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IONIC ALUMINUM(III) IN GENERATOR ELUATE AS AN ERYTHROCYTE-AGGLUTINATING AGENT

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INTRODUCTION

Weinstein and Smoak (1) have reported agglutination of erythrocytes by technetium generator eluate. They suggested that the agglutination was related to contaminating aluminum (Al) in the generator eluate. The present report describes a confirmation and extention of their observation.

MATERIALS AND METHODS

Technetium generator were eluted with 20 ml isotonic saline once daily from the day of their arrival at the laboratory. A 5 mM disodium ethylenediaminetetraacetate (EDTA) solution was prepared in isotonic saline. A gallium generator was eluted every 4th day with 10 ml 5 mM EDTA solution for four times followed 2 weeks later by six consecutive elutions every 20 min the first four of the six with 10 ml isotonic saline and the last two with 10 ml 5 mM EDTA solution.

Agglutination of red cells was performed on glass slides with either aluminum chloride ($AlCl_3$) solution or generator eluate. The $AlCl_3$ solution was made by dissolving $AlCl_3 \cdot 6H_2O$ in isotonic saline. Both washed and unwashed red cells were used. Heparinized venous blood from healthy human subjects was briefly centrifuged. A portion of the packed red cells was washed in ten volumes of isotonic saline four times. One drop of the loosely packed red cells was mixed with two drops of $AlCl_3$ solution or generator eluate on the slide. Macroscopic agglutination of red cells was then visually graded within 1 min. The technetium generator eluate tended to be acidic; the gallium generator eluate, alkaline. To evaluate the effect of pH, pH of portions of the $AlCl_3$ solution and the generator eluate was adjusted with HCl or NaOH solution prior to the slide agglutination. At concentration of 22 μg(Al)/ml, the $AlCl_3$ solution appeared clear at acidic pH, barely opalescent without precipitate at pH 7, and

again clear at pH 9. Al concentration of generator eluate was determined by extraction of the Al with cupferron into methylisobutylketone followed by atomic absorption spectroscopy of the extract at 3093 Å (2,3).

RESULTS

Eluate from certain technetium generator induced agglutination of washed red cells. This agglutination occurred also with eluate from a gallium generator. When Al concentration of the eluate was determined, a higher Al content of the eluate was found to be associated with a greater erythrocyte-agglutinating capability of the eluate. To evaluate whether ionic Al(III) could agglutinate red cells, the slide agglutination was also performed with $AlCl_3$ solution. These results are shown in Table 1. At pH 4-5, the critical Al concentration for the agglutination was approximately 5 μg(Al)/ml with the $AlCl_3$ solution and somewhere between 2 and 12 μg(Al)/ml with the generator eluate.

Positive agglutination was limited to washed red cells and to the use of generator eluate at acidic pH. When unwashed packed red cells with trapped plasma were used, red cell agglutination failed to occur regardless of the Al concentration of generator eluate. Further, the generator eluate failed to induce agglutination of washed red cells when its pH was neutral or alkaline. Table 2 shows the pH dependence of agglutination using $AlCl_3$ solution of varying pH. The erythrocyte-agglutinating capability of the $AlCl_3$ solution was grossly independent of its pH in the pH range 3-5. Lowering pH of the $AlCl_3$ solution to below 3 resulted first in increase then in decrease of the extent of agglutination. When the point of pH about 1 was reached, gross hemolysis occurred. Isotonic saline at acidic pH did not induce red cell agglutination in the absence of $AlCl_3$. Raising pH of the

$AlCl_3$ solution to above 5 uniformly resulted in no agglutination. These findings indicated that cationic Al(III) species in the $AlCl_3$ solution was the agglutinating agent.

The gallium generator was eluted with 5 mM EDTA solution both before and after elutions with isotonic saline as described under Materials and Methods. Al concentration of the saline-eluate has been given in Table 1. Those of the four EDTA-eluate samples obtained prior to elution with saline fell in the range 37-66 μg(Al)/ml. Those of the two EDTA-eluate samples obtained after the elution with saline were 6.4 and 32 μg(Al)/ml, respectively. The fact that Al was eluted from the gallium generator more readily with 5 mM EDTA solution than with isotonic saline indicated soluble ionic nature of the eluted Al. In spite of their high Al content, none of these EDTA-eluate samples induced agglutination of washed red cells at pH 4 and 9.

DISCUSSION

Alumina is used in the construction of the generator column. It is conceivable that alumina had accounted for most of the Al found in the generator eluate. However, this is unlikely since alumina is practically water-insoluble. Unless there is physical defect in the support of the alumina column bed, alumina will not appear in the generator eluate to a concentration greater than 0.2 μg(Al)/ml. On the other hand, soluble ionic Al(III) may possibly form in the column as a result of radiation decomposition of alumina. The finding that Al was eluted from the gallium generator more readily with EDTA solution than with isotonic saline indicates soluble ionic nature of the eluted Al. For these reasons, we believe that the Al found in the generator eluate was in soluble ionic forms of Al(III). Thus by inspection of Table 1 comparing $AlCl_3$ solution and generator eluate,

it appears that the extent of red cell agglutination by the generator eluate did not exceed that expected of the ionic Al(III) content of the eluate. Accordingly, Al contamination in the generator eluate was felt to be a sufficient cause for the red cell agglutination by the eluate. For generator eluate at pH 4-5, the critical Al concentration for the agglutination appears to be in the order of 5 μg(Al)/ml (Table 1). For testing at other pH, the critical Al concentration may be different.

Mechanism of the agglutination seems to involve ionic linking of red cells by cationic Al(III) bridges. In physiologic media, red cells have negatively charged surface due to anionic chemical groups present on their surface (4). Studies of electrophoretic mobility of red cells in aluminum chloride solution indicate binding of cationic Al(III) to red cells with diminution in their surface negative charge (5). Aluminum hydroxide is amphoteric (6). The anionic aluminate ion, $Al(OH)_4^-$, which forms in $AlCl_3$ solution at alkaline pH, will not be expected to agglutinate red cells. The findings shown in Table 2 can be understood qualitatively as a combined result of two opposing trends as the pH was lowered: (i) increasing concentration of cationic Al(III) tended to increase the agglutination, (ii) decreasing surface negative charge of red cells tended to decrease the agglutination.

Necessary conditions for red cell agglutination by Al(III) do not exist *in vivo*. Accordingly, intravascular agglutination of red cells resulting from administration of generator eluate appears highly improbable.

SUMMARY

1. Three technetium generators and one gallium generator were used for the evaluation of aluminum contamination and erythrocyte-agglutinating capability of generator eluate. Aluminum content of the generator eluate was

determined by atomic absorption spectroscopy. Erythrocyte-agglutinating capability of the eluate was evaluated by a slide agglutination method.

2. A higher aluminum content of the generator eluate was found to be associated with a greater erythrocyte-agglutinating capability of the eluate. Positive agglutination was limited to washed red cells and to the use of the eluate at an acidic pH.

3. Slide agglutination using reference aluminum chloride solution showed that ionic aluminum(III) solution at acidic pH could agglutinate red cells with a critical concentration of about 5 μg(Al)/ml at pH 4-5.

4. Aluminum was eluted from the gallium generator more readily with ethylenediaminetetraacetate solution than with isotonic saline indicating that the eluted aluminum was in ionic forms.

5. It was concluded that the contaminating ionic aluminum(III) was a sufficient cause for the red cell agglutination by the eluate. Plausible mechanism of the agglutination was discussed.

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Table 1. Agglutination of Washed Erythrocytes (RBC Aggl) by Aluminum Chloride Solution and by ^{99}Mo-^{99m}Tc and ^{68}Ge-^{68}Ga Generator Eluate in Relation to Aluminum Concentration (Al Conc) of the Solution and the Eluate

Aluminum chloride solution@ (pH 4.2-4.6)		Generator eluate@ (pH 4.0-4.7)								
		Elution**	Technetium						Gallium	
			Amersham/Searle #D-122		New England Nuclear, #602-004		Abbott #T-104		New England Nuclear, #1701	
Al conc (μg/ml)	RBC aggl*		Al conc (μg/ml)	RBC aggl	Al conc (μg/ml)	RBC aggl	Al conc (μg/ml)	RBC aggl	Al conc (μg/ml)	RBC aggl
21.6	4+	1st	26	4+	<0.2	Neg	<0.2	Neg	23	3+
10.8	3+	2nd	17	3+	<0.2	Neg	<0.2	Neg	2.3	Neg
5.4	1+	3rd	16	3+	<0.2	Neg	<0.2	Neg	1.4	Neg
2.7	±	4th	12	2+	<0.2	Neg	<0.2	Neg	0.8	Neg
1.4	Neg	5th	12	2+	<0.2	Neg	<0.2	Neg		

@ In isotonic saline.

* "4+" agglutination corresponds to formation of "very coarse" clumps; "1+" agglutination, to formation of "fine" clumps; and "Neg", to absence of macroscopic agglutination.

** See Materials and Methods.

Table 2. Agglutination of Washed Erythrocytes by Aluminum Chloride Solution of Varying pH

Aluminum concentration	pH										
	1.0	1.5	2.0	3.0	4.0	4.3	4.6	5.0	6.0	7.0	8.0
µg/ml	Visual grading of agglutination*										
5.4	hemolysis&	±	4+	1+	1+	1+	1+	1+	Neg	Neg	Neg
21.6	hemolysis	2+	6+	4+	4+	4+	4+	4+	Neg	Neg	Neg

* See footnote to Table 1.

& Gross hemolysis occurred forming brownish mixture.

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