CASE REPORT

In Vivo and *In Vitro* Positive Interference by Cefpirome in Measurement of Serum Creatinine by the Jaffe Method

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Abstract. We report the case of an 81-year-old female patient with diverticulitis of the colon, whose symptoms were relieved by intravenous administration of cefpirome. However, her serum creatinine levels were falsely increased by the Jaffe method when serum samples were drawn after intravenous administration of cefpirome. The serum creatinine level in the same sample was within the normal range by the enzymatic method in the automated analyzer. *In vitro* experiment demonstrated dose-dependent positive interference of the creatinine level with cefpirome. These results indicate that we should be aware of the positive interfering effect of cefpirome when we measure serum creatinine by the Jaffe method, and that the enzymatic method should be widely used to measure serum creatinine levels to eliminate false reactions due to certain chemicals. (Keio J Med 48 (2): 93–96, June 1999)

Key words: creatinine, Jaffe method, enzymatic method, cefpirome, cephalosporin antibiotics

Introduction

Serum creatinine measurements are important in evaluating glomerular function in routine clinical laboratory studies.¹ Methods of analyzing serum creatinine levels consist of the Jaffe method and the enzymatic method.² The Jaffe method of creatinine analysis was first described in 1886.³ It is based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red Janovski complex, and the absorbance of red Janovski complex is read at 515 nm.³ Although this assay has been widely used, the major disadvantage of the Jaffe reaction is its lack of specificity, with ascorbic acid, pyruvate, acetone, and certain cephalosporin antibiotics having been reported to cause false positive reactions.^{1,2} However, it is not known whether the newly developed antibiotics interfere with the Jaffe reaction.⁴ The second method, the enzymatic method, is based on creatinase digestion of creatinine.^{1,2} The recent availability of pure enzyme reagents has allowed the enzymatic method to be adopted for automated analysis.

We report a patient who showed higher serum creatinine levels according to the Jaffe method than by the

enzymatic method during the period when the patient was receiving cefpirome. We demonstrated a dose-dependent false positive effect of cefpirome on serum creatinine levels by the Jaffe method by adding different concentrations of cefpirome to the patient's serum or pooled human sera. However, this effect could not be observed with other antibiotics such as cefazopran, cefpiramide, or minocycline.

It was shown that the serum creatinine levels should be measured by the enzymatic method while patients are receiving cephalosporin antibiotics such as cefpirome to exclude false positive reactions by the Jaffe method.

Case Report

An 81-year-old female was admitted to the Department of Internal Medicine, Tokyo Electric Power Company Hospital, in October, 1996, because she had experienced left lower quadrant abdominal pain for 3 days. Physical examination revealed tenderness in the left lower quadrant of her abdomen. Laboratory examinations showed polymorphonuclear leucocytosis (white blood cell count 9700/ μ l) and elevated level of C-

rective protein (14.99 mg/dl). Acute diverticulitis of her descending colon was suspected. Her symptoms and abnormal laboratory findings were relieved by intravenous administration of cefpirome, 4 grams per day, combined with bowel rest and intravenous fluid supplement. Multiple colonic diverticula were confirmed by a barium enema on hospital day 14. The patient's clinical course indicated that cefpirome was effective against her colonic diverticulitis.

However, when her blood was drawn after 30 minutes of administration of 2 grams of cefpirome in 100 ml of saline intravenously in the morning on hospital day 2, the serum creatinine level was 3.7 mg/dl by the Jaffe method, although there were no clinical manifestations of renal failure and the serum blood urea nitrogen level was 9 mg/dl. The Jaffe method was performed by mixing 30 μ l of serum with 1 ml of an alkaline solution of 0.05 mol/L sodium picrate and measuring the sample in a Cynchron CX3 (Beckman Instrument Inc., Tokyo, Japan). However, the serum creatinine level of the same sample by the enzymatic method was 0.9 mg/dl. The enzymatic method was carried out in a HR-2300 automated analyzer (Nihon Denshi Co., Tokyo, Japan). Intrinsic creatine and sarcosine in the serum sample are digested by creatinase and sarcosine oxidase as pretreatment in the first reaction of the enzymatic method. Creatinine in the serum sample is digested into creatine by creatinase, and this creatine is then digested by creatinase into sarcosine and urea in the second reaction of the enzymatic method. Sarcosine reacts with sarcosine oxidase, and produces glycine and H_2O_2 , which is quantitated by peroxidase at 550 nm.

We then conducted *in vitro* experiments to confirm the effect of cefpirome against the level of creatinine by the Jaffe method. We tested the patient's serum drawn on the hospital day 2 before she received cefpirome administration and pooled normal human sera as a control. Serum was mixed with different concentrations of cefpirome or other antibiotics and then incubated at 37°C for one hour. The level of serum creatinine was measured by the Jaffe method or the enzymatic method in the HR-2300 automated analyzer. The levels of serum creatinine in both the patient's serum and pooled normal human sera were increased in a dose-dependent manner by the Jaffe method. However, the serum creatinine levels did not change in the same samples tested by the enzymatic method (Fig. 1). Similar effects of the serum creatinine levels by the Jaffe method were not observed with other antibiotics such as cefozopran, cefpiramide, and minocycline (Fig. 2).

Discussion

Serum creatinine can be measured by both the Jaffe method and the enzymatic method.^{1,2} Positive interfer-

Fig. 1 *In Vitro* Effect of Cefpirome on the Serum Creatinine Measurements. The level of serum creatinine was falsely raised by cefpirome in both the patient's serum (●—●) and normal serum (▲—▲) when determined by the Jaffe method. However, the level of serum creatinine was unchanged in either the patient's serum (○---○) or normal serum (△---△) by cefpirome when determined by the enzymatic method.

Fig. 2 *In Vitro* Effect of Other Antibiotics on the Serum Creatinine Measurements. Cefozopran, cefpiramide and minocycline yielded the same results. They had no effect on serum creatinine measurements by either the Jaffe method (●—●) or the enzymatic method (○---○) when mixed with normal serum.

ence with serum creatinine by reactions with picrate and certain chemicals have been reported in the Jaffe method. One group of these interfering chemicals consists of several cephalosporin antibiotics such as cephalothin, cephaloglycine, and cefoxitin.⁵⁻⁷ However, all of

the cephalosporin antibiotics do not have these effects. Cephalexin and cefazolin produce negative or only very slight responses under the same *in vitro* conditions.⁵ In this report, we have added cefpirome as another chemical that positively interferes with creatinine measurement by the Jaffe method.

We showed falsely increased serum creatinine levels *in vivo* when our patient's blood was drawn after 2 grams of cefpirome was intravenously administered, whereas the serum creatinine level in the same sample was within the normal range by the enzymatic method. In addition, we demonstrated a dose-dependent positive interference effect of cefpirome *in vitro* up to 400 µg/ml. This dose-dependent effect of cefpirome is consistent with another report which described the same *in vitro* dose-dependent effect of cefoxitin and cephalothin.⁶ This falsely increased creatinine level by cefpirome was also similar to the *in vitro* effects of cefoxitin and cephalothin.⁶ The maximum blood concentration of cefpirome when 2 grams of cefpirome is intravenously given to healthy volunteers is reported to be 119.3 µg/ml.⁸ Thus, our *in vitro* experiment indicates that administration of 2 grams of cefpirome can cause the false positive effect in serum creatinine measurements by the Jaffe method. While the level of the patient's serum creatinine was 3.7 mg/dl, the results of our *in vitro* experiment showed mild increases in serum creatinine at a concentration of 119.3 µg/ml. This discrepancy may be caused by possible glomerular dysfunction in our aged patient, as suggested by Allen, *et al.*,⁶ although her glomerular filtration rate and serum cefpirome concentration were not determined. Further studies are necessary to confirm this hypothesis by examining the glomerular filtration rates and serum concentrations of cefpirome in patients and normal volunteers.

Other antibiotics such as cefozopran, cefpiramide, and minocycline did not have any effect on the serum creatinine measurements by the Jaffe method in our experiment. It has been reported that the β-lactam ring is not responsible for the Jaffe reaction, as treatment of cefoxitin with β-lactamase did not abolish the positive interference effect.^{4,5} The structure of cefpirome is different from that of cefpiramide and minocycline, but similar to that of cefozopran (Fig. 3). It is suggested that cyclo-pyridinium hydroxide plays a role in causing the positive interference effect in the Jaffe method by reacting with picrate, leading to the creatinine-like reaction. However, further studies are necessary to elucidate the effect of cephalosporin antibiotics, especially in terms of the 3-dimensional structure of these antibiotics.

A surveillance report of laboratory medicine by the Japan Medical Association showed that the proportions of the Jaffe method and the enzymatic method used for serum creatinine measurements were 36% and 59%,

Fig. 3 Structures of the Antibiotics Used in Our Experiments. (A) Cefpirome, (B) Cefozopran, (C) Cefpiramide, (D) Minocycline.

respectively, in 1997.⁹ About one-third of the clinical laboratories in Japan are still determining creatinine levels by the Jaffe method. Therefore, it is necessary to recognize that serum creatinine levels can be falsely raised by the use of cephalosporin antibiotics, including the newly developed antibiotic cefpirome, by the Jaffe reaction. It is recommended that creatinine levels are examined by the enzymatic method in the automated analyzer to exclude false reactions by certain chemicals.

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