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THE SPARING EFFECT OF HEPARIN ANTICOAGULANT ON PLATELETS IN STORED BLOOD

* W. C. NWAFIA ** J. O. ANEKE *** N. EKWURIKE

**Department of Physiology, College of Medicine and Health Sciences Abia State University Uturu Nigeria*
***Department of Haematology/ Immunology University of Nigeria Teaching Hospital (UNTH) Enugu – Nigeria.*
****Department of Haematology and Blood Group Serology, School of Medical Laboratory Technology and Sciences. University of Nigeria Teaching Hospital (UNTH) Enugu - Nigeria*

Summary: The platelet counts were carried out on one hundred and fifty-(150) blood samples stored in six different types of anticoagulants at 4^0C. The samples were apparently healthy volunteers at the University of Nigeria Teaching Hospital (UNTH) Enugu aged between 19 and 40yrs. The ratio of male to female was 7:3 The anticoagulants used were dipotassium ethylene diamine tetra acetic acid (K_2EDTA), sodium flouride (NaF), heparin, sodium citrate (NaC), citrate phosphate dextrose-adenine (CPD –A) and acid citrate dextrose (ACD). The platelet counts were carried out daily for four consecutive days (days 0 – 3) in each of the anticoagulant using an automatic Particle Counter (PCE – 90) of the ERMA PCE series (ERMA INC. TOKYO Japan). The mean values obtained were recorded and statistically tested. There were reductions in the platelet counts in the blood samples stored in all the six anticoagulants. These reductions became more significant as the storage period increased. The greatest effect was observed in the samples stored in sodium fluoride. The highest number of platelets was obtained from heparinised blood. Suggesting that heparin has a sparing effect on platelet number. It is recommended that whenever platelet count cannot be performed within the usual two hours of blood collection, heparin could be used to store the blood. However, it is important that platelet counts should always be performed on the same day of sample collection.

Key Words:Platelet count, Heparin, Stored blood, Anticoagulants.

Introduction

Platelets have been documented to play a vital role in blood coagulation (Mustard et al; 1966; Guyton and Hall, 1996). To adequately play this role, it's important that the quantity of these platelets in blood be maintained regularly within a narrow range of 150 – 400 x 10^9 cells per litre of blood. - (caucasians) (Dacie and Lewis, 1994) and 100 – 400 x 10^9 cells per litre of blood – (Nigerians) Quantitative abnormalities of platelets have been reported to result in many disease conditions e.g. hemorrhage, thrombosis and atherosclerosis (Miller and Weller, 1971; bloom and Thomas, 1981; Sheldon, 1988).

In our tropical environment, blood is commonly stored or preserved in anticoagulants at 4^0C. Anticoagulants are substances or chemicals when added to blood, has the property of retarding or inhibiting coagulation.

Despite documented abnormalities observed in blood stored in anticoagulants, this method still remains the most available, widely accepted and most patronised method of blood storage in the tropics. This is as a result of the absence of modern methods of blood storage.

Abnormalities of blood stored in anticoagulants include tetany of muscles, decreased parked cell volume (PCV), increased mean cell volume (MCV), artificial increase in platelet count (PC) and decreased white blood cell (WBC) count (Goossens et al, 1991; Dacie and Lewis, 1994).

Many platelet abnormalities resulting from storage of blood in anticoagulants have been documented (Bloom and Thomas, 1981; James, 1996; Rock et. al., 1997; Rivers et. al., 1999).

Blood transfusion especially with fresh whole blood is very vital in certain disease states. Often times it is very difficult to obtain this fresh whole blood in the environment under study; hence the only readily available choice are blood stored in anticoagulants. It then becomes very vital that the components of these stored blood especially platelets are maintained relatively constant to prevent possible post transfusion complications especially with blood coagulation. This in the tropical environment under study is near impossible considering the level of technological and scientific advancement.

Thus we studied the preservation of platelet in different anticoagulants in use in blood storage in our environment with a view to suggesting the best anticoagulant for routine platelet preservation.

Materials and Methods

Subjects

The blood samples of one hundred and fifty (150) apparently healthy volunteers between the ages of 19 and 40yrs at the University of Nigeria Teaching

Hospital (UNTH) Enugu were used for the study. There were 105 males and 45 females.

Sample Collection

Nine milliliters (9mls) of venous blood were collected from each subject by venepuncture using aseptic methods. The left median antecubital vein was used for all the subjects to maintain uniformity.

The blood samples were immediately placed into different sterile containers containing specific anticoagulants to be used for the study. Each anticoagulant container was prepared to anticoagulate 1.5mls of blood. Sample were mixed slowly to avoid destroying the blood platelets. Six (6) types of anticoagulants were used for the study-dipotassium ethylene diamine tetra acetic acid (K_2 EDTA), sodium fluoride (NaF), heparin, sodium citrate (NaC), citrate phosphate dextrose – adenine (CPD – A) and acid citrate dextrose (ACD). The final concentration of the six anticoagulants in the 1.5 mls of blood were K_2 EDTA – 3mg, NaF – 48mg, heparin – 25iu, NaC – 6.46mg, CPD-A – 5.5mg and ACD – 5.06mg respectively. The anticoagulated blood samples in the six different containers were stored at 4^0C from where the samples were taken on a daily basis for four (4) consecutive days for platelet count.

Method of Determination of platelet count

The platelet counts were done using an automatic - Particle Counter (PCE – 90) of the ERMA PCE Series (ERMA INC. TOKYO JAPAN). This uses the principle of electric resistance and colorimetric measurement method with a dilution ratio of 1:40,000 for platelets. It has an inbuilt diluter, a Liquid Crestar Display (LCD), and a printout facility for the values obtained Three readings were taken in each case, the mean values and the standard deviations were calculated and recorded. The values obtained were subjected to "student t" test using 100 – 400 x 10^9 / litre as the standard. The P. values of < 0.05 were taken as significant statistically. Comparisons were made between the different days within each anticoagulant group.

Results

TABLE 1

The platelet counts obtained in the six different anticoaguluted blood samples for the four consecutive days are presented as platelet count (x10^9/L) mean ± standard deviation (S.D). The platelet count was reduced in all of them, the reduction in count increasing as the storage time increases. In the NaC sample of day 3, no platelet was seen.

Table 1: Platelet counts in six different anticoagulated blood samples for four (4) consecutive days (x 10^9 cells per microlitre of blood) mean ± s.d

Anticoagulant	Days of Storage			
	0	1	2	3
EDTA	191.6± 92.1	154.7±85.7	121.8±63.5	56.0±53.1
NaF	81.0±62.0	51.4±38.6	29.6±26.4	19.0±25.1
Heparin	195.0±91.5	173.3±86.3	139.6±77.3	84.7±52.2
NaC	174.0±80.2	122.7±69.5	75.5±46.9	ABSENT
CPD-A	197.2±79.1	168.1±76.0	123.4±68.7	65.1±35.1
ACD	176.8±80.3	141.8±74.6	95.1±66.6	38.7±35.5

TABLE 2

The test group values were compared with the normal count of 150x109/L as the standard. There were statistical differences from day 1 to day 3; while significance was only noticed in NaF on day O.

Table 2: Comparison between test group and control group (normal count 150 x 10^9/ul)(mean ±S.E.M)

days of storage	Anticoagulants					
DAYS	K_2EDTA	NaF	Heparin	NaC	CPD-A	ACD
O	191.6±92.1	81.0±62.0	195.0±91.5	174.0±80.2	197.2±79.1	176.8±80.3
P.VALUE	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
1	154.7±85.7	51.4±38.6	173.3±86.3	122.7±69.3	168.1±76.0	141.8±74.6
P.VALUE	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
2	121.8±63.5	29.6±26.4	139.6±77.3	75.5±46.9	123.4±68.7	95.1±66.6
P.VALUE	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
3	56.0±53.1	19.0±25.1	84.7±52.2	ABSENT	65.1±35.1	38.7±35.5
P.VALUE	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

TABLES 3 – 5
Shows intra day's comparisons, using Day O as the control. In table 3, their were significant differences in K_2 EDTA and NaC, table 4 showed no significant difference in only Heparin, while table 5 showed that their were significant differences in all the samples when day 3 was compared with day O.

Table 3: Comparison between Platelet counts of Day O against Day 1 (Values are mean ± S.E.M)

Anticoagulants	Day O	Day1	P – Value
K_2 EDTA	191.6±92.1	154.7±85.7	S
NaF	81.0±62.0	51.4±38.6	N.S
Heparin	195.0±91.5	173.3±86.3	NS
NaC	174.0±80.2	122.7 ±69.3	S
CPD – A	197.2±79.1	168.1±76.0	NS
ACD	176.8±80.3	141.8±74.6	NS

Table 4:*Comparison between platelet counts of day 0 against Day 2 (Value are mean ± S.E.M)*

Anticoagulants	**Day 0**	**Day 2**	**P – Value**
K_2 EDTA	191.6 ±92.1	121.8±63.5	S
NaF	81.0±62.0	29.6±26.4	S
Heparin	195.0±91.5	139.6±77.3	NS
NaC	174.0±80.2	75.5±46.9	S
CPD – A	197.2±79.1	123.4±68.7	S
ACD	176.8±80.3	95.1±66.6	S

Table 5:Comparison between platelet counts of day 0 against Day3 (values are mean ± S.E.M)

Anticoagulants	**Day 0**	**Day 3**	**P – Value**
K_2 EDTA	191.6±92.1	56.0±53.1	S
NaF	81.0±62.0	19.0±25.1	S
Heparin	195.0 ±91.3	84.7±52.2	S
NaC	174.0±80.2	– absent	S
CPD – A	197.2±79.1	65.1±35.1	S
ACD	176.8±80.3	38.7±36.5	S

TABLES 6 and 7 Further intra days comparisons were done using Day I as the control. Their were significant reductions in the platelet counts

Table 6: Comparison between platelet counts of day 1 against Day 2 (Values are mean ± S.E.M)

Anticoagulants	**Day 1**	**Day2**	**P – Value**
K_2 EDTA	154.7±85.7	121.8±63.5	S
NaF	51.4±38.6	29.6±26.4	S
Heparin	173.3±86.3	139.6±77.3	S
NaC	122.7±69.3	75.5±46.9	S
CPD – A	168.1±76.0	123.4±66.6	S
ACD	141.8±74.6	95.1±66.6	S

Table 7: Comparison between platelet counts of day 1 against Day 3 (Values are mean ± S.E.M)

Anticoagulants	**Day 1**	**Day 3**	**P – Value**
K_2 EDTA	154.7±85.7	56.0±53.1	S
NaF	51.4±38.6	19.0±25.1	NS
Heparin	173.3±86.3	84.7±52.2	S
NaC	122.7±69.3	Absent	S
CPD – A	168.1±76.0	65.1±35.1	S
ACD	141.8±74.6	38.7±36.5	S

TABLE 8

Day 2 and Day 3 were also compared. Significant differences were also obtained.

Table 8: Comparison between platelet counts of day 2 against Day 3 (Values are mean ± S.E.M)

Anticoagulants	Day I	Day 4	P – Value
K_2 EDTA	121.8±63.5	56.0±53.1	S
NaF	29.6±26.4	19.0±25.1	NS
Heparin	139.6±77.3	84.7±52.2	S
NaC	75.5±46.9	Absent	S
CPD – A	123.4±68.7	65.1±35.1	S
ACD	95.1±66.6	38.7±36.5	S

Discussion

The determination of platelet count is a very important laboratory test. The quantitative abnormality of platelet is one of the commonest haemostatic defect. Reduction in platelet number (thrombocytopenia) has been documented to result to many abnormalities including haemorrhage, thrombosis, atherosclerosis, etc (Bloom and Thomas, 1981; Sheldon, 1988).

For haemostasis to be accomplished, platelets must adhere to the site of the injury, then aggregate to form a platelet plug which can then arrest the bleeding. The platelet number is an important factor in the formation of the plug which can stop the bleeding if small vessels are injured. Hence the importance of optimum platelet number in the blood especially stored blood for transfusion.

The analysis of the results in the different anticoagulants showed significant decreases after three days of storage (table 2). In the intra group analysis, the result of the 0 day against the 1st day of storage (table 3) in K_2 EDTA and NaC, showed significant differences as against non significant difference in NaF, Heparin, CPD – A and ACD. Still on intra-group analysis, in table 4 (between 0 vs 2nd day of storage) their were significant reductions in platelet counts in most of the anticoagulants. While in table 5 (between 0 vs 3rd day of storage) the reduction in count occurred in all the anticoagulants. This may be due to the fact that enough time has elapsed for significant biochemical changes taking place in the stored blood to manifest as reduced platelet count. This is supported by the report of James (1996) who noted that during storage, storage defects results to loss of membrane glycoprotein I (GPI) and glycoprotein III (GPIII). It also leads to decreased thrombin receptor sites and decline or deterioration in both alpha (α) and dense granules storage pool content. The above are required for the maintenance of a normal platelet membrane structure (John West; 1985), hence their abnormality leads to instability of the platelets membrane, its rupture and finally decreased platelet count.

Further intra-group results analysis (tables 6-8) showed significant decreases in platelet count in all the anticoagulant samples. This is in agreement with the report of Mollison (1979).

It can thus be concluded from the above study that platelets are lost when blood is stored in anticoagulants. This loss is minimal in blood stored in heparin when compared with the other anticoagulants used in this study, hence can be said to have a sparing effect on platelets. It is recommended that whenever platelet count cannot be performed within the usual two hours of blood collection, heparin can be used to store the blood.

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