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Application of Receptor Assay for the Detection of Cephalosporin Residues in Live Pigs

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Cephalosporin is very popular antibiotics in clinical fields. However, recently, the safety of cephalosporins has been issued because of its potential toxic effects; neutropenia, thrombocytopenia, agranulocytosis, hepatopathy and neuropathy (1). Therefore, it is very important to minimize antibiotic residue in foods for public health.

A variety of rapid screening tests have been developed and applied for determining drug contamination of the farm animal products. Previously, we have developed ELISA method which has two advantages; it is very fast compare to conventional methods (e.g. bioassay of TLC method), and it can detect antibidotic residues in tissues of live animals (2, 3, 4, 5, 6, 7). Here, we report the application fo receptor assay for the detection of cephalosporin residue in live pigs which is rapid and more sensitive than previous methods.

Materials and Methods

Materials

Animal used in this study were sixty healthy pigs weighing an average of 65kg with no previous history of antibiotic treatment. Injectable cefazolin (400 mg/ml, cefazolin sodium, Yuhan Cefazolin) was obtained from Yuhan Corporation (Seoul, Korea), ceftiofur (500 mg/ml, ceftiofur sodium, LG Accent®) was obtained from LG Chem, Ltd. (Seoul, Korea), cephalexin (180 mg/ml, cephalexin sodium, Ceporex Injection) was obtained from Eagleprocess Inc. (Seoul, Korea). Charm II test kit for β-lactams, manufactured by Charm Science Inc. (MA, USA), was purchased from Daiki Science Co., Ltd. (Seoul, Korea)

Drug administration and Sampling

Cefazolin was administered intramuscularly to each of 20 pigs at the dose of 15 mg per kg of body weight per day for three consecutive days. Ceftiofur was administered intramuscularly to each of 20 pigs at the dose of 5 mg per kg of body weight per day for three consecutive days. Cephalexin was administered intramuscularly to each of 20 pigs at the dose of 9 mg per kg body weight per day for five consecutive days.

Blood samples were collected from all pigs before administration of the drugs and on days 1, 2 and 3 after the last cefazolin and ceftiofur injection. From the pig treated with cephalexin, blood samples were collected on days 1, 2, 3, 4 and

5. 10 ㎖ of blood from jugular vein was collected in EDTA-treated tube and centrifuged at 1750 ×g for 10 minutes to seperate plasma.

Preparation of standard curves

Stock standard solutions of cefazolin, ceftiofur and cephalexin were prepared at a concentration of 1000 ㎍/㎖ using USP standards in distilled water. These stock solutions were further diluted with blank porcine plasma to prepare 0, 50, 100, 200, 400 and 800 ㎍/㎖ working standard solutions. Standard curves of each antibiotic were constructed using the standard solutions fortified into plasma to determine the detection limit and quantity of Charm kit.

Analysis of cephalosporins in plasma

Charm Ⅱ receptor assay for cephalosporins was done in duplication of each plasma samples following a manufacturer' s protocol. Briefly, 300 ㎕ of plasma was pipetted into a test tube at room temperature. And 6 ㎖ MSU extraction buffer was added and mixed throughly (the sample was diluted 1:20). At this time, pH of sample should be fallen within range of 7.2~7.5. A binding receptor (transpeptidase receptor in a form of tablet) was pushed through blister pack into an empty test tube. And, 300 ㎕ of distilled water was added to the tablet. 2 ㎖ of dilluted sample (or internal standard solution) was pipetted into the test tube. And then, the tube was placed in incubator at 55℃ for 2 minutes. The test tube was removed from incubator. And [^{14}C]-labeled Penicillin G, in a form of tablet, was pushed through blister pack into the test tube. The tube was placed in incubator at 55℃ for 2 minutes, and the tube was centrifuged for 3 minutes at 1750×g (setting 3.5× 1000 rpm on IEC Centra CL-2 centrifuge). After centrifuge, the extract was poured off and edge of tube was blotted on absorbent towel. Three hundred microliters of disterilized water was pipetted into a test tube and the pellet was broken up by mixing well. Three milliliter of scintillation fluid was added to the test tube, caped and mixed. The test tube was counted in analyzer Charm Ⅱ for 1 minute. The value was read by count per minute (CPM) on [^{14}C] channel of analyzer and the count was compared with that of the internal standard to determine positive or negative. Samples with cpm count higher than that of the internal standard were considered to be negative(cephalosporin drug free), and those with cpm count lower than that of the internal standard were considered as positive. In this analysis, 10 samples were processed simultaneously and the assay was completed within 10 minutes.

Results and Discussion

1. Standard curves

Standard curves were constructed by using USP standards of each cephalosporins, which was spiked into blank porcine plasma and plotted the response (cpm) versus concentrations These standard curve showed the linearity upto 400ppb for cefazolin (Fig. 1), upto 200ppb ceftiofur (Fig. 2) and upto 500ppb cephalexin (Fig. 3).

2. Detection of cephalosporins in live pigs.

The plasma concentrations of cephalosporins were elucidated by time after drug administration. The concentration of the last day of withdrawal period (cefazolin 1st day, ceftiofur 2nd day and cephalexin 4th day after the last injection respectively) were about 40 ppb which were calculated on the each standard curve. So, we set the concentration of internal standard solution to 50ppb. Each samples that give a sample/internal standard cpm ratio (B/Bs ratio) less than 1 were considered positive and lager than 1 were considered negative. All 60 plasma samples of non-treated pigs showed negative for all cehpalosporin. Cephalosporins were detected until the last day of withdrawal period. Whereas, cefazolin (Table 1) and ceftiofur (Table 2) wasn't detected after withdrawal time. Thus useful method for the detection of cephalosporin residues (cefazolin, ceftiofur, cephalexin).

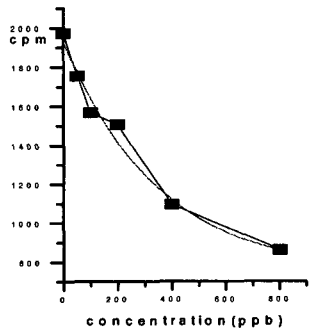

Fig. 1. Standard curve of cefazolin

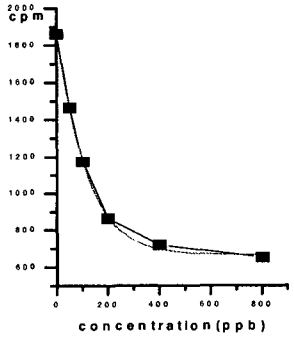

Fig. 2. Standard curve of ceftiofur

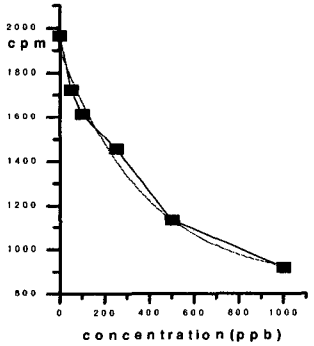

Fig. 3. Standard curve of cephalexin

Table1. Diminishing profile of cefazolin in plasma during withdrawal period

Withdrawal (days)	No. of Positive	No. of Negative	B/Bs ratio (Mean±SE)
Control*	0	20	1.09±0.009
1	0	20	1.074±0.008
2	0	19	1.015±0.24

* Blood was collected before administration of cefazolin.

Overall, the receptor assay is rapid and sensitive method which can detect cephalosporin less than 500ppb. Moreover, the assay method can be applied to the live animals.

Table 2. Depletion profile of ceftiofur in plasma during withdrawal period

Withdrawal (days)	No. of Positive	No. of Negative	B/Bs ratio (Mean±SE)
Control*	0	20	1.256±0.014
1	19	0	0.617±0.021
2	6	14	1.034±0.021
3	0	20	1.161±0.013

* Blood was collected before administration of ceftiofur.

Table 3. Depletion profile of cephalexin in plasma during withdrawal period

Withdrawal (days)	No. of Positive	No. of Negative	B/Bs ratio (Mean±SE)
Control*	0	20	1.053±0.006
1	7	12	0.939±0.05
2	6	10	0.802±0.092
3	1	15	0.817±0.094
4	3	16	0.961±0.051

* Blood was collected before administration of cephalexin

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