Comparison of Glucose Concentrations among Plasma Samples Obtained from Various Types of Antiglycolytic Agents

การเปรียบเทียบระดับน้ำตาลในพลาสมาซึ่งได้จากสารต้านการสลายกลูโคสหลายชนิด

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ABSTRACT

The decreasing of plasma glucose concentrations when whole blood is stored at room temperature as a result of glycolysis. Lithium heparin (LH) plasma samples have been used recently in urgent cases for simultaneously measuring glucose and routine biochemical testing. Previous studies introduced several antiglycolytic agents and combined with LH to clinical chemistry testing. There were many arguments on the efficiency of these agents. Plasma glucose levels were compared among LH alone and LH plus various antiglycolytic agents, glyceraldehyde, tris-bromoacetate, or D-mannose. This study found that blood samples treated with LH plus D-mannose and LH plus tris-bromoacetate effectively preserved glucose level for up to 8 hours at room temperature while LH alone and LH plus glyceraldehyde preserved glucose level for up to 2 hours. Combination between LH and D-mannose is the most appropriate preservation of blood for both glucose and electrolyte analysis.

บทคัดย่อ

เมื่อเก็บเลือดที่อุณหภูมิห้อง ระดับน้ำตาลในเลือดจะลดลงอย่างรวดเร็ว อันเป็นผลจากขบวนการสลายกลูโคสของเซลล์ ปัจจุบันมีการใช้สารกันเลือดแข็งลิเทียมเฮปารินในงานเร่งด่วน เพื่อตรวจระดับน้ำตาลและสารชีวเคมีอื่นๆในหลอดเดียวกัน หลายการศึกษาก่อนหน้านี้ใช้สารต้านการสลายกลูโคสหลายชนิดร่วมกับลิเทียมเฮปาริน ซึ่งยังมีข้อถกเถียงถึงประสิทธิภาพของการรักษาระดับน้ำตาลในเลือด คณะวิจัยจึงเปรียบเทียบการเปลี่ยนแปลงระดับน้ำตาลจากเลือดที่เก็บโดยลิเทียมเฮปารินที่ไม่มีสารต้านการสลายกลูโคสและลิเทียมเฮปารินที่ผสมกับสารต้านการสลายกลูโคสชนิดต่างๆ จากผลการศึกษาพบว่า เลือดที่เก็บในส่วนผสมลิเทียมเฮปารินกับดีแมนโนส และลิเทียมเฮปารินกับทริส-โบรโมอะซิเตตมีประสิทธิภาพรักษาระดับน้ำตาลในเลือดได้นาน 8 ชั่วโมงที่อุณหภูมิห้อง ส่วนลิเทียมเฮปารินชนิดเดียวและลิเทียมเฮปารินกับกลีเซอรอลดีไฮด์รักษาระดับน้ำตาลในเลือดได้นานเพียง 2 ชั่วโมง ทั้งนี้ส่วนผสมลิเทียมเฮปารินกับดี-แมนโนส มีความเหมาะสมที่สุดในการรักษาเลือดสำหรับการตรวจวิเคราะห์ทั้งกลูโคสและอิเล็กโทรไลท์

Keywords : Antiglycolytic agent, Blood glucose, Lithium heparin
คำสำคัญ : สารต้านการสลายกลูโคส น้ำตาลในเลือด ลิเทียมเฮปาริน

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Introduction

In generally, it is impractical to assay all several samples in the clinical chemistry laboratory immediately. Especially, glucose is requested as one of several tests to be determined in a single specimen. The limitation is blood glucose decrease considerably and rapid when whole blood is kept at room temperature without preservative resulting glycolysis. (Sazama, Arthur et al. 1979). Commonly procedures are used to minimize the error introduced by the metabolism of glucose by blood cells; rapid separation of the cells from plasma or serum within 30 minutes of collection (Stahl, Jorgensen et al. 2001) but it is unfeasible when sample size must be restricted. In another way, using the whole blood anticoagulant in combination with various antiglycolytic agents such as, D-mannose (Nakashima, Takei et al. 1987), sodium fluoride (NaF) (Chan, Swaminathan et al. 1989), and glyceraldehyde (Landt 2000) for preservation of plasma glucose concentrations are in current use. NaF has been the most widely used for preservation of blood glucose concentration. However, the NaF alone is slow but effective in preserving blood glucose for three day, having no effect in the first hour but slowing glycolysis considerably by second hour, and more or less completely inhibiting it by the fourth hour (Chan, Swaminathan et al. 1989). Recently, Shi found that rapid separation of lithium-heparinized blood is superior to fluoride alone for blood glucose measurements (Shi, Seeley et al. 2009).

Nowadays plasma glucose measurement using whole blood with anticoagulants lithium heparin (LH) was used in case of urgent samples that could be used in the routine biochemical testing as diabetic profile. However, it had still argued in preservation of glucose concentration and some biochemical measurements. Therefore, the more effective antiglycolytic agent that could preserve glucose in blood sample completely, immediately, and maintain during transportation and analysis are challenged. The mixtures of antiglycolytic agents and LH were considerable efficiency in routine biochemical testing. The aims of this study were to compare the levels of glucose in plasma that preserved with NaF, LH, LH with various antiglycolytic agent-contained tubes such as tris-bromoacetate, glyceraldehyde, and D-mannose at room temperature and to evaluate theirs effect in electrolyte profile; sodium, potassium, and chloride level during various time-points.

Materials and Methods

Subjects and procedures

Fasting blood samples were collected from a total of 20 healthy and diabetic patient-volunteers (aged 20-60 years). From individual, the 20 ml of blood were taken and divided into 6 tubes which each tube contained; 1) 2-ml of NaF/K_3EDTA (NaF) commercial tube (Greiner, bio-one, Austria) for only glucose measurement, 2) 4-ml of LH, 3) 4-ml of LH plus tris- bromoacetate (BA), 4) 4-ml of LH plus glyceraldehyde (GA), 5) LH plus D-mannose (MA), and 6) 2 ml of the residual blood was obtained in a plastic tube for preparing serum. For studying the time effect, LH with antiglycolytic agent-tubes was divided into five aliquots for various time-points at 0, 2, 4, 6, and 8 hours, respectively. The concentrations of antiglycolytic agents are recommended by the previous studies; 10 mmol/l glyceraldehyde (GA) (Landt, 2000), 9.8 mmol/l of tris-bromoacetate (BA), (Marbach 1977), and 16.7 mmol/l of D-mannose (MA) (Nakashima, et al. 1987). All samples were

stored at room temperature (24-26°C) and centrifuged (3,000 rpm for 5 minutes) at each certainly time-point.

Biochemical analysis

Each aliquot of plasma was measured glucose and electrolyte in sequence with duplicated determination. Glucose concentration obtained from NaF plasma and electrolytes obtained from serum were used as reference value for each analytes. Each sample was simultaneously analyzed glucose using hexokinase G6P-DH method, sodium (Na^+), potassium (K^+), and chloride (Cl^-) ion using I.S.E. indirect potentiometry method. All measurements were performed on Cobas cIII automated chemistry analyzer (Roche Diagnostics, GmbH Mannheim, Germany) using the manufacturer's reagents, calibrator (cfas), and control materials (Precinorm, Precinorm L, and Precipath).

To avoid variability, all samples in the same time-point were simultaneously analyzed in the same batch following by serum and then each plasma sample. Each batch was immediately analyzed and finished within 30 minutes.

Data analysis

Differences in mean values of these plasma anticoagulant plus antiglycolytic agents were calculated at each time-point in the percentage of glucose decreasing level. The comparison of considered significance, *P*-value < 0.05 was analyzed by one-way ANOVA statistical method.

Results and discussion

The intra-assay coefficient of variation (CV) for plasma glucose was 1.0% at a quality control level of 92 mg/dl and 1.1 % at a quality control level of 249 mg/dl; for sodium (Na^+) was 1.6 % at 113.5 mmol/l and 1.9 % at 133.4 mmol/l; for potassium (K^+) was 2.1 % at 3.3 mmol/l and 2.1 % at 5.9 mmol/l; for chloride (Cl^-) was 2.4 % at 79.6 mmol/l and 2.3 % at 108.8 mmol/l. The percentage of the recovery and precisions of the Cobas cIII were performed by mixing two levels of control materials at ratio 1:1, and glucose measurement were analyzed by the Cobas Integra 600 (Roche Diagnostics, GmbH Mannheim, Germany) as the reference analyzer. The recovery of plasma glucose, Na^+, K^+, and Cl^- were 101.5%, 100.8, 100.5, and 101.4; at expected values were 170.5 mg/dl, 143.5, 4.6, and 101.4 mmol/l, respectively.

The means and percentages of decreasing level of glucose concentrations in various antiglycolytic agents at each time-point were shown in Table1 and Figure 1. Sanders and Deadman (Sanders and Deadman 1985) reported that NaF was able to preserve glucose in whole blood for 48 hours whereas Chan and colleagues (Chan, Swaminathan et al. 1989) found NaF effective preserved blood glucose for at least three days and the rate of decreasing level in glucose concentration was lower than heparin-treated samples at room temperature.

Our study showed the glucose concentrations in mixture of LH and D-mannose were the least decreasing levels at every time-point. These results were correlated to the report of Nakashima, et al (Nakashima, Takei et al. 1987) who found D-mannose is a specific inhibitor of glycolysis and can be used to stabilize the glucose in blood collecting tube. The inhibition of glucose consumption indicates the preservative action of D-mannose on glucose in blood.

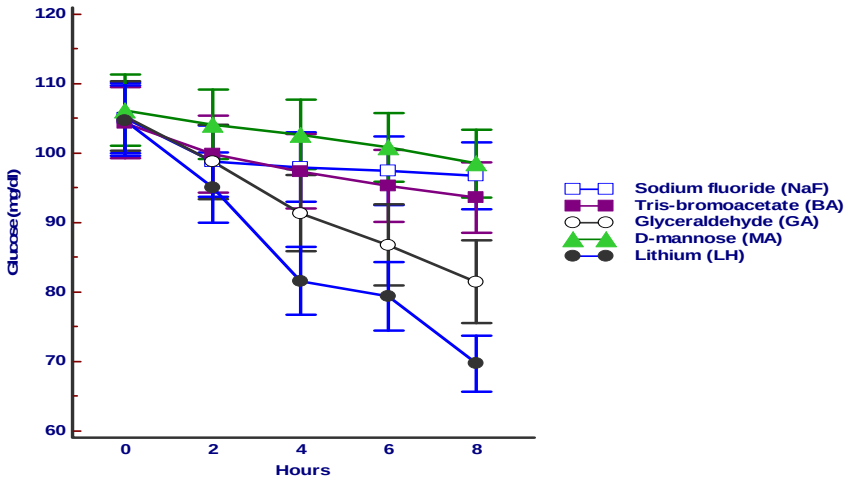

Figure 1 Mean glucose (mg/dl) of plasma from various antiglycolytic agent contained tubes at 0-8 h at room temperature, n=20, (NaF, sodium fluoride; LH, lithium heparin (alone); BA, lithium heparin plus tris- bromoacetate, GA, lithium heparin plus glyceraldehyde; MA, lithium heparin plus D-mannose)

Table 1 Mean glucose (mg/dl) and percentage of decreasing of plasma glucose from various antiglycolytic agents at time-points, 0-8 hours (n=20)

	NaF		**LH**		**BA**		**GA**		**MA**	
Hours	Mean ±SEM	Decreasing (%)	Mean ±SEM	Decreasing (%)	Mean ±SEM	Decreasing (%)	Mean ±SEM	Decreasing (%)	Mean ±SEM	Decreasing (%)
0	105.1 ± 5.05	0.0	104.7 ± 5.11	0.0	104.4 ± 5.13	0.0	105.4 ± 5.04	0.0	106.2 ± 5.10	0.0
2	98.9 ± 5.16	5.9	95.1 ± 5.03	9.2	99.9 ± 5.55	4.3	98.8 ± 5.39	6.3	104.2 ± 5.01	1.9
4	98.0 ± 5.02	6.8	81.6 ± 4.91	22.1	97.4 ± 5.33[a]	6.7	91.4 ± 5.47	13.3	102.7 ± 4.96	3.3
6	97.5 ± 4.93	7.2	79.4 ± 4.99	24.2	95.3 ± 5.13[a]	8.7	86.8 ± 5.82	17.6	100.9 ± 4.96	5.0
8	96.8 ± 4.84	7.9	69.8 ± 4.03	33.3	93.6 ±5.05[a]	10.3	81.5 ± 5.97	22.7	98.6 ± 4.88	7.2
Range	70-137		46-139		65-139		50-139		72-138	
p-value	0.775		<0.001[b]		0.632		0.025[b]		0.844	

[a] Visible haemolysis as 1^+ to 2^+ in 5, 8, and 12 samples at 4, 6, and 8 hours, respectively.

[b] Significant Difference (*p*-value <0.05)

Even though the previous studies of Chan and the others (Chan, Ho et al. 1992) found that blood glucose preserved with NaF is slow, having no affect in the first hour more or less completely a inhibiting it by the fourth hour. Our study showed the

MA tube was superior to the NaF because the rate of decreasing of NaF is higher than MA tube.

Additional, plasma glucose levels in BA tube decreased gradually, the percentage of decreasing levels increased from 4.3% to 10.3% for up to 8 hours. Our results indicated that BA tube has effectively preserved glucose levels inferior to the LH plus D-mannose (MA tube) and NaF tube.

According to the mean differences and percentage of decreasing of plasma glucose, we found, during the first 2 hours, all of antiglycolytic agents are effective. However, it is impractical in clinical laboratory if blood samples are delayed for transportation and processing. Therefore, the effective antiglycolytic agents are necessary to preserve glucose concentration. The results of MA and BA are more effective than GA and LH for up to 8 hours when compared to NaF. (*P*-values are 0.844, 0.775, and 0.632 respectively). Even if, the visual hemolysis is occurred by BA but the plasma glucose level could be well preservation. While, the unpreserved sample (LH) was rapidly decreased by 9.2 % to 33.3% and 10 mmol/l of GA were likely decreased by 6.3 % to 22.7% for 8 hours. Both of LH and GA are ineffective; could preserved glucose level for up to 2 hours at room temperature by significance (*P*-value < 0.05).

Stability of blood glucose levels were reported by several studies, Nakashima et al. (Nakashima, et al. 1987), Chan et al. (Chan, Ho et al. 1992; Chan, Swaminathan et al. 1989) and Landt (Landt 2000), however, these authors examined the changes in only first few hours. But this work is the first report of antiglycolytic agent preserved glucose level for up to 8 hours at room temperature. That is adequate time to delay transportation and sample preparation process.

Serum were not measured glucose level because the glucose content in serum sample was approximately 0.2 mmol/l (3.6 mg/dl; 95% confidence interval from 0.14 to 0.25 mmol/l) lower than in plasma sample (Lippi, Salvagno et al. 2006).

The limitation of this study is the using of initial plasma glucose levels as low concentrations that obtained from 20 fasting diabetic patients. This was due to the patients were not have complications and maintained themselves in good control of plasma glucose levels. The high concentration of glucose up to 180 mg/dL should be further investigations.

For more information, the changes of electrolyte profile, sodium (Na^+), potassium (K^+), and chloride (Cl^-) levels from the various types of antiglycolytic agents were evaluated. The blood samples treated with NaF cannot be analyzed Na^+, K^+ and some enzymes; because of interfering and it is also incompatible with automated multi-channel instruments analyzer (Young, Thomas et al. 1972).

The mean of Na^+, K^+, and Cl^- for each time period of storage at room temperature after blood sampling are shown in Table 2, 3, and 4 respectively. These results showed all of plasma samples can be used for electrolyte analysis when compared to the serum value (*p*-value ranged from 0.071 to 0.979), except the BA plasma for measurement of K^+ (*p*-value = 0.009). Because the BA plasma showed slightly hemolysis at 4 -8 hours, this was not recommended for potassium assay. The effect of BA related to cellular releasing from damaged red blood cells when stored whole blood at room temperature at several time (Stankovic and Smith 2004).

Table 2 Mean (mmol/l) sodium of blood samples preserved with various antiglycolytic agents at time-points, 0-8 hours (n=20)

Sodium Concentration, mmol/l Mean (SD)						
Hours	**Serum**	**LH**	**BA**	**GA**	**MA**	***p-value***
0	126.7 (1.15)	125.7 (1.39)	123.9 (0.33)	125.9 (1.15)	125.0 (0.69)	0.082
2	-	126.8 (0.93)	125.0 (0.75)	126.5 (1.15)	125.5 (0.76)	0.141
4	-	126.3 (1.68)	125.2 (0.65)[a]	126.6 (1.51)	125.6 (0.98)	0.725
6	-	126.2 (1.31)	124.9 (0.74)[a]	126.0 (1.52)	125.8 (1.09)	0.791
8	-	126.4 (1.69)	125.3 (0.43)[a]	126.5 (1.58)	125.2 (1.26)	0.589
p-value	-	0.876	0.275	0.942	0.960	

[a] Visible haemolysis as 1^+ to 2^+ in 5, 8, and 12 samples at 4, 6, and 8 hours, respectively.

[b] Significant Difference (p-value <0.05)

Table 3 Mean (mmol/l) potassium of blood samples preserved with various antiglycolytic agents at time-points, 0-8 hours (n=20)

Potassium Concentration, mmol/l Mean (SD)						
Hours	**Serum**	**LH**	**BA**	**GA**	**MA**	***p-value***
0	3.74 (0.25)	3.67 (0.20)	3.50 (0.09)	3.67 (0.22)	3.45 (0.10)	0.237
2	-	3.56 (0.22)	3.91 (0.15)	3.74 (0.21)	3.51 (0.09)	0.152
4	-	3.45 (0.22)	4.54 (0.38)[a]	3.74 (0.21)	3.58 (0.16)	0.001[b]
6	-	3.39 (0.22)	5.09 (0.49)[a]	3.88 (0.27)	3.71 (0.26)	<0.001[b]
8	-	3.38 (0.28)	5.08 (0.85)[a]	4.28 (0.63)	3.90 (0.32)	<0.001[b]
p-value	-	0.260	0.009[b]	0.071	0.209	

[a] Visible haemolysis as 1^+ to 2^+ in 5, 8, and 12 samples at 4, 6, and 8 hours, respectively.

[b] Significant Difference (p-value <0.05)

Table 4 Mean (mmol/l) chloride of blood samples preserved with various antiglycolytic agents at time-points, 0-8 hours (n=20)

Chloride Concentration, mmol/l Mean (SD)						
Hours	**Serum**	**LH**	**BA**	**GA**	**MA**	***p-value***
0	86.5 (2.21)	86.1 (2.57)	85.0 (0.42)	86.7 (1.98)	89.6 (0.50)	0.056
2	-	85.1 (3.14)	85.5 (0.29)	87.2 (2.35)	89.7 (0.25)	0.038[b]
4	-	86.3 (2.27)	85.9 (1.10)[a]	87.2 (2.33)	89.3 (0.81)	0.075
6	-	86.1 (2.15)	85.5 (0.65)[a]	86.4 (1.94)	89.3 (0.40)	0.027[b]
8	-	86.5 (2.24)	86.7 (0.17)[a]	86.4 (2.41)	88.8 (0.12)	0.229
p-value	-	0.936	0.155	0.979	0.580	

[a] Visible haemolysis as 1^+ to 2^+ in 5, 8, and 12 samples at 4, 6, and 8 hours, respectively.

[b] Significant Difference (p-value <0.05)

Remark: NaF, sodium fluoride; LH, lithium heparin (alone); BA, lithium heparin plus tris-bromoacetate, GA, lithium heparin plus glyceraldehyde; MA, lithium heparin plus D-mannose

Conclusions

Blood samples treated with lithium heparin plus D-mannose and lithium heparin plus tris-bromoacetate effectively preserved glucose level for up to 8 hours at room temperature while lithium heparin alone and lithium heparin plus glyceraldehyde preserved glucose level for up to 2 hours. Combination between lithium heparin and D-mannose is the most appropriate preservation of blood for both glucose and electrolyte analysis.

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