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CHANGES OF pH OF BLOOD DILUTED WITH PLASMA SUBSTITUTES IN VITRO

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Abstract

Changes of pH of dog's arterial blood (7.41) by in vitro dilutions (1:1, 1:3, 1:7, 1:15) with five types of modified fluid gelatin (MFG) solution and four types of dextran solution were observed. In general, dilution with MFG solution (4 %) decreased pH of blood relatively greater than that with dextran solution, namely pH of blood was below 7.21 at 1:15 dilution. Dilution with the more concentrated MFG solutions (7 % and 10 %) induced the more severe decrease of pH. Dilution with dextran in 5 % glucose solution induced great decrease of pH which was below 6.80 at 1:7 dilution, while dilution with dextran in saline, lactated Ringer's or acetated Ringer's solution caused slight decrease of pH of blood and remained above 7.31 at 1:15 dilution.

INTRODUCTION

The previous studies[1,2] reported that pH of blood remained unchanged mostly until blood would be diluted at 1:7 with plasma substitute solutions anaerobically in vitro. On the other hand, acute hemodilution in vivo with dextran solution induced marked decrease of pH of blood[3], while hematocrit value was reduced at 1/4-1/6 of control and metabolic and respiratory conditions were maintained normally. It was obvious that hemodilution reduced buffering capacity of blood by losing hemoglobin and plasma protein and subsequently pH of the blood became unstable against enviromental agitation[4]. However, this should not induce acidosis or alkalosis essentially. Therefore it was anticipated that metabolic acidosis, which was often observed in exsanguinated patients treated with massive infusion of plasma substitute, should be caused by other mechanism. Scrutinizing the previous study[1], however, $\times 16$ dilution with dextran in saline (pH=5.00) caused a slight decrease in pH of blood (7.44−7.35), while the same dilution with saline (pH=5.62) alone did not cause noticeable changes in pH of blood.

Furthermore we observed recently that in vitro hemodilution with

4 % modified fluid gelatin (MFG) in saline in the same fashion as with 6 % dextran-75 in saline produced the more pronouced decrease in pH of arterial blood, particularly with the more concentrated MFG solution (e. g. 7 % or 10 %). These data reserved attention that acidity of plasma substitute may also influence upon changes of pH of blood after hemodilution.

In this study anaerobical dilutions of blood with various plasma substitutes were performed serially in vitro and changes of pH the blood were observed.

MATERIAL AND METHODS

Blood was withdrawn into a heparinized syringe (10 ml) from healthy, mongrel dog anesthetized lightly with pentobarbital sodium by arterial puncture. Immediately, 5m l of this blood was transfered anaerobically into 10 ml syringe, each containing 5 ml of test solution.

The solutions tested were as followed:

1) MFG solution, 4 % in saline, pH=7.13 (MFG-G): — Gelofusin®
2) MFG solution, 3.5 % in saline, pH=6.80 (MFG-H): — Haemaccel®
3) MFG solution, 3 % in saline, pH=5.74 (MFG-P): — Plasmagel®
4) Dextran-75, 6 % in saline, pH=5.17
5) Dextran-75, 6 % in lactated Ringer's solution, pH=6.14
6) Dextran-75, 6 % in acetated Ringer's solution*, pH=6.76
7) Dextran-75, 6 % in 5 % glucose solution, pH=4.00
8) MFG-G solution**, 7 % in saline, pH=7.02
9) MFG-G solution**, 10 % in saline, pH=7.00
10) 5 % glucose solution, pH=4.03

The syringe was rotated for mixing blood and solution. Then 5 ml of the mixture was transfered into another syringe which contained 5 ml of the same solution, etc., until dilution of 1 : 15 was obtained. pH of undiluted and diluted blood were measured at 37°C with an Instrumentation Laboratory pH meter model 113, pH of the tested solution were measured at 25°C with Hitachi-Horiba D-5 glass electrode pH meter. The accuracy of these dilutions were checked in duplicate by hematocrit deterimations. All blood samples in syringes were kept in ice water and all determination was completed in duplicate within 2 hours. The entire procedure was performed five times on different days.

* Electrolyte composition of acetated Ringer's solution as followed: Na — 130 meq/l, K — 4 meq/l, Cl — 109 meq/l and acetate — 28 meq/l.

** Chemical structure and physical character of MFG in those solutions were essentially same as those of 4 % MFG solution. These solutions were specially manufactured and kindly supplied from Toyo Johzo Co., Ohoshita, Shizuoka Pref., Japan.

Statistical analysis on comparison between grades of dilution or groups were done using F-test or analysis of variance.

RESULTS

Means of pH values obtained from experiments and their standard deviations are shown in Table 1. and 2.

TABLE 1.

Changes of pH of blood diluted with MFG and dextran solutions
mean values of pH and standard deviations

Diluent solution and its pH	Undiluted blood	Dilution			
		1:1	1:3	1:7	1:15
M. F. G. solution					
H: 6.80±0.04	7.41±0.02	7.36±0.03	7.28±0.01	7.18±0.03	7.06±0.03
G: 7.13±0.01	〃	7.36±0.03	7.32±0.04	7.26±0.03	7.21±0.03
P: 5.74±0.14	〃	7.11±0.05	< 6.80	—	—
Dextran solution					
in physiological saline 5.17±0.12	〃	7.43±0.03	7.42±0.02	7.37±0.03	7.32±0.02
in lactated Ringer's 6.14±0.04	〃	7.44±0.02	7.43±0.02	7.39±0.03	7.31±0.02
in acetated Ringer's 6.76±0.04	〃	7.44±0.02	7.43±0.02	7.39±0.03	7.33±0.03
in 5 % glucose 4.03±0.05	〃	7.16±0.02	6.96±0.04	< 6.80	—

TABLE 2.

Changes of blood diluted with three types of MFG solution
mean values of pH and standard deviations

Diluent solution and its pH	Undiluted blood	Dilution			
		1:1	1:3	1:7	1:15
M. F. G.-G solution					
4 %: 7.13±0.01	7.41±0.02	7.36±0.03	7.32±0.03	7.26±0.03	7.21±0.02
7 %: 7.02±0.01	〃	7.32±0.03	7.25±0.03	7.17±0.02	7.08±0.01
10 %: 7.00±0.01	〃	7.24±0.04	7.12±0.02	7.05±0.01	7.00±0.01
5 % Glucose solution 4.59±0.29	〃	7.20±0.01	6.98±0.01	6.86±0.04	< 6.80

In general, MFG solution produced the relatively greater decrease of pH of blood by dilution than dextran solution, except dextran in 5 % glucose solution.

MFG solutions:

pH of blood was decreased to 7.11 at 1:1 dilution with MFG-P solution and below 6.80 at 1:3 dilution. Decreases in pH of the blood diluted with MFG-H solution and -G solution were identical at 1:1 dilution. At 1:15 dilution with MFG-G solution pH was decaeased to 7.21 and contrary with MFG-H pH decreased to 7.01 which was significantly lower than the former ($p < 0.01$).

Changes of pH of blood diluted with three types of MFG-G solution (4, 7 and 10 %) were presented in Table 2. Hemodilution with the more concentrated MFG solution showed the tendency to decrease pH more extensively in every grades of dilution. Particularly at 1:7 dilution, the changes of pH of blood were significantly different each other ($p < 0.01$), namely -0.15 with 4 % MFG-G solution, -0.24 with 7 % and -0.36 with 10 %. However, there was no significant difference between decreases of pH at 1:15 dilution with 7 % and 10 % MFG-G solution.

Dextran solutions:

Hemodilution with 6 % dextran-75 in saline, lactated or acetated Ringer's solution showed a little increase in pH of blood at 1:1 dilution and then a small decrease at 1:7 dilution ($p < 0.01$). Mode of changes of pH of blood, however, were essentially same following serial dilutions with those three solutions. Although hemodilutions with those three dextran solutions decreased pH down to 7.31-7.33 at 1:15 dilution, their decreasing grades of pH were definitely less compared to that with 6 % dextran-75 % in 5 % glucose solution ($p < 0.01$). Hemodilution with 6 % dextran-75 % in 5 % glucose solution showed marked decreases in pH, namely 7.16 even at 1:1 dilution and below 6.80 at 1:7 dilution.

To explain the much more decrease in pH of blood diluted with 6 % dextran-75 in 5 % glucose solution, hemodilution in vitro with 5 % glucose solution without dextran were peformed in the same fashion. Decrease in pH of blood was mostly identical as that with 6 % dextran in 5 % glucose solution, namely pH of the blood was 7.20 at 1:1 dilution and 7.69 at 1:3.

DISCUSSION

A few factors were proposed to explain that hemodilution with MFG solution caused the greater decrease in pH of blood than that with dextran solutions (except dextran in 5 % glucose solution). First of all, it was impressed that MFG solution would react strongly as acidic buffer solution. This possibility was realized by alkaline titration of the solu-

tion, namely 0.1 ml of 1 N NaOH solution produced 2.7 unit increase of pH in 30 ml MFG-H solution and 1.7 unit increase in MFG-G solution. Contrary the similar titration produced 6.0 unit increase in dextran in 5 % glucose solution. Free amino-acids in the solution which would be liberated from MFG molecule were anticipated to increase acidity of the solution. However mostly negligible amount of amino acids had been found in the solution — as shown in Table 3.

TABLE 3.

Free amino acids in MFG-H and -G solution
(analysed by YANAGIMOTO aminoacid autoanalyzer)

	MFG-G (μ mol/ml)	MFG-G (μ mol/ml)
Aspartic acid	0.011	0.056
Serine	trace	0.030
Glutamic acid	0.016	0.088
Proline	trace	0.019
Glycine	0.049	0.117
Alanine	trace	0.049
Others	trace	trace

Recidual succinate after re-polymerization of degradated gelatin was also anticipated to take a part in acidification of the solution. Free succinate, however, had been undetectable in the solution by chemical analysis. Nevertheless it might be possible that massive infusion of such acidic MFG solution aggrevated the original acidosis in some of the patients.

Unionizable dextran per se should not effect on pH of dextran solution or pH of blood mixed with dextran solution. Therefore acidity of solvent for dextran seemed important to change pH of blood following hemodilution in vitro. It was amazing that the hemodilution with dextran in 5 % glucose solution decreased greatly pH of the blood while the other dextran solutions did not change until dilution extended to 1 : 15. The crucial factor caused such decrease in pH should be acidity of 5 % glucose solution. Incidentally hemodilution with 5 % glucose solution in vitro was done in the same fashion and identical decrease of pH was observed. The decrease in pH of blood diluted with 5 % glucose solution would be explained from that the solution was absolutely acidic because formic acid, levulinic acid[5,6] 5-hydroxy-methylfural[7] and

etc. had been formed from glucose in heat sterilizating process of the solution. Therefore the change in pH after may be recognized simply a result of mixing of two solutions with different pH.

A number of speculation on the metabolic acidosis after following hemodilution with plasma substitute in vivo have been offered. Takaori and Safar[3] reported that metabolic acidosis following hemodilution would be induced by reduction of buffer base, such as hemoglobin and plasma protein. DeWall et al[8] suggested that such metabolic acidosis would be casued by excess lactate following increase of glycolysis. Mackenzie[9] suggested that the diminished oxygen carrying capacity of the blood produced hypoxia in tissues and consequently induced lactoacidemia. Recently Takaori[10] showed that metabolic acidosis following hemodilution with massive infusion of plasma substitute or saline was induced simply by imbalance between sodium, chloride and bicarbonate ions. According the above data, however, it was indicated that acidity and buffering capacity of solution took a part in decrease of pH of blood following hemodilution in vitro and even in vivo.

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