

The Effect of Storage of Blood Specimens on Erythrocyte Transketolase Activity and Thiamine Pyrophosphate Effect

Wilmar Musram, dr; Evi Setiadi, dr

Abstrak

Penelitian ini dimaksudkan untuk mengetahui apakah darah dapat disimpan pada suhu -20°C selama 7 dan 14 hari tanpa terjadi perubahan berarti pada aktivitas transketolase (TK) darah dan pada efek tiamin pirofosfat (TPP). Subjek penelitian terdiri dari 20 orang mahasiswa kedokteran yang tidak menunjukkan tanda-tanda dan gejala-gejala defisiensi vitamin. Dengan menggunakan metode Dreyfus diperoleh hasil sebagai berikut : Aktivitas TK darah tanpa dan dengan penambahan TPP *in vitro* pada darah segar berturut-turut $83,95 \pm 9,94$ UI ($\bar{X} \pm 1SD$) dan $92,90 \pm 11,06$ UI; sesudah penyimpanan selama 7 hari pada suhu -20°C $83,70 \pm 9,89$ UI; dan $92,60 \pm 11,14$ UI dan sesudah penyimpanan 14 hari pada suhu -20°C $83,75 \pm 9,99$ UI dan $92,70 \pm 11,06$ UI. Efek TPP pada darah segar $10,75 \pm 4,02\%$ ($\bar{X} \pm 1SD$), sesudah penyimpanan selama 7 dan 14 hari pada suhu -20°C berturut-turut $10,70 \pm 4,09\%$ dan $10,79 \pm 4,23\%$. Perhitungan statistik dengan analisis varians menunjukkan bahwa tidak ada perbedaan bermakna antara aktivitas TK darah maupun efek TPP darah segar dan darah yang disimpan pada suhu -20°C selama 7 dan 14 hari ($p > 0,05$). Dengan demikian dapat disimpulkan bahwa dengan cara yang kami lakukan darah dapat disimpan pada suhu -20°C selama 14 hari tanpa terjadi perubahan pada aktivitas TK darah efek TPP. Hasil penelitian ini sesuai dengan pendapat Brin, bahwa hemolisis tidak mengalami perubahan pada suhu -20°C paling sedikit selama 3 bulan. Demikian juga sesuai dengan penemuan Smeets dkk. bahwa hemolisis dapat disimpan pada suhu -20°C selama 4 minggu tanpa kehilangan aktivitas TK atau perubahan pada efek TPP. Akan tetapi hasil penelitian kami berbeda dengan yang didapatkan oleh Puxty dkk., yaitu bahwa aktivitas TK sel darah merah dan efek TPP menurun bermakna setelah penyimpanan hemolisis pada -20°C selama 14 hari. Demikian juga ditemukan oleh Bayoumi dan Rosalki bahwa aktivitas TK sel darah merah sebelum dan sesudah penambahan TPP *in vitro* berturut-turut menurun 10% dan 16% setelah penyimpanan hemolisis pada suhu -18°C selama 14 hari. Perbedaan ini mungkin disebabkan oleh karena baik Puxty maupun Bayoumi menentukan aktivitas TK dalam hemolisis dengan metoda yang lain, yaitu dengan mengukur kegiatan oksidasi NADH.

Abstract

The objective of this study was to know the effect of storage of blood specimens at -20° for 7 to 14 days on the blood transketolase (TK) activity and on the thiamine pyrophosphate (TPP) effect. The subjects of the study consisted of 20 medical students, who had no signs or symptoms of vitamin deficiency. With the method proposed by Dreyfus the following results were obtained: The blood TK activity of fresh specimens without and with the *in vitro* addition of TPP were respectively $83,95 \pm 9,94$ IU and $92,90 \pm 11,06$ IU ($\bar{X} \pm 1SD$); those of specimens after storage for 7 days at -20°C respectively $83,70 \pm 9,89$ IU and $92,60 \pm 11,14$ IU and those of specimens after storage for 14 days at -20°C respectively $83,75 \pm 9,90$ IU and $92,70 \pm 11,06$ IU. The TPP effect of fresh specimens was $10,75 \pm 4,02\%$ ($\bar{X} \pm 1SD$); those of specimens after storage for 7 and 14 days respectively $10,70 \pm 4,09\%$ and $10,79 \pm 4,23\%$. Statistical analysis showed no significant difference between the blood TK activity as well as the TPP effect of fresh specimens compared to specimens stored at -20°C for 7 and 14 days ($p > 0,05$). From the results obtained could be concluded that with the method and procedure used, specimens may be stored at -20°C for 14 days without significant change in the blood TK activity and the TPP effect. The results of this study agreed with those obtained by Brin who found that hemolysed specimens were stable for at least 3 months at -20°C ; and with those obtained by Smeets et al. who found that the hemolysates may be stored at -20°C for 4 weeks without any loss of activity or change in the TPP effect. However, they were different from the findings of Bayoumi and Rosalki and from those of Puxty et al. who found that the erythrocyte TK activity and the TPP effect of washed erythrocytes stored at 18° - 20°C decreased significantly by day 14. These different results may be due to the different method used by Bayoumi and Puxty who determined the TK activity in hemolysates by measuring the rate of NADH oxidation.

Keywords : Erythrocyte transketolase activity, TPP effect, Vitamin B₁ deficiency.

INTRODUCTION

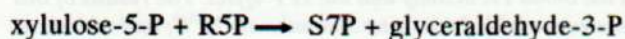
Vitamin B₁ (thiamine) deficiency presently rarely presents itself as beri-beri. However, subclinical deficiency of this vitamin remains a problem, especially in South East Asian countries, where the diet is known to be very high in carbohydrate. Thiamine as thiamine pyrophosphate (TPP) is an essential coenzyme for 2 important reactions in the metabolism of carbohydrates. Consequently when the diet is rich in carbohydrate the requirement for vitamin B₁ is high.

In the blood thiamine is primarily found in the blood cells as the pyrophosphate. In the plasma thiamine is present in the free form, probably as the inactive transport form. It is phosphorylated when entering the cell, including the nucleated erythrocytes in the bone marrow.¹

The ideal method for the determination of the thiamine status is by measuring the thiamine content of the tissues. Blood is a tissue that can be obtained relatively easy, but its thiamine content is not constant and fluctuates with the thiamine intake. Besides, the measurement of thiamine itself is relatively difficult to be performed routinely.²

TPP is the coenzyme for several enzymes present in the erythrocytes, such as transketolase (TK) which catalyses the formation of sedoheptulose-7-phosphate (S7P) from ribose-5-phosphate (R5P) and xylulose-5-phosphate. In thiamine deficiency the activity of this enzyme is decreased and addition of TPP will increase the activity. "TPP effect" or the percentage increase of TK activity produced by the addition of TPP increases with the severity of the deficiency. This TPP effect is specific for thiamine deficiency, is sensitive and relatively easy to determine and it is therefore a convenient method for clinical and field studies.³

The estimation of TK activity with the method proposed by Dreyfus (4) is based on the production of S7P from the reaction it catalyses :



The red color produced from the reaction of S7P with cysteine and sulfuric acid is measured spectrophotometrically. The TK activity is expressed in International Units (IU), defined as the number of micromoles of S7P formed per minute per liter of blood. The normal value for the TPP effect proposed by Dreyfus is 9 - 10%.⁵

Brin (quoted from Puxty⁵) reported that hemolysed specimens are stable for at least 3 months at -30°C. According to Smeets et al (quoted from Puxty⁵) hemolysates may be stored at -20°C for 4 weeks without any loss of TK activity or change in the TPP

effect. However, Puxty et al. found that the erythrocyte TK activity and the TPP effect decreased significantly on storage at -20°C for 14 days.⁵ Bayoumi and Rosalky also found a decrease of 10% and 16% prior to and following the addition of TPP respectively on storage at -18°C for 14 days.²

MATERIALS AND METHOD

The method proposed by Dreyfus⁵ was used for this study. The erythrocytes were hemolysed by storing the specimens overnight at -20°C. For the determination of S7P, the amounts of H₂SO₄ used, were adapted from Ismadi.⁶ The subjects of the study consisted of 20 medical students, who had no signs or symptoms of vitamin deficiency.

Reagents were prepared from chemically pure chemicals and deionised water.

Buffer-electrolyte solution 0.143 M pH 7.4 : 4 parts of 0.9% NaCl, 206 parts of 1.15% KCl, 1 part of 3.82% MgSO₄, 20 parts of phosphate buffer.

TPP 0.002 M in buffer-electrolyte solution : 0.09216 g TPP (Sigma, MW 460.8) in 100 ml buffer-electrolyte solution pH 7.4.

R5P 0.018 M : 0.49338 g R5P disodium anhydrous (Sigma, MW 274.01) in 100 ml water.

S7P standard solution 1 μmol/2 ml.

Sulfuric acid-water mixture (12 : 5 v/v).

Procedure

Venous blood was collected in heparin-containing tubes. For each specimen 3 sets of tubes (set 0, 7 and 14) were prepared; set 0 for the fresh specimens, set 7 and 14 for the specimens after storage at -20°C for 7 and 14 days respectively. Each set consisted of 1 unknown blank tube (UB), 2 TK tubes and 2 TPP tubes. The UB tube and TK tubes each contained 0.1 ml of a buffer-electrolyte solution and 0.1 ml of whole blood, while the TPP tubes 0.1 ml of TPP solution and 0.1 ml of whole blood. The tubes were capped with parafilm and stored at -20°C to hemolyse the blood. The tubes of set 0 were taken from the freezer the next morning and after agitating the contents, placed in a water bath of 37°C for 30 minutes. To the UB tubes was then added 0.2 ml of a 15% TCA solution; to the TK and TPP tubes 0.2 ml of R5P solution. After thoroughly mixing the contents, the tubes were reincubated in a waterbath for 30 minutes at 37°C. To the TK and TPP tubes were then added 0.2 ml of TCA solution to stop the TK activity. All the tubes were thoroughly agitated, then centrifuged at 3000 rpm for 10 minutes. From each tube 0.02 ml of supernatant was transferred to

other tubes and 1 ml of sulfuric acid mixture was added. A standard (St.) and standard blank (StB) were also prepared. The St tube contained 0.02 ml of S7P standard solution (1 μ mol/2 ml) and 1 ml of sulfuric acid mixture; the StB tube 0.02 ml of saturated benzoic acid and 1 ml sulfuric acid solution. The tubes were capped, the contents thoroughly mixed and heated in a waterbath at 80°C for 15 minutes. They were then allowed to cool to room temperature. To each tube was added 0.02 ml of 3% cysteine HCl solution; the tubes were then agitated and left overnight. The optical density was read the next morning at 520 and 540 nm. Each TK and TPP tube was read vs. its UB and the St. vs. the StB. From the difference of the OD at 520 and 540 nm (Δ OD) the amount of S7P formed was calculated. The TK activity was expressed in International Units (IU), equivalent to the number of μ moles S7P formed per liter per minute (1 IU = 1 μ mol S7P formed/liter/min.).

$$\text{Blood TK activity} = \frac{\text{OD sample}}{\text{OD standard}} \times 100 \text{ IU}$$

The TPP effect was calculated with the following equation :

$$\frac{B - A}{A} \times 100\%$$

A = TK activity in the TK tube

B = TK activity in the TPP tube

The data obtained from set 0, 7 and 14 were compared using the variant analysis.

RESULTS AND DISCUSSION

The results (Table 1) showed no significant difference between the TK activity either with or without addition of TPP in set 0, 7 and 14 ($p > 0.05$).

Table 1. Blood TK activity and TPP effect of set 0, 7 and 14.

Set	TK activity (IU)	TK activity + TPP (IU)	TPP effect (%)
0 (n=20)	$83,95 \pm 9,94$	$92,90 \pm 11,06$	$10,75 \pm 4,02$
7 (n=20)	$83,70 \pm 9,89$	$92,60 \pm 11,14$	$10,70 \pm 4,09$
14 (n=20)	$83,75 \pm 9,99$	$92,70 \pm 11,06$	$10,79 \pm 4,23$

Set 0 : fresh specimens

Set 7 : specimens stored at -20°C for 7 days

Set 14 : specimens stored at -20°C for 14 days

These results agreed with those obtained by Brin, who found that TK activity in hemolysed specimens were stable when stored at -20°C for 3 months. They also agreed with findings of Smeets et al. who found that the hemolysates may be stored at -20°C for 4 weeks without any loss of activity or change in TPP effect (quoted from Puxty⁶). However they were different from the findings of Puxty et al. who found that the erythrocyte TK activity and TPP effect decreased significantly after storage of the hemolysates at -20°C for 14 days.⁵ Bayoumi and Rosalki also found that the erythrocyte TK activity prior to and following the in vitro addition of exogenous TPP fell by 10% and 16% respectively after storage at -18°C for 14 days.² These different findings may be caused by the difference in method used by Puxty and Bayoumi. They used erythrocyte hemolysates and a method based on the rate of NADH oxidation, while in the method proposed by Dreyfus TK activity was determined by measuring S7P formed.

CONCLUSIONS

From our results may be concluded that blood specimens can be stored at -20°C for 14 days without significant change in erythrocyte TK activity or TPP effect. Further studies should be done to get information on the effect of storage for longer periods of time.

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