, MD 20814-5044

RE: First Quarter Report - 9/1/89 - 11/30/89
2nd Year of Navy Contract N0014-88-C-0755

Dear Captain Melaragno:

Work on this project is still progressing and the results are extremely encouraging.

The purpose of this study was to find out if blood that had been thawed could be stored in a refrigerator for periods longer than 72 hours without excessive hemolysis.

Currently, we have drawn 44 donors and have studied 26 of these for better methods of storage. Ten more are currently being studied. Each unit of blood is frozen and thawed following the methods outlined in the Navy SOP with no changes. After the wash is finished, the red cells are concentrated by centrifugation, and distributed equally between four smaller plastic bags joined as a quadruple unit and attached in place of the usual receiving blood bags. Thus all red cell samples are handled identically to that point. Various preservative solutions are then introduced to the red cells and the hematocrit is adjusted to approximately 45% with the solution. Saline is added to make up the difference if the amount of anticoagulant is insufficient. The red cells in Bag #1 are reconstituted with autologous thawed plasma (containing the anticoagulant CPDA-1). This is felt to be the equivalent of whole blood and therefore serves as one of the controls. The other control is bag #4 which was reconstituted with the standard 0.2% glucose, 0.9% NaCl preservative. This is the preservative medium that is now approved for 72 hour storage at 4C. The test solutions were placed in either bags #2 or #3. At biweekly intervals, samples are removed aseptically from these bags and analyzed for plasma hemoglobin, Na+, K+, Cl- and glucose. The results are shown in Table 1.

To simplify the table, pH total hemoglobin and hematocrit have not been included, but these have shown no significant changes in any of the studies performed to date. They will however be included in the final report. As can be seen from our results to date, plasma hemoglobin levels, although certainly raised over that of normal blood, are at an acceptable level of 200 mg/dl or less in blood samples stored in all three anticoagulants: ACD, CPD, and

CPDA-1. These results are slightly higher than the hemolysis found when the red cells are stored in their own plasma. The additive solutions AS-1 which is a replica of the SAG solution used by Fenwal Laboratories and AS-3 which is the additive solution used by Cutter in their Nutracel preservative solution do not give anywhere near as good results. The plasma hemoglobin levels at the end of 21 days in those specimens at 5 to 700 mg/dl and at 28 days the results are even higher. A Student t-test was performed on these results and the values are shown in Table 2. This shows that anticoagulant ACD, CPD, and CPDA-1 are comparable both at 21 and 28 days and are significantly different from samples 4 and 5 which are the AS-1 and AS-3 solutions respectively. We thus feel from the basis of these studies very confident in stating that almost any of the standard anticoagulants, if added to red cells after washing will preserve the red cells for a significant period of time. Because of its availability we have decided to use CPDA-1 as the solution that will be tested in the in vivo portion of the study. We are still somewhat puzzled at the poor preservation produced by the additive solutions. The studies of both Heaton and Moore would seem to indicate that these should be better preservatives than we found, however, they did no comparison between the older coagulants and the additives and simply made the assumption that what is new is better. Currently the changes in each preservative plus the ATP and 2,3-DPG levels are being measured on single units of blood, thus an entire unit of blood from each donor is frozen, thawed, reconstituted with the appropriate preservative and samples are being taken for electrolytes, plasma hemoglobin, ATP, 2,3-DPG and all other studies.

We currently have recruited ten donors and are anticipating drawing blood in January and February for the in vivo studies. The Technetium survival studies will be done and we will look at red cell viability after 2, 3, and 4 weeks of storage. From the amount of hemolysis seen in our in vitro study, it would appear that beyond four weeks, good survival is unlikely.

We have slight concerns about the plasma hemoglobin levels. The plasma hemoglobin at the end of three weeks is significantly elevated, however, the original Navy SOP calls for centrifuging the unit and removing the supernatant plasma before transfusion. This should not represent a problem to the average trauma patient. Further a number of studies have been done by various individuals to show that if the patients kidneys are in good order, a moderate amount of plasma hemoglobin is not toxic. However it would be best removed. It is also possible that the survival studies will show that 14 days is all that the blood may be stored before it losses its viability. If this is the case of course the plasma hemoglobin levels are much lower at 14 days and would produce even less change in the patient's electrolyte values.

We are continuing to finish the in vitro work, and as I said previously, have begun to bleed the donors for the in vivo studies which will be performed in the next 6 month period.

Sincerely,

Byron A. Myhre, M.D., Ph.D.
Professor of Pathology
Chief, Clinical Pathology

BAM:pw
Attachment

cc: L. Yaffee, Commanding Officer

Table 1
First Quarter Report
2nd Year of Navy Contract N0014-88-C-0755

Days	Plasma Hb	Cl^-	Na	Glucose	K^+
ACD					
n = 7					
0	52.7 ± 37.3	116.6 ± 2.3	165.4 ± 5.2	281.4 ± 8.0	3.18 ± 0.63
7	97.9 ± 69.8	121.9 ± 3.2	154.4 ± 18.1	289.9 ± 30.0	17.2 ± 5.6
14	163.8 ± 83.1	121.9 ± 2.3	146.3 ± 6.9	265.3 ± 6.9	29.3 ± 3.9
21	216.7 ± 81.1	125.9 ± 2.8	144.1 ± 5.2	261.1 ± 8.6	37.1 ± 4.6
28	362.1 ± 174.9	124.6 ± 1.9	139.1 ± 7.8	251.4 ± 9.7	43.0 ± 4.9
CPD					
n = 7					
0	80.3 ± 45.2	117.9 ± 3.6	162.4 ± 3.2	263.4 ± 13.6	3.9 ± 0.9
7	108.0 ± 61.4	120.7 ± 4.2	155.7 ± 4.2	249.2 ± 17.7	21.4 ± 2.9
14	127.5 ± 62.3	122.4 ± 4.6	151.6 ± 2.3	236.7 ± 18.5	30.4 ± 5.2
21	207.8 ± 124.4	125.9 ± 6.1	146.4 ± 5.2	230.7 ± 24.2	38.9 ± 6.4
28	382.0 ± 358.6	124.1 ± 4.7	142.9 ± 8.4	222.7 ± 22.8	42.9 ± 6.6
CPD-A1					
n = 4					
0	45.9 ± 14.1	110.8 ± 1.5	165.0 ± 5.4	332.3 ± 14.7	3.9 ± 0.89
7	77.5 ± 34.4	118.8 ± 2.5	153.0 ± 1.2	316.3 ± 17.5	22.5 ± 3.4
14	137.4 ± 75.9	119.8 ± 2.9	149.8 ± 11.7	299.0 ± 11.6	34.0 ± 5.0
21	189.8 ± 114.7	125.0 ± 4.4	143.0 ± 4.3	292.5 ± 6.0	40.8 ± 6.0
28	359.8 ± 335.5	123.0 ± 2.9	137.0 ± 3.4	277.0 ± 9.5	46.5 ± 6.6
AS-1					
n = 6					
0	60.8 ± 17.9	124.8 ± 2.6	169.2 ± 5.9	316.5 ± 7.3	3.5 ± 0.9
7	204.5 ± 96.6	131.5 ± 3.3	158.8 ± 5.0	302.3 ± 10.8	17.0 ± 5.7
14	564.5 ± 274.6	132.5 ± 3.0	151.3 ± 7.0	280.8 ± 8.6	27.3 ± 4.2
21	734.0 ± 583.7	137.5 ± 3.1	145.6 ± 3.8	272.7 ± 13.6	33.3 ± 4.5
28	1328.8 ± 797.0	135.2 ± 2.9	140.5 ± 6.1	265.8 ± 15.1	37.5 ± 3.9
AS-3					
n = 8					
0	106.5 ± 16.3	107.3 ± 11.0	157.0 ± 7.0	232.8 ± 46.2	6.0 ± 4.0
7	375.6 ± 158.5	110.3 ± 9.2	145.6 ± 8.5	216.1 ± 45.9	25.4 ± 14.6
14	410.0 ± 229.5	114.6 ± 7.9	138.3 ± 10.5	204.1 ± 41.4	36.1 ± 19.3
21	509.8 ± 243.9	117.1 ± 8.2	134.8 ± 12.0	197.4 ± 43.4	39.7 ± 15.8
28	921.8 ± 530.4	120.1 ± 14.3	135.1 ± 10.2	184.0 ± 33.6	39.2 ± 6.4

Table 2
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Comparison of Plasma Hemoglobin values at 21 and 28 days.

Sample comparison	p value 21 day storage	28 day storage
ACD vs CPD	0.9	0.886
ACD vs CPDA-1	0.987	0.656
ACD vs AS-1	0.0092	0.039
ACD vs AS-3	0.0098	0.013
CPD vs CPDA-1	0.926	0.838
AS-1 vs AS-3	0.415	0.445