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Bromocresol Dye-binding Methods Underestimate Serum Albumin Value in Patients Treated with High Dose of Penicillin G

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Authors' contributions

Author HF planned the research and wrote this manuscript. Author YS measured the albumin measurements. Author KO advised the clinical features in the research. Author SN advised all the research in the clinical sites and in vitro experiments. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Original Research Article

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ABSTRACT

Background: Various factors contribute to the discrepancies observed between the bromocresol green (BCG) and bromocresol purple (BCP) assays of serum concentration. Either a BCG or a modified BCP assay is a routine laboratory for albumin measurement in Japan. High-dose of penicillin G underestimates serum albumin level using a modified BCP method *in vitro*. Therefore, we examined the serum albumin level in the patients treated with high-dose of penicillin G and also performed the experiments on co-incubation with plasma, or albumin product and penicillin G solution *in vitro*.
Methods: The medical records of 71 patients treated with high-dose of Penicillin G collected between 2009 and 2012 were reviewed for age, gender, biochemistry (total protein, albumin and potassium), underlying diseases and usage of albumin product. Patients were divided into 2 groups: BCG group (N = 38) and a modified BCP group (N = 33). We compared serum albumin levels between two groups. We performed the

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experiments on co-incubation with albumin product or human plasma and penicillin G solution *in vitro*.
Results: Serum albumin levels using a modified BCP assay decreased during the treatment with high dose penicillin G (-0.4 ± 0.1 g/dL), while serum albumin levels by a BCG method did not decrease (0.06 ± 0.05 g/dL). Although only one patient revealed hypoalbuminemia (<2.0 g/dL) by a BCG method (2.6%), ten patients revealed hypoalbuminemia by a modified BCP method (33%). Penicillin G underestimated plasma albumin levels using a modified BCP methods (% underestimation: 42.9 ± 0.0%) more than those using a BCG method (% underestimation: 10.6 ± 0.0%) in co-incubation experiments *in vitro*.
Conclusion: High-dose of penicillin G might cause the underestimation of serum albumin levels using bromocresol dye-binding methods.

Keywords: Underestimation; hypoalbuminemia; antibiotics; infectious endocarditis.

1. INTRODUCTION

Various factors such as globulins, oxidative condition and so on, contribute to the discrepancies observed between the bromocresol green (BCG) and bromocresol purple (BCP) assays of serum concentration [1-3]. Serum globulins contribute to the discrepancies of serum albumin concentration measured by a BCG method compared with a BCP method [1]. Duly et al reported that a BCP method is superior to a BCG method for the assay of albumin [4]. However, serum albumin values measured by a BCP method were affected by oxidative condition of albumin, mercaptalbumin or non-mercaptalbumin [2]. Therefore, a modified BCP was established with correction about reaction difference by oxidative condition [2]. As a result, either a BCG or a modified BCP assay is a routine laboratory for albumin measurement in Japan [2-5]. Serum albumin measured by an immunoassay is accurate, but is not common in a routine laboratory, because high cost and reaction time are required. We previously reported the process on the use of hypertonic albumin product in our hospital [6]. After 2010 year when a modified BCP method was changed from a BCG method, the use of the hypertonic albumin product increased [6]. Nibu et al also indicated the difficulty in the indication of albumin use by a different measurement between a modified BCP method and a BCG method [6].

Various roles of plasma albumin are maintenance of colloid oncotic pressure, delivery of amino acids, drugs [8]. The two BCP binding sites on albumin molecules were reported [9]. These sites included a warfarin binding site and one of two ibuprofen binding sites [9]. High dose of penicillin G underestimates serum albumin level using a modified BCP method in vitro [4]. Because penicillin G binds the albumin [10], the binding of penicillin G may inhibit the binding of BCP. There are few clinical reports on the relationship between serum albumin level and penicillin G treatment [5]. Ono M et al. reported that only two cases treated with high dose of penicillin G revealed the discrepancies between a BCG and a modified BCP method [5]. Therefore, we examined the serum albumin levels among the patients treated with high dose of penicillin G in one Tokyo metropolitan hospital. In addition, we studied the effects of co-incubation with albumin product or human plasma and penicillin G solution *in vitro* on the albumin values. We firstly showed that penicillin G also underestimated plasma albumin values using a BCG method *in vitro*.

2. PATIENTS, MATERIALS AND METHODS

2.1 Patients

Tokyo Metropolitan Bokutoh Hospital is located in Eastern Tokyo. We retrospectively reviewed the medical records of 71 patients treated with high dose of Penicillin G collected between 2009 and 2012 were reviewed for age, gender, biochemistry (total protein, albumin and potassium), underlying diseases and usage of albumin product. Patients were divided into 2 groups: BCG group (N = 38) and a modified BCP group (N = 33). We compared serum albumin levels between two groups. In our hospital, serum albumin levels were measured by a BCG method until July 2010 (BCG group) and they were measured by a modified BCP method from August 2010 (BCP group). Patients undergoing surgery were excluded from this study. The patients with various underlying diseases (Table 1) received high dose of penicillin G ($\geq$ 18 million units daily, $\geq$ 5 days) intravenously.

2.2 Materials

Penicillin G was purchased from Meiji Seika Pharma Co. (Tokyo, Japan). Penicillin G was easily soluble in 0.9% saline solution (Terumo Co. Ltd., Osaka, Japan), which was used as a vehicle in the experiments. 5% Albumin product was purchased from CSL Behring KK (Tokyo, Japan). Whole blood packed in citrate-phosphate-dextrose-adenosine (CPDA)-containing bags (KBS-400CA; Kawasumi Co. Ltd., Tokyo, Japan) was also obtained from the volunteers. Human plasma was obtained from the whole blood.

2.3 Methods

In clinical laboratory, serum albumin levels were analysed by both a BCG method and a modified BCP method (Kainos Laboratories Inc., Tokyo, Japan) on an auto-analyser (Biomajestry 6050, JEOL Ltd., Tokyo, Japan) [2,11]. To minimize the redox states, A BCP method was modified by addition of 5,5`-dithiobis 2-nitorobenzoic acid, resulting in no difference between mercaptalbumin and nonmercaptalbumin [2].

In *in vitro* testing procedure, we measured albumin values in the plasma or albumin product incubated with saline (control), or penicillin G solution. Albumin product or human plasma was co-incubated with penicillin G solution ($10X10^4$ units/mL) in 1/10 dilution (Albumin product or human plasma: penicillin G solution = 9:1; final concentration $1X10^4$ units/mL) for 24 hours at 37 degrees. After incubation, albumin values were measured by a BCG method and a modified BCP method, routine laboratories in Japan [2,11]. In this study, we defined percentage underestimation as follows, % underestimation = albumin value co-incubated with saline - albumin value co-incubated with penicillin G solution) ÷ albumin value co-incubated with saline X 100 (%)

All subjects provided informed consent to participate in the study, and the study design was approved by the ethics review board of our institution.

2.4 Statistical Analysis

We compared the differences among the two groups by using Wilcoxon's analysis. Data are expressed as group means ± standard errors of the mean or medians with interquartile ranges. Rates of hypoalbuminemia were compared by using the chi-square test with Yates's

correction. All statistical calculations were performed using JMP version 8.0 software (SAS Institute, Inc., Cary, NC), and significance was defined as $p < 0.05$.

3. RESULTS

3.1 Serum Levels of Albumin in Patients Treated with High Dose of Penicillin G

As shown in Table 1, no difference of age and gender, and underlying diseases between two groups were noted. There was no significant difference of total protein, albumin and potassium before the treatment with penicillin G between BCG and BCP groups, as shown in Table 1. However, serum albumin levels measured by a modified BCP method during the treatment or after the treatment were significantly lower than those measured by a BCG method. In a modified BCP method, serum albumin levels were significantly lower than those before and after the treatment. In a BCG method, there were no significant changes of serum albumin levels in the clinical course. In addition, serum potassium levels during the treatment were significantly higher than those before the treatment in each group. The rate of hypoalbuminemia (<2.0g/dL) in the BCP group was significantly higher than that in the BCG group (Table 1).

Non-high dose of penicillin G (<18 million million units daily) did not decrease serum albumin measured by a modified BCP method during the treatment (serum albumin before treatment: 2.7±0.2 g/dL, serum albumin during treatment: 2.7±0.2 g/dL, serum albumin after treatment: 2.7±0.4 g/dL, N=11, respectively). Non-high dose of penicillin G (<18 million million units daily) did not change serum albumin measured by a BCG method during the treatment (serum albumin before treatment: 3.0±0.1 g/dL, serum albumin during treatment: 3.0±0.1 g/dL, serum albumin after treatment: 3.1±0.2 g/dL, N=15, respectively).

3.2 Effects of Co-incubation with Albumin Product or Human Plasma and Penicillin G Solution *in vitro*

The albumin values from albumin product or human healthy plasma co-incubated with penicillin G solution (final concentration $1X10^4$ units/mL) for 24 hours at 37 degrees were shown in Table 2. In co-incubation of albumin product and penicillin G solution, the albumin values from a BCG method were not affected by co-incubation (% underestimation = 1.7±0.4 %), while those from a modified BCP method revealed significantly lower (% underestimation = 7.6±0.5 %). On the other hands, co-incubation with human plasma and penicillin G solution significantly underestimated the albumin values from both methods, as shown in Table 2.

Table 1. Clinical features and biochemistry of patients

	BCG group N=38	BCP group N=33
Age	57	66
Gender (M/F)	20-89	35-88
Underlying diseases	23/15	21/12
Infectious endomyocarditis		
Central nervous infection	10	9
Skin or muscular infection	10	7
(phlegmon etc.)	9	10
Pneumonia	8	
Other	1（infectious aortitis）	7
Pre-treatment		
Total protein (g/dL)	6.5（0.2）	6.3（0.2）
Albumin (g/dL)	3.1（0.1）	2.9（0.1）
Potassium (mEq/L)	4.0（0.1）	3.9（0.1）
During the treatment	6.5（0.1）	6.2（0.1）
Total protein (g/dL)	3.2（0.1）	2.5（0.1）*
Albumin (g/dL)	4.4（0.1）	4.4（0.1）
Potassium (mEq/L)	6.8（0.1）	6.7（0.2）
Post-treatment	3.3（0.1）	2.8（0.1）
Total protein (g/dL)	4.3（0.1）	4.3（0.1）
Albumin (g/dL)		
Potassium (mEq/L)		
rate of hypoalbuminemia	1/38	10/33
<2.0 g/dL	2.6%	30.3%

Data represent the means with the standard errors in parentheses.
**: p<0.05 vs. pretreatment*

Table 2. Effects of co-incubation with penicillin G on the albumin values from albumin product and human plasma

	% underestimation
Albumin product	
BCG method (N=5)	1.7 (0.4)
Modified BCP method (N=5)	7.6(0.5)*
Human plasma	
BCG method (N=5)	10.6 (0.0)*
Modified BCP method (N=5)	42.9(0.0)*

Data represent the means with the standard errors in parentheses.
**: p<0.05 vs. co-incubation with saline (albumin product: 4.60±0.03 g/dL from a BCG method, 4.46±0.07g/dL from a modified BCP method, human plasma: 3.02±0.02g/dL from a BCG method, 2.80±0.00 g/dL from a modified BCP method)*

4. DISCUSSION

Because administration of penicillin G intravenously increased serum potassium [12-13], its slow drip infusion may increase the slight level of serum potassium. In this study, serum potassium increased slightly during the treatment with high-dose of penicillin G in both two groups (Table 1). In a modified BCP method, serum albumin levels decreased during the

treatment with high-dose of penicillin G in comparison with those before and after the treatment, while no changes of serum albumin levels in the clinical course in a BCG method. These data showed the possibility that a modified BCP method might underestimate the serum albumin levels. Binding of penicillin G to the albumin molecules in the blood might inhibit the binding of BCP to albumin molecules. Fortunately, albumin products were not used during the treatment with high-dose of penicillin G. Because serum albumin levels <2.0 g/dL do not maintain the colloid osmotic pressure, Italian guideline showed the albumin use against the symptom by hypoalbuminemia (<2.0 g/dL) [14]. In a modified BCP method, the rate of hypoalbuminemia <0.2 g/dL was approximately 30%. Because there were no symptoms concerning the hypoalbuminemia such as edema, ascites, we considered that albumin infusion was unnecessary.

Mechanism by which serum albumin levels were underestimated using a modified BCP method might be an inhibition effect of penicillin G on binding of BCP to albumin [5]. Ono M et al. reported that various concentrations of penicillin G (1-5 X 10^4 units /mL) underestimated the plasma albumin values from 5 g/dL to 2 g/dL *in vitro*, but did not examine the experiments on the albumin product [4]. Then, we showed the difference of albumin values between albumin product and human plasma. In addition, we, but not Ono et al, used saline as control. Therefore, we could show the differences of albumin values measured by a BCG method between saline and penicillin G solution. These data suggested that underestimation by penicillin G for albumin measurement might be not only an inhibition of binding of bromocresol to albumin but also the other mechanisms. Because the degree of underestimation by a BCG method was lower than that by a modified BCP method, serum albumin values measured by a BCG method did not reveal underestimation in clinical settings. Since globulins are cross-reacted with serum albumin in a BCG method, we speculated that globins might increase in the inflammatory status, resulting in no decreased serum albumin value during the treatment with high dose of penicillin G. To obtain accurate serum albumin level in the patients is very important in the evaluation of nutrition, their outcome [14-15]. Therefore, further examination on mechanism underestimated by penicillin G should be performed. For examples, we described these in the limitation, as follows.

In limitation, there were no data about serum albumin measured by two dye-binding methods or an immunoassay, since our clinical studies were retrospectively examined. We showed the possibilities that serum albumin measured by a BCG method might reveal underestimation in *in vitro* experiment. If serum albumin can be determined by an immunoassay, we will clarify the underestimation by a BCG method. Unfortunately, we have no clinical laboratory environment measuring serum albumin immunologically.

In *in vitro* experiments, we used human healthy plasma and albumin product. We had no data about plasma from patients with various infectious diseases.

5. CONCLUSION

High-dose of penicillin G might cause the underestimation of serum albumin levels using bromocresol dye-binding methods.

CONSENT

All subjects provided informed consent to participate in the study.

ETHICAL APPROVAL

The study design was approved by the ethics review board of our institution.

COMPETING INTERESTS

The authors have declared that no competing Interests exists.

REFERENCES

1. Xu Y, Wang L, Wang J, Liang H, Jiang X. Serum globulins contribute to the discrepancies observed between the bromocresol green and bromocresol purple assays of serum albumin concentration. Br J Biomed Sci. 2011;68(3):120-125.
2. Muramoto Y, Matsushita M, Irino T. Reduction of reaction differences between human mercaptalbumin and human nonmercaptalbumin measured by the bromocresol purple method. Clin Chim Acta. 1999;289(1-2):69-78.
3. Michelis R, Kristal B, Snitkovsky T, Sela S. Oxidative modifications impair albumin quantification. Biochem Biophys Res Commun. 2010;401(1):137-142.
4. Duly EB, Grimason S, Grimason P, Barnes G, Trinick TR. Measurement of serum albumin by capillary zone electrophoresis, bromocresol green, bromocresol purple, and immunoassay methods. J Clin Pathol. 2003;56(10):780-781.
5. Ono M, Aoki Y, Masumoto M, Hotta T, Uchida Y, Kayamori Y, Kang D. High-dose penicillin G-treatment causes underestimation of serum albumin measured by a modified BCP method. Clin Chim Acta. 2009;407(1-2):75-76.
6. Nishimura S, Yamamoto E, Daibo K, Fujimoto S, Moriyama A, Hazama Y, Hoshino M, Fujita H. Development of Clinical Index for Appropriate Hyperoncotic Albumin Use. Int J Pharamacol. 2012;10(1):DOI:10.5580/2b89.
7. Nibu K, Nomaguchi Y, Kubota K, Yoshiura Y, Kumagawa M. Effects of promoting appropriate albumin usage in a university hospital. Jpn J Transf Cell Ther. 2008;54(3):378-385.
8. Colombo G, Clerici M, Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Redox albuminomics: Oxidized albumin in human diseases. Antioxid Redox Signal. 2012;17(11):1515-1527.
9. Ito S, Yamamoto D. Identification of two bromocresol purple binding sites on human serum albumin. Clin Chim Acta. 2010;411(19-20):1536-1538.
10. Sudlow G, Brikett DJ, Wade DN. Further characterization of specific drug binding sites on human serum albumin. Mol Pharmacol 1976;12(6):1052-1061.
11. Am J Kidney. 11.K/DOQI clinical practice guidelines for nutrition in chronic renal failure. Dis. 2000;35 (suppl): S1-S140.
12. Mercer CW, Logic JR. Cardiac arrest due to hyperkalemia following intravenous penicillin administration. Chest. 1973;64(3):358-359.
13. Thiele A, Rehman HU. Hyperkalemia caused by penicillin. Am J Med. 2008;121(8):e1-2.

14. Don DR, Kaysen G. Serum albumin: relationship to inflammation and nutirition. Semin Dial. 2004;17(6):432-437.
15. Forse RA, Shitzgal HM. Serum albumin and nutritional status. JPEN J Parenter Enteral Nutri. 1980;4(5):450-454.

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