The Effect of Reflex Closure of the Esophageal Groove on Bioavailability of Oral Sulfamethoxazole-Trimethoprim in Ruminating Calves

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ABSTRACT. The effect of reflex closure of the esophageal groove induced by the accustomed milking procedure on absorption of sulfamethoxazole (SMX)-trimethoprim (TMP) combination was examined, using calves trained to suck through a nipple-bucket. The experiment was carried out with a two-way cross-over design in which 2 groups of 5 calves of 6 weeks old were used. SMX-TMP combination was administered to the calves in each group through a nipple-bucket or an esophageal catheter. Tmax of SMX after nipple-bucket dosing was about 40 min shorter than that after catheter dosing. Cmax and AUC of SMX after the nipple-bucket dosing were 7.5 times greater and 6.9 times larger than those after the catheter dosing, respectively. Plasma TMP concentration was measurable only after the nipple-bucket dosing. These results suggest that a large portion of the drugs administered by the nipple-bucket dosing, which induces closure of the esophageal groove, were transferred directly into the abomasum resulting in higher bioavailabilities of the drugs. — KEY WORDS: bioavailability, esophageal groove, ruminating calf, sulfamethoxazole, trimethoprim.


For oral administration of drugs to calves, various methods such as via a bucket, a bottle or an esophageal catheter have been used. For calves more than 6 weeks of age, however, oral administration of some drugs does not produce efficient blood concentrations due to the ruminal function. Suckling calves have the ability of reflex closure of the esophageal groove, which allows passage of liquid ingesta from the cardiac orifice directly to the abomasum. The purpose of this study is to elucidate the effects of reflex closure of the esophageal groove on drug absorption in ruminating calves. This report describes the bioavailability of sulfamethoxazole (SMX)-trimethoprim (TMP) combination in calves, comparing pharmacokinetic parameters after oral administration through a nipple-bucket with those through an esophageal catheter.

MATERIALS AND METHODS

Animals: Thirteen healthy Holstein calves, 11 males and 2 females, were purchased at one week of age from farms near the institute, and kept in individual pens. For the first 4 weeks of their life, they were given a lukewarm solution (37–38°C) containing an adequate amount of a commercial milk replacer (SYNTHEThIC FEED LABORATORY Co., Ltd., Japan) twice a day, at 9:00 and 17:00, by means of a nipple-bucket (31 stainless bucket with a rubber teat). From the fourth week onward, they were given 21 of lukewarm water instead of the milk replacer solution, twice a day using the nipple-bucket. Water, timothy hay, and calf starter (SYNTHEThIC FEED LABORATORY Co., Ltd., Japan) were given ad libitum throughout the study. The calves were used for the experiment when they were 6 weeks old, and at this time the body weight of the animals ranged from 65 to 92 kg with a mean of 77 ± 9 kg.

Drugs: A commercial formulation of sulfamethoxazole (SMX) and trimethoprim (TMP) combined powder (Shinoral San®, Shionogi Pharmaceutical Co., Ltd., Japan) was used for oral administration. It contained 3 g of SMX and 1 g of TMP in 150 g of the powder. SMX sodium (purity of 100%, Shinomin Soda®, Shionogi Pharmaceutical Co., Ltd., Japan) was used for intravenous injection.

Experimental design: The experiment was carried out with a two-way cross-over design which was a routine method for a bioequivalent test. Ten calves (8 males and 2 females) were randomly divided into 2 groups of 5 calves. The calves in each group were given the combined powder at a dose of 600 mg/kg (SMX: 20 mg/kg, TMP: 4 mg/kg) in 1 l of lukewarm solution (37–38°C) using the nipple-bucket (nipple-bucket dosing), or through a rubber catheter (140 cm in length, 15 mm in diameter) inserted into the esophagus (catheter dosing). It took about 2 min to administer the solution to the calves by both methods. The calves were fasted 16 hr before and 2 hr after dosing. After 7 days, for the drug wash-out, dosing was repeated by the alternative method. For evaluation of the relative F value, the residual 3 calves were given SMX sodium intravenously at a dose of 20 mg/kg. SMX sodium was dissolved in 20 ml of saline followed by filtration through a membrane filter (FP 030/3, Schleicher & Scuell Co., Ltd., Germany) and injected into the jugular vein of the calves. All the drug administrations were carried out at 9:00.

Blood samples (10 ml) were drawn before drug administration and at 5, 15, 30, 45, 60 min and 1.5, 2, 3, 4, 6, 8 hr after oral dosing, and at 0.5, 1, 2, 4, 6, 8 hr after intravenous dosing. After centrifugation, plasma was transferred into a polyethylene tube and stored at -20°C until analysis.

Analytical methods: Plasma drug concentrations were determined in supernatants from 2 ml of the sample following a twofold deproteinization with 5 ml of ethyl alcohol. The supernatant was transferred into a 50 ml flask containing 2 ml of n-propyl alcohol and evaporated to
dryness. The residue was dissolved in 10 ml of methyl alcohol, and the solution was poured into an Alumina B cartridge (Mltpore Co., Ltd., U.S.A.). The efflux solution was collected to determine TMP concentration. Three ml of purified water was poured into the cartridge and the efflux solution was collected for SMX analysis. Each efflux solution was evaporated to dryness. To the residue, 2 ml of mobile phase was added and the solution was injected into an HPLC (Japan Spectroscopic Co., Ltd., Japan); mobile phase: acetonitrile/water/acetic acid mixture (33/67/0.1), flow rate: 1.0 ml/min, detection wavelength: 270 nm. For TMP: Inertsil C8 (GL Sciences, Co., Ltd., Japan), acetonitrile/0.1% phosphoric acid mixture (9/91), 1.0 ml/min and 240 nm.

Recoveries of SMX and TMP were 91 ± 4% and 81 ± 5% (each n=3), respectively. The detection limit was 0.1 µg/ml for SMX and 0.05 µg/ml for TMP.

Pharmacokinetic calculations: Plasma peak concentration (Cmax), and time to reach the peak (Tmax) were read from the individual data. The area under the concentration-time curve (AUC) from time 0 to the last sample point (at 8 hr after dosing) was estimated by the trapezoidal method [17]. The absorption-rate constant (ka) and elimination-rate constant (ke), during 4–8 hr after dosing) were determined by Damping Gauss-Newton analysis [12]. The elimination half-life (t1/2) was calculated by the formula of 0.693/ke, and the relative F of SMX was calculated by dividing the mean AUC after oral dosing by the mean AUC obtained after intravenous injection of SMX sodium.

Statistics: Data are shown as the mean ± SD. Statistical analyses were performed by the paired t-test at a significance level of P<0.05.

RESULTS

Before the first and second drug administrations, SMX and TMP were not detected in the calves. After the nipple-bucket dosing, Tmax of SMX (1.13 ± 0.36 hr) was about 40 min shorter than that (1.85 ± 0.24 hr) after the catheter dosing (Fig. 1 and Table 1). Significant differences were found in both Tmax and ka between the two oral administrations. Cmax of SMX was 25.6 ± 5.7 µg/ml after the nipple-bucket dosing and 3.4 ± 1.1 µg/ml after the catheter dosing. The Cmax after the nipple-bucket dosing was 7.5 times greater. AUC (103.6 ± 13.3 µg-hr/ml) after the nipple-bucket dosing was 6.9 times larger than that (15.1 ± 5.6 µg-hr/ml) after the catheter dosing. AUC of SMX sodium after intravenous injection was 157.6 ± 1.1 µg-hr/ml. The relative F was 66% for the nipple-bucket dosing and 10% for the catheter dosing. No significant differences were found in either t1/2 or ke between the two oral administrations.

As shown in Fig. 2, plasma TMP level was measurable only after the nipple-bucket dosing; Tmax was 0.90 ± 0.36 hr and Cmax was 0.36 ± 0.17 µg/ml. No significant difference was found in Tmax between SMX and TMP after the nipple-bucket dosing.

DISCUSSION

Calf Feeding: The recent practice of calf keeping is to allow calves to suck colostrum for at least 3 days and afterwards to drink lukewarm water containing milk replacer twice a day until 4 or 8 weeks old, with calves being
provided with roughage and concentrated feed (calf starter) ad libitum from around the 4th week. These practices are considered to accelerate growth of the rumen and obtain a better feed efficiency. It was reported that fully functional ruminal activities were established by 6 weeks of age in calves under normal feeding conditions [2, 3, 7].

Rumen Bypass: The reflex closure of the esophageal groove in calves allows the passage of milk or liquid ingesta from the cardiac orifice directly to the abomasum. This bypass is considered to protect calves from intoxication by ammonia or lactic acid which is produced in the rumen from milk. The reflex was suggested to be conditioned only by feeding procedures adopted to milk feeding, and not by chemical and physical stimuli with sucking [10, 11]. The reflex induced by the accustomed feeding procedure was reported to cause efficient closure of the esophageal groove up to 16 weeks of age [1], but interruption of the procedure seems to cause a losing of the ability to elicit the reflex within a few weeks. For the above reasons, the feeding procedure used in this study could stimulate early development of the rumen functions, whilst keeping the zility of reflex closure of the esophageal groove.

Oral Medication to Calves: Oral administration of some drugs to calves does not produce efficient blood concentrations [9]. The inefficient absorption of drugs after oral administration was thought to be caused by lack of ruminal motility in ruminating animals [4, 13], degradation by rumen flora in ruminating ones [4, 6, 14], a long transient time through the rumen, and a low absorption rate from the rumen mucosa [4]. In the pharmacokinetic studies on oral chloramphenicol in calves [4, 5], maturational changes in the rumen functions have been suggested as contributing to large variations in drug absorption with age, and a therapeutically effective level of chloramphenicol was obtained only after direct intra-abomasal administration. From a therapeutic point of view, therefore, for oral drug administration to calves, to attain efficient levels of drugs in blood and tissues, it is necessary that they are transferred directly into the abomasum through the rumen bypass route.

In the present study, SMX-TMP combination administered by the catheter dosing to the ruminating calves could not produce efficient plasma concentrations of either drug. The poor availability of SMX after the catheter dosing could be explained by the reason that only a small portion of the drug was transferred into the small intestine, where the drug would be absorbed efficiently. Since the intraruminal environment is acidic, an acidic drug such as SMX, but not a basic drug such as TMP, could be absorbed slowly through the ruminal mucosa, and this would explain why TMP was undetected after the catheter dosing.

Whereas, after the nipple-bucket dosing, Cmax and AUC of SMX were, respectively, with 7.5 and 6.9 times greater than those after the catheter dosing. TMP being detected only after the nipple-bucket dosing. As an explanation of the higher plasma concentrations of the drugs, it was supposed that a large portion of the suspension was transferred directly into the abomasum through the esophageal groove induced by the nipple-bucket dosing.

It was reported that oral administration of drugs could sometimes induce partial closure of the esophageal groove and some portions of drugs were transferred into the abomasum bypassing the rumen [4, 8], but this is of less practical value, because of inconsistency of the phenomena. Thompson and Black [15] reported that after oral administration by various methods, ampicillin was only detected in the plasma of calves given the drug with milk, and suggested that ampicillin should be given to suckling calves dissolved in milk and fed according to the accustomed feeding procedure which induces closure of the esophageal groove. For some drugs, however, administration with milk was reported to inhibit absorption from the gastrointestinal tract [16, 18]. The present study suggests that oral administration of a drug dissolved in lukewarm water according to the accustomed feeding procedure could produce efficient blood concentration in calves capable of reflex closure of esophageal groove, avoiding the adverse effects of forestomach functions and feed composition on drug absorption.

REFERENCES