Galactomannan Antigenemia after Infusion of Gluconate-Containing Plasma-Lyte

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Abstract

We demonstrated that sodium gluconate was the factor causing false-positive galactomannan (GM) antigenemia of Plasma-Lyte hydration solution. Infusion of sodium gluconate-containing solution but not gluconate-free Plasma-Lyte resulted in positive serum GM antigenemia. Serum GM concentrations also correlated with volume and *in vitro* concentrations of GM within gluconate-containing solutions of infused Plasma-Lyte.
GM antigen is an important biomarker for early non-culture diagnosis of invasive pulmonary aspergillosis in immunocompromised patients (8-10, 13). False-positive GM test results have been reported in patient serum or bronchoalveolar lavage (BAL) fluid in association with antibiotics, infant feeding, and some enteric bacteria (1, 2, 4, 12, 23). Plasma-Lyte infusion more recently has been reported to cause false-positive antigenemia (7, 15, 21).

Plasma-Lyte is a sterile, nonpyrogenic electrolyte solution, containing sodium chloride, potassium chloride, magnesium chloride, sodium acetate trihydrate and bicarbonate ions with or without sodium gluconate. It is used for fluid resuscitation, hydration in seriously ill patients (6, 11, 17, 20), and for extended storage of platelets or peripheral blood stem cell products (3, 16, 19).

Hage et al. first described Plasma-Lyte as a cause of false-positive results for Aspergillus GM in BAL fluid (7). Surmont and Stockman then reported a patient receiving gluconate-containing Plasma-Lyte as the cause of false-positive serum GM (21). These two reports hypothesized that GM generated from A. niger during the industrial fermentation process of sodium gluconate was present in Plasma-Lyte solution, resulting in false-positive reactions. Racil and colleges subsequently reported a study where
healthy volunteers receiving Plasma-Lyte demonstrated false-positive circulating GM lasting as long as 24 h (15). Whether sodium gluconate is the cause of false-positive GM reactions is not known.

We therefore conducted a series of experiments to test the hypothesis that the mechanism of false-positive GM reactions in Plasma-Lyte was the presence of sodium gluconate. Rabbits, weighing 2.6 to 3.3 kg (Covance Research Products, Inc., Denver, PA), monitored under humane care (14), received intravenous infusions of different formulations and lots of gluconate-containing Plasma-Lyte A or Plasma-Lyte 148, and a solution without gluconate Plasma-Lyte 56 via a Silastic tunneled central catheter (22). GM concentrations and GM indices (GMIs) were determined by using GM sandwich enzyme-linked immunosorbent assay (Platelia™ Aspergillus EIA; Bio-Rad, France). Serial serum GM levels measured in rabbits were analyzed using Mann-Whitney or Kruskal-Wallis tests. A two-tailed p-value of <0.05 was considered to be statistically significant.

In order to understand the kinetics of circulating Plasma-Lyte-associated GM antigen, rabbits received intravenous infusions of single doses and multiple doses. The single dose of 30 ml/kg of Plasma-Lyte solution was infused over 1 h. The multiple dose study consisted of 20 ml/kg Plasma-Lyte infusions (1 ml/min) daily for 7 d following by a 30 ml/kg
bolus infusion on day 8. Serum specimens were collected at 0.17, 0.25, 0.5, 1, 2, 3, 4, 12, 14, 16, 24, 30, 36, and 48 h postinfusion for single and multiple dose studies, following the last infusion on day 8.

Assays were also conducted in vitro for the presence of GM antigen in different formulations and lots of gluconate-containing Plasma-Lyte solution and Plasma-Lyte without gluconate. Among the six lots of Plasma-Lyte A with sodium gluconate (502 mg/100 ml) the GMIs ranged from 3.28±0.09 to 7.26±0.01. Sodium gluconate-containing Plasma-Lyte 148 (502 mg/100ml) in vitro expressed the highest concentrations of GM antigen (GMI 8.17±0.15). By comparison, the GMI of solutions without sodium gluconate (Plasma-Lyte 56) did not cause positive GM results (GMI 0.16±0.01; p<0.001). Three to six tests for GMI were performed on each batch of Plasma-Lyte solutions.

We then compared serum GMI kinetics following infusion of Plasma-Lyte with and without sodium gluconate. The expression of circulating GM antigen in rabbits following intravenous infusions of 30 ml/kg of gluconate-containing Plasma-Lyte A (n=4) or 148 (n=4) solutions with in vitro GMIs of 7.26±0.01 or 8.17±0.15 over 1 h are presented in Figure 1, panel A as combined concentration-time curve. Following gluconate-containing Plasma-Lyte infusion, the serum GMI underwent significant changes over
time (p<0.0001, Kruskal-Wallis test). By comparison, there were no significant changes in serum GM in rabbits receiving solution without gluconate Plasma-Lyte 56 (n=4) (Figure 1, panel B).

We then studied serum GMI kinetics following repeated administration of Plasma-Lyte with or without gluconate over 7 d. Gluconate-containing Plasma-Lyte resulted in an accumulation of circulating GM to a peak GMI of 9.74 ± 0.75 after 1 h (Figure 1, panel C). The serum GMI value remained positive for more than 36 h (0.54±0.03) following administration of Plasma-Lyte solution containing sodium gluconate while there were no changes in the kinetics of GM antigen in serum following multiple administration of solution without sodium gluconate Plasma-Lyte 56 (GMI 0.16±0.01; Figure 1, panel D). Serum GM levels remained at very low concentrations (GMI 0.12±0.03).

We further sought to demonstrate whether there is a direct relationship between serum GMI with infused volume and in vitro GMI of Plasma-Lyte solution. Following 10 ml/kg of Plasma-Lyte A infusion (GMI 4.18 ± 0.01) (n=4), the serum GMI value increased to 2.47 ± 0.32 in 10 min. (Figure 2, panel A). With increased volume to 30 ml/kg of Plasma-Lyte A (GMI 4.18 ± 0.01), the serum GMI value significantly increased over time to 3.68 ± 0.38 (n=6) (Figure 2, panel B; p < 0.0001, Kruskal-Wallis test).
Formulations of sodium gluconate-containing Plasma-Lyte solutions expressed distinctively high levels of GM antigen in vitro and in vivo. By comparison, Plasma-Lyte formulations without sodium gluconate did not cause false-positive results in vitro or false-positive antigenemia. Thus, there was a direct relationship between GM concentrations in gluconate-containing Plasma-Lyte and GMI levels in serum. The persistent antigenemia for ≥24 h after infusion was similar in animals reported here and human volunteers reported by Racil et al. (15).

To our knowledge, this is the first study to demonstrate that sodium gluconate in Plasma-Lyte is directly related to the cause of false-positive antigenemia. The present study demonstrates that Plasma-Lyte solutions that contain gluconate also contain high levels of GM antigen and cause false-positive circulating GM results after parenteral administration. Small molecular weight organic acids, such as gluconate, are produced by a fermentation process involving Aspergillus niger and A. terreus (5, 18). Calcium gluconate is also used in medical settings and may potentially be a cause of false-positive antigenemia. GM is released into the fermentation solution and is likely carried through the fractionation process for medical grade gluconate. Although Plasma-Lyte is sterile, GM still persists in the gluconate-containing solution.
In summary, this study demonstrates the role of gluconate in the GM antigenicity of Plasma-Lyte solution. Intravenously administered sodium gluconate-containing but not gluconate-free Plasma-Lyte demonstrates a concentration and rate dependent serum GM signal. Awareness of this source of false-positive GM antigen may allow for improved interpretation by clinical microbiology laboratories and improved patient care.

REFERENCES

Figure 1. Expression of galactomannan antigen following intravenous infusion of different formulations and lots of Plasma-Lyte solution. Panel A: Combined concentration-time curve of single dose of 30 ml/kg of gluconate-containing Plasma-Lyte A (lot #C738476; GMI 7.26 ± 0.01) and 30 ml/kg of gluconate-containing Plasma-Lyte 148 (lot #C730549; GMI 8.17 ± 0.15) given over 1 h. Panel B: Single dose of 30 ml/kg of solution without gluconate Plasma-Lyte 56 (GMI 0.16 ± 0.01) administered over 1 h. Panel C: Combined concentration-time curve after multiple doses of gluconate-containing Plasma-Lyte A (lot #C738476) and Plasma-Lyte 148 (lot #C730549) infusions given over 1 h performed on day 8. Panel D: Multiple infusions of solution without gluconate Plasma-Lyte 56 given over 1 h performed on day 8. Values are presented as means ± SEMs. (*, p < 0.0001; Kruskal-Wallis test).

Figure 2. Expression of galactomannan antigen following intravenous infusion of gluconate-containing Plasma-Lyte A (lot #C689893; GMI 4.18 ± 0.01). Panel A: 10 ml/kg administered over 10 min. Panel B: 30 ml/kg...
infusion over 1 h. Values are presented as means ± SEMs. (¶, p = 0.0002, Mann-Whitney test; *, p < 0.0001; Kruskal-Wallis test).