Assessment of RBCs membrane protective activity of citicoline and eicosapentanoic- decosahexanoic acid in osmotic fragility model

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(Received 6/5/2013, Accepted 9/6/2013)

الخلاصة

ان للكثير من المواد العضوية خاصية مدعمة لاستقرارية الاغشية الخلوية مما قد يحمي كريات الدم الحمراء من التحلل بسبب جهود الضغط التناضحي المسلط من المحلول المحيط يمكن تقييم الاثر الواقي لهذه المواد ضد تحلل الكريات بواسطة تحليل التكسر التناضحي .

من هذه المواد العضوية التي تزيد سعة تحمل التكسر التناضحي هو السايتكولين والاحماض اكاربوكسيلية الغير مشبعة المتعددة أي بي أي- دي اج أي ان. خلال هذا التقييم ظهر ان كلا من السايتوكولين والاحماض يؤدي الى حماية تكسر الخلايا الحمراء الى مدى اوسع من المحلول الاساس المقارن على الرغم من ان التغير لم يكن معتدا بمقياس التقييم الاحصائي الارتباطي الا انه ذو اثر كبير بمقياس للفارق الاحصائي في مدى تغير التركيز الملحي بين 0.4 و0.5 عند $P<0.5$.

ومن عموم النتائج فان السايتكولين واي بي اي اج اي احدثا فرقا مهما في ثباتية الخلايا الحمراء ضد تغيرات الضغط التناضحي للمحلول بين تركيز 0.4-0.5 .

وقبل التوصية باستعمال مثل هذه المركبات العضوية في الوقاية من تحلل كريات الدم الحمراء فمن الضروري اجراء تقييمات على نطاق اوسع .

Abstract

Different organic compounds possess a good membranes stabilizing effects that protect cells like erythrocytes from strains exerted by change in medium osmolarity . These changes in tonicity could predispose RBCs for hemolysis . This disorder could be assessed by osmotic fragility test . From those compounds that widen osmotic fragility test values are citicoline (membrane phospholipids precursor) and polyunsaturated fatty acids EPA-DHA.

Assessment of these compounds on osmotic fragility test revealed that both of citicoline and EPA-DHA will protect RBCs from hemolysis for a wider range than values of the control blank. Although these effects were statistically not significant on considering statistical correlation test, however, the differences were important on considering t test for the range of saline concentration between 0.4-0.5 at $P<0.05$. From the overall results, both citicoline and pufa induced a significant change in statbility of RBCs membrane upon exposure of osmotic effects in the saline concentration between 0.4-0.5. Further evaluation of citicoline and EPA- DHA effects may be necessary before recommending the use these compounds in protection against hemolytic diseases.

Keywords: osmotic fragility test, citicoline, EPA-DHA, hemolytic Anemia.

Introduction

Many disorders of erythrocytes membrane can attenuate ability of RBCs in overcoming osmotic stress like hereditary spherocytosis, hemolytic anemias, sickle cell anemia, thalassemia major and conditions that exert oxidative stresses on cells lipid bilayer can induce rupture of erythrocytes and cause hemolytic sequalae (1). Human red blood cells RBCs have the ability to minimize strain exerted on their cell membranes by their concave shape and specific lipid constituents of their membranes that enable RBCs resistant to rupture under higher osmotic stress like hypotonic conditions. RBCs will swell under hypotonic blood conditions to make intracellular osmolarity as similar as to that of extracellular(2) . However , under genetic or oxidative disorders above or

under abnormal hypotonicity this ability of RBCs is lacking giving rise to fragile RBCs that eventually will hemolyse when for example traversing splenic network .

As many disorders can cause RBCs to lack one or more of their plasma membrane constituents, supplement of some essential lipid bilayer supporting agents like unsaturated fatty acids (eicosopentanoic EPA and decosohexanoic DHA acids) and adenosine analoque citicoline are expected to increase RBCs resistance for strains exerted by hypotonic or stressful conditions since these compounds could optimize structural integrity of plasma membrane of many cell types (3) .

Citicoline is a phosholipid precursor that stabilizes cell membrane (Chemical IUPAC Name 2-[[[5-(4-amino-2-oxo-pyrimidin- l-yl)-3,4- dihydroxy – oxolan -2-yl] methoxy –hydroxy-phosphory l]oxy-hydroxy – phosphory l] oxyethy l –trimethy l – azanium). It has the structure:

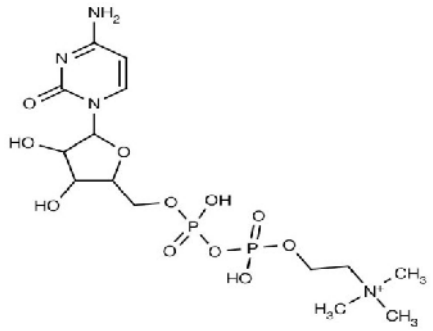

Figure (1) citicoline structure, a membrane phospholipids precursor (4) In regard to polyunsaturated fatty acids (pufa) and omega-3 pufa they have the following properties:

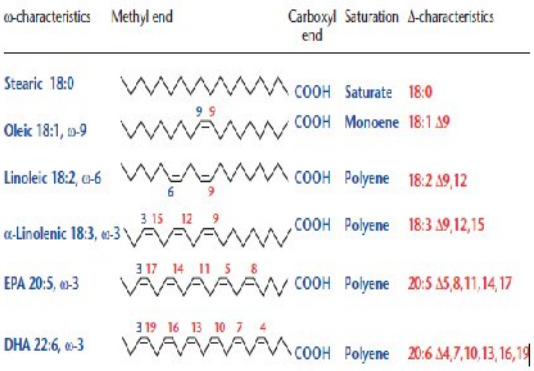

Figure (2) the structure of different types of pufa (5).

Osmotic fragility is a test to measures red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. It has a normal range: Hemolysis begins 0.45% and complete 0.35%.

The sooner hemolysis occurs, the greater the osmotic fragility of the cells .

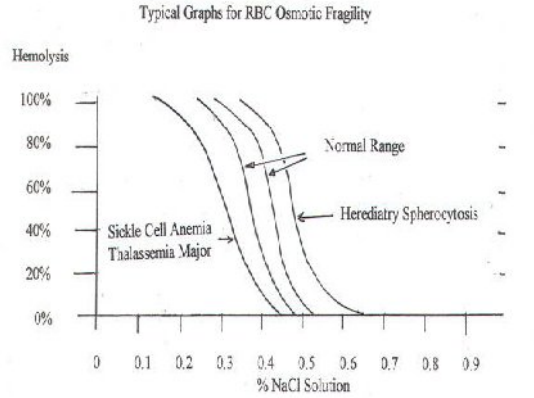

Figure (3) the normal range of human RBCs fragility values as compared with sickle cell anemia and spherocytosis.

Materials and Methods

The research was carried out at Therapeutic Research Unit College of Medicine Kufa University.
Collection Medium: EDTA blood collection tubes
Minimum: 5 ml whole blood.
Specimen: whole blood
Rejection Criteria: Hemolyzed specimen.
Methodology: Spectrophotometer .At 540 nm .

Table (1) Steps of serial dilutions of blood samples for testing RBCs fragility.

Test tube	1%Nacl (ml)	D.W.(ml)	Final conc.(%)
1	10.0	0.0	1.00
2	8.5	1.5	0.85
3	7.5	2.5	0.75
4	6.5	3.5	0.65
5	6.0	4.0	0.55
7	5.0	5.0	0.50
8	4.5	5.50	0.45
9	4.0	6.0	0.40
10	3.5	6.0	0.35
11	3.0	7.0	0.30
12	2.0	8.0	0.20
13	1.0	9.0	0.10
14	0.0	10.0	0.00

1- Preparation of test tubes and blood samples.
2-Then every volume was divided into 2 tubes so that 28 tubes were obtained.
3- 50 micron of whole blood was added to each tube.
4- The tubes were left at R.T for 30 min at 2500 rpm.
5- Mixing by using the vortex.
6-Centrifugation for 5 minutes at 2500 rpm
7- Measuring the absorbance in the tubes by using spectrophotometer (540nm).
8-calculate the % of hemolysis =
(Abs of tube /Abs of tube 14)* 100% :
Normal Range
Hemolysis begins 0.45% and complete 0.35%

Results

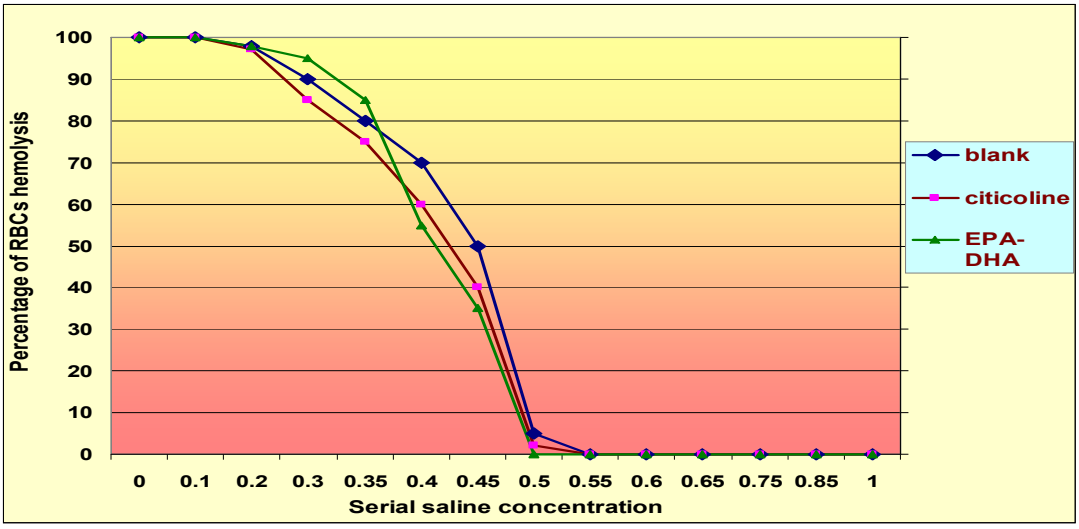

Figure (4) osmotic fragility values of human RBCs against serially increasing saline concentration for both citicoline and EPA-DHA in comparism with the blank (untreated) solution.

There was an obvious left graphic shift (increased RBCs resistance for more hypotonic solution) as compared with blank.However this effect is significant not along the whole dilution range (correlative assessment) other than saline concentration between 0.4-0.5 at P<0.05.

Discussion

It was noticeable from the result graph that both citicoline and EPA-DHA induced a protective effect for RBCs against hypotonic swelling exerted on their plasma membranes due to the aggressive hypotonic medium.

However that change in RBCs osmotic fragility was only statistically significant for citicoline and EPA-DHA at P<0.05 on considering NaCl concentration between 0.4-0.5. This minor changes in osmotic fragility, although limited but they could safe a large proportion of blood cells from hemolysis in critical conditions like hereditary spherocytosis .

It is expected that both citicoline and those unsaturated fatty acids will incorporate with RBCs plasma membrances and give them more flexibility and strength that prevent them from rapture. Omega -3 fatty acids like EPA-DHA expand the spectrum of osmotic fragility test for human RBCs similar studies agreed with this attribution (6),(7) . Another studies showed that citicoline as a part of phospholipids will stabilize cell membranes and protect against stress as well (8). These RBCs stabilizing effects may be beneficial in treatment of patients with hemolytic conditions and heridetary and aquired spherocytosis.

Conclusion

Citicoline and EPA-DHA had a beneficial erythrocyte protective effect against hemolytic stimuli like hypotonicity. This property could make them a good candidate for use in thalassemia, sickle cell anemis and spherocytosis after being further evaluated.

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