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Chemiluminesence study of Leukocytes Function in Blood Samples in Different Anticoagulants and Different Periods of Storage

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Abstract

The in vivo function of polymorphonuclear leucocytes (PMNs) may be divided into three parts: the ability of the cells to circulate normally, the ability of the cells to localize at areas of inflammation, and the ability of the cells to carry out phagocytic and bactericidal activity at the inflammatory site**.** The study aimed to study the effect of different anticoagulants for different duration of time on the leukocytes activity, and study the effect of storage of blood in different temperatures on the polymorphonuclear leukocytes activity.

Leucocytes function was evaluated using Luminol –dependent Chemiluminesence techniques , Barium sulphat crystals was used to stimulate the oxidative burst in leukocytes of whole blood. Chemiluminesence functional activity was measured immediately after blood collection after 24, 48, and 72 hour of storage at 4℃ .

This study revealed that the blood storage for longer than 24 hours caused a marked inhibitory effect on the induction of luminol dependent Chemiluminesence of leukocytes in blood collected in acid citrate dextrose (ACD) , citrate phosphate dextrose (CPD) , heparin and sodium citrate anticoagulants. Regarding the effect of storage of blood in different temperature on polymorphonuclear leukocytes activity the results showed that the activity has decrease in both temperature at storage time (24-72) hr. and greater decrease occurred when blood storage at room temperature. In vitro testing of leukocytes maintain normal function at least during the first 24 hour after collection under standard blood bank conditions. Chemiluminesence inhibition after 48 hour and 72 hour of storage was associated with leukocytes morphological changes.

Keywords Chemiluminesence, storage time, leucocytes function

دراسة وظيفة كريات الدم البيض بطريقة التألق الكيميائي في عينات الدم في مضادات تخثر المختلفة وفترات زمنية مختلفة للتخزين

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الخلاصة

يمكن تقسيم وظيفة الخلايا متعددة النواة(كريات الدم البيضاء) الى ثلاثة محاور:قدرتها على الدوران والتواجد في جهاز الدوران بشكل طبيعي , قدرتها على التجمع في اماكن الالتهاب , قدرتهاعلى تنفيذ البلعمة وقتل البكتريا في الموقع الالتهابي. هدفت الدراسة إلى دراسة تأثير مضادات التخثر المختلفة لفترة زمنية مختلفة على نشاط الكريات البيض ، ودراسة تأثير تخزين الدم في درجات حرارة مختلفة على نشاط الكريات البيض المتعددة النوى .تم تقيم وظيفتها باستخدام تقنيات التألق الكيميائي المعتمد على اللومينول بلورات سلفات الباريوم استحدمت لتحفيز الاحتراق التاكسدي في كريات الدم البيضاء . قابيلة التألق الكيميائي تم قياسها مباشرة بعد جمع الدم , وبعد 24 ساعة , وبعد 48 ساعة , وبعد 72 ساعة من تخزين الدم بدرجة حرارة 4 درجة مئوية. كشفت هذه الدراسة أن تخزين الدم لأكثر من 24 ساعة تسبب في تأثير مثبط ملحوظ على استحثاث التألق الكيميائي المعتمد على اللومينول من الكريات البيض التي تم جمعها في كل انواع موانع النخثر المستخدمة في الدراسة , فيما يتعلق بتأثير تخزين الدم في درجات حرارة مختلفة على نشاط الكريات البيض متعددة النوى ، أظهرت النتائج أن النشاط قد انخفض في 4 درجة مئوي ودرجة حرارة الغرفة في وقت التخزين (24-72) ساعة

وحدث انخفاض أكبر عند تخزين الدم في درجة حرارة الغرفة. اختبار قابلية الكريات البيض في المختبر في الحفاظ على وظيفة عادية على الأقل خلال أول 24 ساعة بعد جمع الدم تحت ظروف بنك الدم القياسية. ارتبط تثبيط التألق الكيميائي بعد 48 ساعة و 72 ساعة من التخزين بتغيرات شكلية في الكريات البيض.

Introduction

The *in vivo* polymorphnuclear leucocytes (PMNs) functions may be separated into connect broadly: the capability of the cells to circulate ordinary, localize at inflammation areas, and the skills of the bactericidal and phagocytic roles.

It has generally been thought that PMNs rapidly lose their capacity to circulate *in vivo* after being stored in vitro for more than a few hours. Has customary been feeling digress PMNs positively do in their faculty to arrogance *in vivo* after being stored *in vitro* for relating to than a some high noon[1][2]. As a result, most

centers use only freshly collected PMNs for clinical granulocyte transfusions. PMNs transfusion programs would be greatly facilitated if it were possible to maintain in vivo function during in vitro storage. As a caution, most skillfully centers render a reckoning for unassisted freshly unruffled PMNs for clinical granulocyte transfusions. PMNs transfusion programs would be copiously facilitated if it were practical joker to argue in vivo play the part on in vitro storage.

The granulocytes transfusion success for neutropenic patients in all directions sepsis is determined by a count and the magnitude similar to granulocytes transfused [1]. According to different studies the granulocyte function will suffer from progressive impairment caused by storage [1,2,3,4,5,6,7] make obtaining more information concerning granulocytes maintenance is mandatory[2].

The respiratory burst refer to phagocyte response with a chain of biochemical and cytophysiological events upon chemical stimulation or interaction with particulate stimuli. Consumption more oxygen that of the converted into excited oxygen species which produce light when oxidized. Measurement of Chemiluminesence (CL) ,which yield from react of reactive oxygen species with certain substances because exited them electronically[3], is indicator for phagocytic function(4) .Using the luminol, oxidized substrate, will increase the Chemiluminesence (CL) response by thousands fold. Aim of the study to study the effect of different anticoagulant for different duration of time on the polymorphonuclear leukocytes activity and to study the effect of storage of blood in different temperatures on the polymorphonuclear leukocytes activity.

Materials and Methods

Venous blood samples (10 ml) were obtained from healthy adults, the samples were separately mixed with sodium citrate, acid citrate dextrose (ACD) , citrate phosphate dextrose (CPD) and heparin as anticoagulants and stored in a blood bank refrigerator at 4 ℃. At various time during storage each sample was thoroughly mixed before, 0.02 ml of blood was taken for chemiluminesence measurement. The remainder of the samples

returned to the blood bank refrigerator. The total leukocytes count and leukocytes differential count and morphology were studied.

In present study, the function of polymorphonuclear leukocytes in whole blood collected in various anticoagulants available for routine blood bank use was studied by the luminol dependent CL techniques [12]. The leukocytes of stored blood were activated by BaSO4 crystals to burst luminol dependent
CL[4].

Chemicals and Reagents:

Luminol solution was prepared by dissolving 20 mg of luminol (sigma chemical Co.) in 2 ml of 0.2 M NaOH. This stock solution was diluted up to 100 ml with deionized water prior to use.

Chemiluminesence inducer:

In order to activate leukocytes to burst luminol dependent CL, a medium of the following composition (mM) was used: 165 NaCl, 15 Tris HCl ,2.25 CaCl2 , and 25 BaSO4 , (pH 7.4) was in this medium in suspension form [5].

Chemiluminesence Measurement:

This was carried out using the principle of oxidation of luminol by reactive oxygen species produced during phagocytosis in phagocytic cells to increase the amount of measurable light[5]. Luminol dependent Chemiluminesence (CL) in stimulated polymorphonuclear leukocytes was measured in an ultra-sensitive photon counting system built in the department of physiology, college of medicine – University of Basrah.

The reaction mixture consisted of 2 ml CL inducer and 0.2 ml of luminol in a 5 ml vessel. To this mixture 0.02 ml whole blood was added and agitated to mix well before it was poured into the measuring cuvette of the photon counting system; where the temperature is kept at 37℃. CL continuously recorded until the CL peaked and demonstrated a definite decline .All the results were taken in relative to standard arbitrary units.
Number of granulocytes were measured at different time of storage. The result of CL activity measured for 100 cells allow for statistical comparisons.

$$CL\ activity\ = \frac{\textbf{CL peak high}}{\textbf{No. of cells}} \times 100$$

$$\%\ inhibition = (test - control)/control$$

Statistically analysis

Data was expressed as Mean ±SD and was compared using Anova: Single Factor and Anova: Two-Factor without Replication. P-value<0.05 was considered statistically significant. Microsoft Excel was used for the analysis of the data.

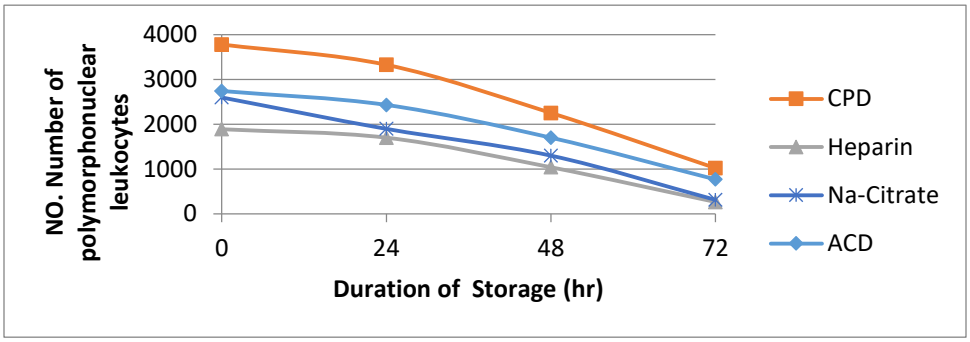
Results:

Immediately after collection , the total polymorphonuclear leukocytes were counted, Following 24 hr. of storage the absolute number of morphologically normal polymorphonuclear leukocytes decreased by only 11.33 % in blood stored in ACD , 12 % in blood stored in CPD and 10 % in blood stored in heparin , whereas those collected in sodium citrate showed a greater decrease 26.98 % (Table 1) . After 3 days of the storage 71.97- 88 % of the all the original morphologically normal leukocytes were lost. Bar chart (1) shows these results

Table. 1 :Changes in the number of morphologically normal polymorphonuclear leukocytes during storage at 4 ℃ in different anticoagulants for different duration of storage

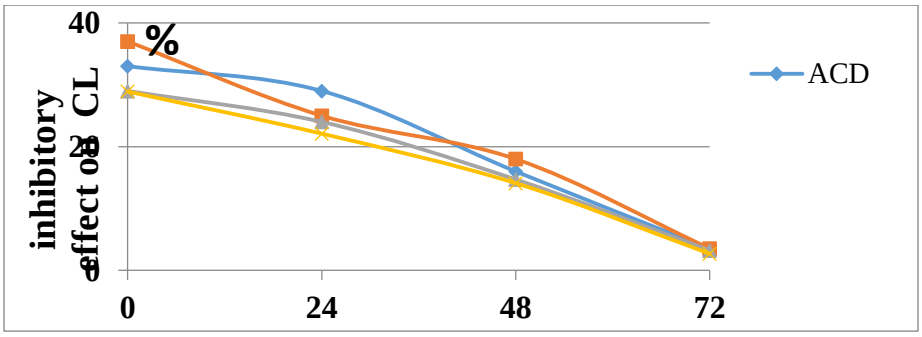
Duration of Storage (hr)	ACD		CPD		Heparin		Na-Citrate	
	No.	% of inhibition	No.	% of inhibition	No.	% of inhibition	No.	% of inhibition
0	2744	-	3783	-	1890	-	2599	-
24	2433	11.33	3329	12	1701	10	1898	26.98
48	1701	38.01	2254	40.42	1040	44.97	1300	49.98
72	769	71.97	1021	73	265	85.97	312	88

NO. Number of leukocytes, (%) percentage loss of normal leukocytes.



Bar chart (1): effect of storage time at 4 ℃ in different anticoagulants on polymorphonuclear leukocytes count.

The maximum Light output of the luminol dependent CL of whole blood, induced by $BaSO_4$ for all anticoagulants was seen at zero time. Table (2) represents the induced CL functional activity of the leukocytes as a percent of the control for the blood samples stored in each anticoagulant at different time intervals. Mean values of triplicate recording were represented. Storage of blood for 48 hr. and 72hr. produced a marked and progressive inhibitory effect on luminol dependent CL the results has been shown in bar chart (2).



Bar chart (2): Inhibitory effect on the Luminol dependent CL at different storage time at 4 ℃ **in different anticoagulants**.

Table (2): The induced CL activity of polymorphonuclear leukocytes as a percent in different times of storage in various anticoagulants and stored at 4 ^{0} C. measures for same no. of cells (100 cells).

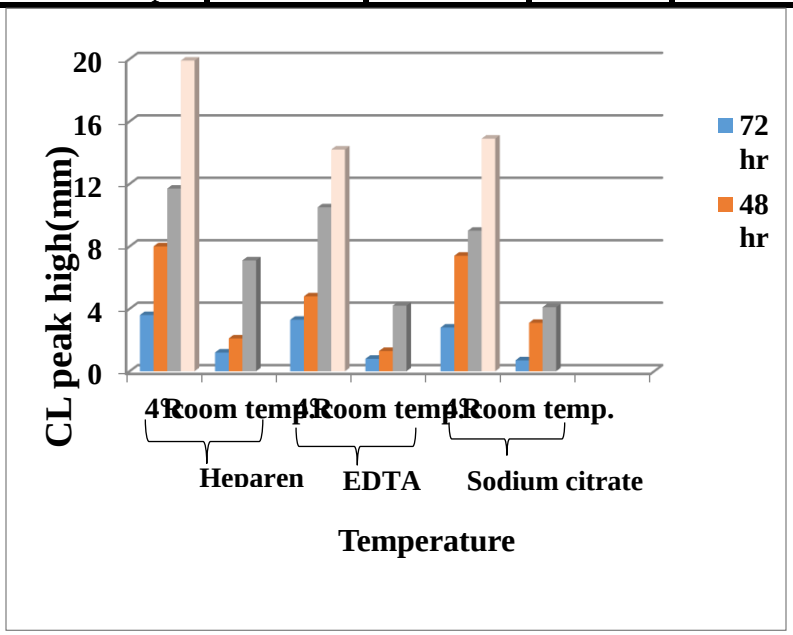
Anticoagulant	Duration of the storage(hr)			
	0	24	48	72
ACD	33±7.3	29±6.6	16±6.1	3.5±1.3
CPD	37±9.1	25±5.7	18±7.3	3.55±1.7
Heparin	29±8.8	24±6.1	14.7±7.4	3.25±1.8

Na.citrate	28.9±7.6	22.1±5.7	14.1±11	2.76±1.6

Table (3) shows the granulocyte functional activity for 100 cells in arbitrary units for variant anticoagulant (CL peak high (mm)) at storage time at 4°c and at room temperature. The peak height has been decreased with time in all cases.

Table(3): Granulocyte functional activity for 100 cells in arbitrary units for various anticoagulants (CL peak high(mm)) storage time at 4°c

Anticoagulant	Temperature	Duration of storage (hr)			
		0	24	48	72
Heparin	4°c		11.7±0.4	8±0.6	3.6±0.7
	Room temp.	19.9±0.3	7.1±0.9	2.1±0.7	1.2±0.8
EDTA	4°c		10.5±1.9	4.8±1.7	3.3±1.5
	Room temp.	14.2±1.6	4.2±0.7	1.3±0.6	0.8±0.3
Sodium citrate	4°c		9±1.4	7.4±1.7	2.8±1.3
	Room temp.	14.9±4	4.1±1.6	3.1±1.8	1.5±0.7



Bar chart (3): Granulocyte functional activity for 100 cells in arbitrary units for variant anticoagulants (CL peak high(mm)) storage time at 4°c and room temperature.

Discussion:

The present study shows that blood storage under standard bank conditions has a marked inhibitory effect on the luminol dependent CL induced by BaSO4 of human blood polymorphonuclear leukocytes. However the CL of polymorphonuclear leukocytes from all anticoagulants was similar immediately after collection (at zero time) and remained essentially unchanged during the first 24 hrs.of storage. Blood stored longer than 24 hr. showed a marked decrease in the CL functional activity of polymorphonuclear leukocytes available for transfusion (Table 2).

In recent years CL has emerged as an important tool in the assessment of the oxidative burst of granulocytes which thought to be closely linked to bactericidal activities in phacocytic cells[4][6][7] . The inhibitory effect of storage on BaSO4 induced CL noticed in this study may be due to the progressive abnormal morphological changes in polymorphonuclear leukocytes during storage (Table 1). The absolute number of polymorphonuclear leukocytes available for transfusion remained essentially unchanged during the first 24 hr. of storage. Blood stored longer than 24 hr. would yield a progressive decrease in the number of the morphologically normal polymorphonuclear leukocytes.

Polymorphonuclear leukocytes morphologically changes and functional deterioration induced by storage were reported [8,9.10,11]. The present study indicates that polymorphonuclear leukocytes maintain nearly normal in vitro activity during the first 24 hrs. of storage .The activity falls markedly and progressively after 72 hrs. of storage at 4 ^{0}C . Thus short –term storage of polymorphonuclear leukocytes may be of importance for the quality transfusion.

Investigating the effect of time , different anticoagulant and different temperature on the viability of human PMNs with a variety of *in vitro* function tests, showed that in vitro function and the ability of the PMNs to induce CL at various anticoagulant and at 4℃ and room tempt(RT) stored up to 72 hours . Activity of the cells was only minimally impaired

after similar 24-hrs storage. With storage times longer than 24 hrs, cells were progressively impaired after 24 to 72 Storage time (hours) at different temperature of 4℃ and RT.

With storage at either RT or 4°C for 72 hrs storage used in this study (at different anticoagulant; storage of whole blood) cells stored at 4°C show (table 4) permanent cell damage in compare to the 24 hrs of storage. In contrast, cells stored longer than 24 hrs at RT have sustained irreversible damage as reflected in reduced CL functional at all-time points. This would suggest that PMN viability is better maintained at 4°C than at RT but further temperature studies are necessary to determine if 4°C is indeed optimal. This model is useful for assessing the *in vitro* function of stored PMNs. These studies demonstrate that PMNs stored in whole blood for 72 hrs at 4°C showed acceptable permanent impairment in their ability to circulate and only a moderate impairment in their ability to localize in vivo at sites of inflammation. Storage for 48 hrs or longer at RT resulted in significant impairment by all anticoagulant used.These observations suggest that it may be possible to store granulocytes for at least 1-2 days for transfusion.

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