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Application of Receptor Assay for the Detection of Sulfamonomethoxine(SM) and Sulfadimethoxine(SD) Residues in Live Pigs

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Currently, approximately 80% of the food animals in the world receive medication (1). In the near future, nearly all the farm animals in the world will be treat with a chemotherapeutic and prophylactic agent (2). In Korea, Department of Veterinary Service, Ministry of Agriculture & Forestry has conducted a National Residue Program (NRP) since 1986 to investigate drug residues in livestock products.

Recently, the safety of sulfonamides has been issued because of its potential toxic effects; hypersensitivity reactions, hepatic changes causing jaundice, renal damage and or so. Therefore, it is very important to minimize antibiotic residue in foods for the health of human beings.

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 (3, 4, 5, 6, 7, 8). Here, we report the application fo receptor assay for the detection of sulfonamide residues in live pigs which is rapid and more sensitive than previous methods.

Materials and Methods

Materials

Animal used in this study were forty healthy pigs weighing an average of 65kg with no previous history of antibiotic treatment. Injectable SM (20 W/v%/ml, monosulfa-200) was obtained from SF Scientific Feed (Seoul, Korea), SD (400 mg/ml, sulfadimethoxine sodium, sulfa-40 injection) was obtained from Green Cross Veterinary Product Co., Ltd (Seoul, Korea). Charm II test kit for sulfa drug, manufactured by Charm Science Inc. (MA, USA), was purchased from Daiki Science Co., Ltd (Seoul, Korea).

Drug administration and Sampling

SM was administered intramuscularly to each of 20 pigs at the dose of 75 mg per kg of body weight on first day. The dose of 37.5 mg per kg of body weight was administered for three consecutive days. SD was administered intramuscularly to each of 20 pigs at the dose of 110 mg per kg of body weight on the first day. The dose of 55 mg per kg of body weight was administered for four consecutive days.

Blood samples of SM were collected from 20 pigs before administration of the drugs and on days 1, 4 and 5 after the last injection. Blood samples of SD were collected from 20 pigs before administration of the drugs and on days 1, 4, 6, 8 and 14 after the last injection. Ten ㎖ 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 SM and SD 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, 300, 400 and 500 ㎍/㎖ 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.

Detection of Sulfa drugs

Charm Ⅱ receptor assay for sulfanomide was done in duplication of each plasma samples following a modificated manufacturer' s protocol. Briefly, 100 ㎕ of plasma was pipetted into a test tube at room temperature. And 300 ㎖ MSU extraction buffer was added and mixed throughly (the sample was diluted 1:3000). 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. 4 ㎖ of dilluted sample or internal standard solution was pipetted into the test tube. And [^{14}C]-labeled Sulfa drugs, in a form of tablet, was pushed through blister pack into the test tube. The tube was placed in incubator at 65℃ for 3 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 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 sulfonamide which was spiked into blank porcine plasma and plotted the response (cpm) versus concentrations. These dose-response curves of SM (Fig. 1) and SD (Fig. 2) were shown to be curvilinear and semi- quantitative from less than 0 ppb upto 500 ppb. Therefore, receptor assay method is sensitive enough for edtecting sulfonamide residue less than 500ppb.

2. Detection of sulfonamides in live pigs

The plasma concentrations of SM and SD were elucidated by time after drug administration. The concentration of the last day of withdrawal period (SM 5th day and SD 14th day after the last injection respectively) were about 500 ppb which were calculated on the each standard curve. So, we set the concentration of internal standard solution to 500ppb. 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 samples of SM and SD tested positive on day 1 of withdrawal. All samples of SM showed negative reaction after day 5 of withdrawal time (Table 1). 16 out of 18 samples of SD showed negative reaction after day 14 of withdrawal time(Table 2).

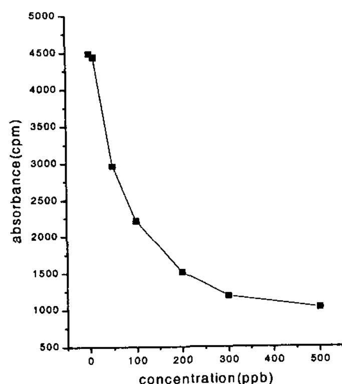

Fig. 1. Standard curve of sulfamonomethoxine(SM)

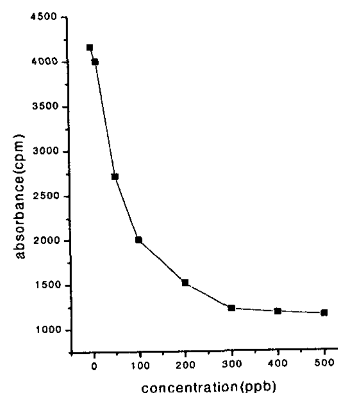

Fig. 2. Standard curve of sulfadimethoxine(SD)

Table 1. Depletion profile of sulfamonomethoxine in plasma during withdrawal period

Withdrawal (days)	No. of Positive	No. of Negative	B/Bs ratio (Mean±SE)
Control*	0	20	3.494±0.204
1	18	2	0.936±0.02
4	3	16	1.138±0.083
5	0	16	1.381±0.179

*Blood was collected before administration of sulfamonomethoxine

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

Withdrawal (days)	No. of Positive	No. of Negative	B/Bs ratio (Mean±SE)
Control*	0	18	2.997±0.313
1	18	0	0.672±0.054
4	18	0	0.699±0.055
6	11	3	0.638±0.103
8	3	12	1.129±0.18
14	2	16	2.11±0.238

* Blood was collected before administration of sulfadimethoxine

According to our results, the modified charm Ⅱ receptor assay can be applied to determine residues of sulfa drug antibiotics (Sulfamonomethoxine and Sulfadimethoxine) in live pigs.
This might be the useful method for screening tissue residue of sulfa drugs in swine plasma before slaughter since the results can be obtained in a day.

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