

Application of ELISA for the Detection of Sulfamethazine Residue in Live Cattle

H. J. Lee, M. H. Lee* and In K. Han¹

College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University
103 Seodundong, Suwon 441-744, Korea

ABSTRACT : Sulfamethazine has been widely used in swine for prevention or treatment of infections. Recently, the safety of the drug to consumers has been questioned because of carcinogenic effects. To prevent unwanted drug residues entering the human food chain, both government authorities and industries have established extensive control measures. The demands for reliable, simple, sensitive, rapid and low-cost methods for residue analysis of foods are increasing nowadays. In this study, we established a rapid prediction test for the detection of cattle with violative tissue residues of sulfamethazine. The recommended therapeutic dose of sulfamethazine (withdrawal time, 15 days) was administered to each of 10 cattle. Blood was sampled before drug administration and during the withdrawal period. The concentration of sulfamethazine in plasma, determined by a semi-quantitative ELISA, was compared to that of an internal standard (10 ppb). The absorbance ratio of internal standard to sample (B/Bs) was employed as an index to determine whether drug residues in cattle tissues were negative or positive. That is, a B/Bs ratio less than 1 was considered residue positive and if larger than 1 was considered negative. All 10 plasma samples from non-treated cattle showed negative to sulfamethazine. Sulfamethazine was detected in plasmas of treated cattle until Day 7 of withdrawal period. The present study showed that the semi-quantitative ELISA could be easily adapted in predicting residues of sulfamethazine in live cattle. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 3 : 378-381)

Key Words : Live Animal Screening Test, Sulfamethazine, Elisa, Cattle, Plasma

INTRODUCTION

With the ever-growing world population, animal production practices have become more intensive and efficient, and accompanied by increasing demands for drug treatments. Currently, approximately 80% of all food animals receive medication for part or most of their lives (Sternesjo et al., 1995). In the near future, nearly all animals produced in the world for food will have received a chemotherapeutic and prophylactic agent of some type (Booth, et al., 1988). A survey in 1993 of all violative carcasses in the United States revealed that the drugs most frequently causing residues were penicillin (20%), streptomycin (10%), oxytetracycline (10%), and sulfamethazine (9%) (Paige, 1994). In Korea, Department of Veterinary Service, Ministry of Agriculture & Forestry has since 1986 conducted a National Residue Program (NRP) to investigate drug residues in livestock products from slaughtering establishments and from import shipments at the port of entry. In 1997, a total of 45,000 samples comprising 10,000 beefs, 23,000 porks, and 11,000 poultry meats were analyzed for five antibiotics (penicillins and tetracyclines) and six sulfonamides, the results showing violative residues of tetracyclines, sulfonamides, and aminoglycosides in beef and pork

meat.

Recently, the safety of sulfamethazine to consumers has been questioned because of its potential toxic effects (Witkamp et al., 1992). Some studies reported sulfamethazine contamination of market milk in the United States and Canada. This information and the report that sulfamethazine induced hyperplasia and adenomas in rat thyroid glands (Littlefield et al., 1994), caused concern about the disposition of sulfamethazine in lactating dairy cows (Paulson et al., 1994). Therefore, it is very important for public health to prevent antibiotic residue in foods. Reliable, simple, sensitive, rapid and low-cost methods for detecting residues in foods are needed (Mitchell et al., 1998). Rapid tests providing same-day results, should also be able to detect antimicrobials in biological fluids, obtained preferably from live animals, which then can be used by primary producers in the field or in abattoirs. A variety of enzyme immunoassays have been developed and adopted for detecting the generic groups of chemical residues in milk, urine, blood, and meat samples (Szekacs, 1994; Gardner et al., 1996). ELISA has become the most popular method for chemical residue detection in food due to its extreme sensitivity, simplicity and ability to screen large numbers of samples (Clifford, 1985; Szekacs, 1994; Gardner et al., 1996).

We developed the sulfamethazine residue detection method for application to live animals by examining the drug depletion profile from blood during the withdrawal period. The method established can be

* Address reprint requests to M. H. Lee. Tel: +88-31-290-2741, Fax: +88-31-293-0084, E-mail: vetlee@snu.ac.kr.

¹ College of Agriculture & Life Sciences, Seoul National University, 103 Seodundong, Suwon, 441-744, Korea.

Received October 23, 2000; Accepted December 6, 2000

applied to live animals at farms or at slaughterhouses before slaughtering.

MATERIALS AND METHODS

Materials

Ten female Holstein cattle at 7 to 8 months old (mean weight 200 kg) were used.

Sulfa-33 Injection (330 mg/ml sulfamethazine sodium) was obtained from Korea Microbial Company (Seoul, Korea). ELISA kits for sulfamethazine, manufactured by Idetek, were purchased from Korea Media Ltd (Seoul, Korea).

Drug administration and samples

Sulfamethazine was administered intramuscularly to the cattle at 100 mg per lb body weight per day on first day and at 50 mg per lb body weight per day on the following four days. Blood samples were collected before administration and on days 1, 3, 5, 7, 10, and 14 after the last injection. Ten ml of blood from each cattle was collected in heparinized tubes and centrifuged at $4500 \times g$ for 10 minutes to collect the plasma.

Preparation of standard curves

Stock standard solution of 1000 $\mu\text{g/ml}$ of sulfamethazine was prepared using USP standards in phosphate buffer solution (10 mM, pH 7.2). These stock solutions were further diluted with plasma (Sigma Chemical Co.) to prepare 1, 2, 5, 10, 20, 50, 100, 500, and 1000 $\mu\text{g/ml}$ working standard solutions. Standard curve of sulfamethazine was constructed using the standard solutions fortified into plasma to estimate the detection limit for the ELISA kit.

Analysis of sulfamethazine in plasma

ELISA for sulfamethazine was applied to each plasma sample in duplicate using the methodology described by Boison et al. (1995), but modified from milk screening to plasma screening. Briefly, 250 μl of the internal standard solution (equivalent to 10 ppb sulfamethazine) was pipetted into a test tube containing immobilized sulfamethazine antibodies. The plasma (250 μl , diluted 1:10 w/PBS) was pipetted into individually labeled tubes. An equal volume of tracer solution (enzyme conjugate, lyophilized horseradish peroxidase labeled sulfamethazine conjugate with preservative) was added, and the test tubes were incubated at room temperature for 3 minutes with continuous shaking. The excess sample and conjugate reactants were then washed out with saline. A colour developer (0.5 ml, enzyme substrate) made up of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and hydrogen peroxide in citrate buffer was added to the test tubes. The mixture was incubated at room

temperature for 3 minutes with shaking. Diluted sodium dodecyl sulfate solution (0.5 ml) was added to each test tube to stop the reaction. The absorbance was read at the wavelength of 405 nm with a photometric detector (Idetek Reader, Awareness Technology, Inc., USA, operated in the 0.9 ratio mode) and compared with that of the internal standard (10ppb). Samples with absorbance greater than that of the internal standard were considered to be negative (sulfamethazine free), and those with absorbance less than that of the internal standard were considered as positive. For accuracy, no more than 5 samples were processed simultaneously, and the assay was completed within 10 minutes (Cullor et al., 1994; Boison et al., 1995).

RESULTS

Standard curve and detection limits

The standard curve of sulfamethazine was constructed to determine the detection limit of the drug, which was found to be less than 10 ppb in both phosphate buffer solution and serum, based on the B/Bo ratio of 0.8 in the ELISA system (figure 1).

Detection of sulfamethazine in plasma from live cattle

Results of analysis for sulfamethazine in plasma are shown in table 1. Since the absorbance ratios of

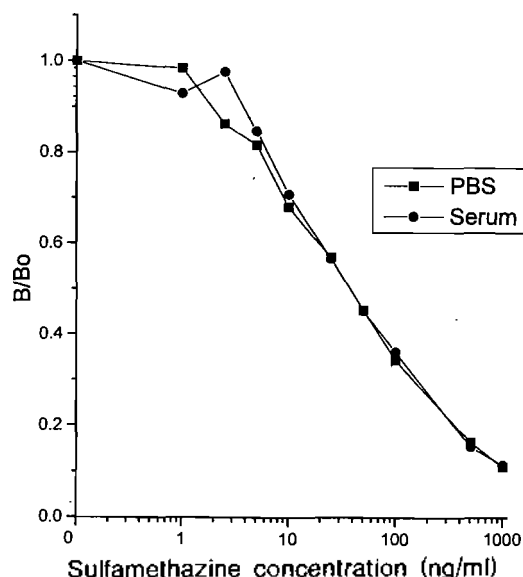


Figure 1. Standard curves of sulfamethazine in phosphate buffer solution and serum. Detection limit of sulfamethazine was calculated as less than 10 ppb. The detection limit of ELISA kit was decided with the point of B/Bo ratio 0.8. B/Bo: Absorbance ratio of standard (B) and saline or control serum (Bo).

the control group were greater than 1.0, the concentrations of sulfamethazine in the diluted plasma ($\times 10$) of this group were determined as less than 10 ppb. All samples tested positive on day 1 of withdrawal. On day 5, 9 of 10 samples reacted as positive. The number of positive samples was 3 on day 7 of withdrawal. On day 10 of withdrawal, all samples showed negative reaction (B/Bs ratio ≥ 1.0).

DISCUSSION

To prevent unwanted drug residues entering the human food chain, both government authorities and industries have established extensive control measures (Sternesjo et al., 1995). A variety of rapid screening tests have been developed and applied for determining drug contamination of animal products on farm and at slaughterhouse.

The Swab Test On Premises (STOP), a non-specific microbial inhibition test, has been used in abattoirs in the United States and Canada for more than 10 years to screen antibiotic residues in tissues from slaughtered animals (Korsrud et al., 1995). Since the STOP test requires overnight incubation, test results are not ready until the following day. It is only conducted on tissues of slaughtered animals (Boison et al., 1995). In the United States, however, a variant of STOP, the Live Animal Swab Test (LAST), which tests urine of live animals, is used to screen for antibiotic residues. This test, like STOP, requires overnight incubation, and test results are not ready until the following day. Bourne et al. (1978) reported plasma and urine concentrations of unchanged sulfamethazine and of 3 metabolites following intravenous administration of sodium sulfamethazine to young ewe lamb. The results indicated that determination of the plasma concentration or urinary output of sulfamethazine can be substituted for tissue residue

analysis to determine contamination of carcasses above specified tolerance limits. Paulson et al. (1994) studied cows that were dosed orally with sulfamethazine for 5 consecutive days (220 mg/kg of body weight on day 1 and 110 mg/kg on days 2-5); sulfamethazine in the blood decreased to less than 100 ppb at 4 days after last doses and to less than 10 ppb at 7 days after the last doses. The concentrations of sulfamethazine in milk were approximately one-fifth the concentrations of sulfamethazine in blood. Sweeney et al. (1993) developed the model to predict the number of days for sulfamethazine concentration to fall below 0.1 ng/g of tissue in various organs of pigs from the urine concentration of sulfamethazine. This predictor model provided the practical basis for current SOS test in which swine urine is used for screening sulfonamide residues in animal tissue in federally inspected abattoirs of the United States, Canada and Korea. As a correlation between residue level in tissue and urine has been established, the urine residue is used as an indicator of sulfamethazine in animal tissue (Boison et al., 1995). With the consideration of administered dosage, plasma concentrations profiles of sulfamethazine in our study were similar to those in the above studies. As blood is a central pool of drug distribution to body compartments (Booth et al., 1988), it may be possible to predict the tissue residues of drugs in tissue by examining the blood drug depletion profile during the withdrawal period (Boison et al., 1995; Korsrud et al., 1995; Lee et al., 2001; Lee et al., 2000; Lee et al., 2000).

According to our results, the developed method can be adapted easily for use in prediction of tissue residues of sulfamethazine in live cattle by screening blood plasma with the modified ELISA test kits. The veterinary inspector in the abattoir may be able to use this method to screen for sulfamethazine in plasma of live cattle in holding pens prior to slaughter and obtain same-day results. Thus, cattle reacting positive can be held in the pens and should be retested before they are slaughtered.

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

Withdrawal (days)	No. of positive	No. of negative	B/Bs ratio (Mean \pm SE)
Control*	0	10	1.475 \pm 0.160
1	10	0	0.290 \pm 0.158
3	10	0	0.570 \pm 0.192
5	9	1	0.856 \pm 0.143
7	3	7	1.248 \pm 0.226
10	0	10	1.504 \pm 0.202
14	0	10	1.588 \pm 0.178

* Blood was collected before administration of sulfamethazine. Concentration of sulfamethazine in plasma was analyzed using LacTek ELISA kit. B is absorbance of sample and Bs is absorbance of internal standard (10 ppb).

ACKNOWLEDGEMENT

This work was supported by the Brain Korea 21 Project, a grant of Agricultural Research and Promotion Center, and a grant from the Veterinary Research Institute, College of Veterinary Medicine, Seoul National University.

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