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Sustained release granules of sulfamethoxazole for chicken utilizing frictional power of gizzard

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Abstract

We designed a novel sustained release preparation for chicken which resides in the gizzard and releases drug gradually following crushing due to the power of the gizzard. Considering the ease of administration, we selected hard granules consisting of a model drug, sulfamethoxazole (SMX), and polymers as the dosage form. When SMX was administered at a dose of 300 mg/kg as granules consisting of SMX (50%), EC (47.5%) and HPC (2.5%), the AUC value reached 72% of that in SMX powder. Moreover, the effective plasma concentration of SMX was maintained for approx. 4 days, which is 8-times longer than in the case of ordinary administration of SMX powder. The results suggest the possibility of development of sustained release dosage forms utilizing the frictional power of the gizzard.

Keywords: Sustained release granule; Sulfamethoxazole; Chicken; Ethylcellulose; Hydroxypropylcellulose; Frictional power; Gizzard

1. Introduction

The chicken has a unique digestive system which is composed of a crop and two stomachs (Fig. 1). Swallowed food is stored in the crop for a while and passes through the glandular stomach which secretes digestive fluid and is then crushed to paste in the gizzard before moving into the small intestine which is considered as a major absorption site. Usually, the gizzard contains small stones for some months which assist crushing of grain (Ewing, 1963) and so the frictional power of the gizzard can be estimated as very strong.

Previously, we have examined the transit of granules containing barium sulfate in the digestive organs of chicken under fasting and non-fasting conditions using roentgenography (Hamamoto et al., 1989). The results showed that the granules administered to chickens under non-fasting conditions remained in the crop for about 1 h, then

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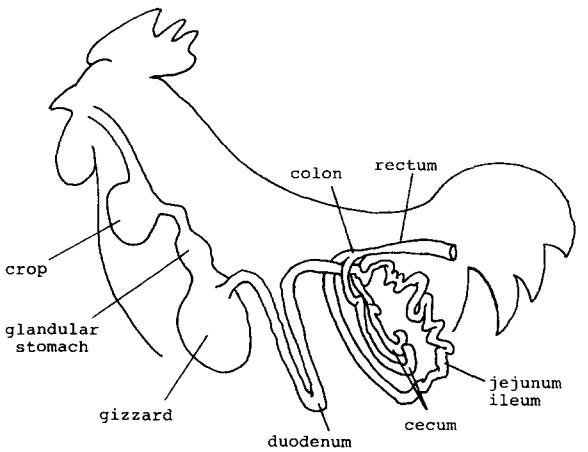


Fig. 1. Structure of digestive organ in chicken.

passed through the glandular stomach within 5 min, and stopped in the gizzard. Finally, the granules were crushed by the strong frictional power of the gizzard within a short time period (15–40 min), since the granules were not hard enough to remain in the gizzard. On the other hand, chicken gizzard contains a number of small stones for a long time in order to aid its digestion as stated above. This suggests that very hard lumps are able to remain in the gizzard.

Therefore, we designed a novel sustained release preparation for chicken which resides in the gizzard and releases drug gradually following crushing. Considering the ease of administration, we selected granules consisting of a drug and polymers, as the dosage form. Ethylcellulose (EC) which is utilized as a coating material for tablets and in preparation of a microcapsules (Kato and Nemoto, 1978; Appel and Zentner, 1991; Kristl et al., 1991) was used as a vehicle and hydroxypropylcellulose (HPC) as an adjuvant.

In this study, very hard granules were prepared using sulfamethoxazole (SMX) as a model drug, which is administered practically to chickens, and their dissolution behavior was measured by the rotatory basket method with a combination of plastic beads. Moreover, the sustained release effect of these granules in vivo was examined by comparing the bioavailability parameters after oral administration of the powder with those in the granules.

2. Materials and methods

2.1. Roentgenographic study

In order to observe the transit of a very hard balls in chicken alimentary tract, 30 metal balls (2–4 mm in diameter) were administered orally to two chickens under fasting or non-fasting conditions. Roentgenograms of the chickens were recorded at different intervals until 30 h after administration using a Hitachi DR-155–13 X-ray generator of 2-peak rectifying system (Hitachi Medical Corp., Tokyo), at 64 kV and 200 mA.

2.2. Preparation of granules

Granules of diameter 3.0–4.0 mm (> 96%) were prepared by drying a mixture of SMX (Sigma Co. Ltd, St. Louis) and ethylcellulose (EC: Tokyo Kasei Kogyo Co. Ltd, Tokyo, 45–55 cps) alone or with addition of hydroxypropylcellulose-H (HPC: Nippon Soda Co. Ltd, Tokyo). At first, 1–2 g of polymer was dissolved in approx. 17–34 ml of ethanol, then mixed with 1 g of SMX to yield a soft mass. The soft mass was allowed to stand in air for approx. 6 h to dry and then the granules were prepared by rounding the small pieces of the mass according to the method for production of pills. The granules were dried in air for 24 h and then dried under vacuum for 6 h at 60°C.

2.3. In vitro drug release test

The in vitro dissolution behavior of granules was monitored using a modified JP XII rotatory basket method in which 10–30 polystyrene beads (6 mm in diameter) were added in the basket. Granules (25 mg as SMX) were introduced into 500 ml of pH 3.6 phosphate buffer solution (mean pH value in the stomach of chicken) at 37 ± 0.5°C. The speed of rotation of the basket was set at 100 or 200 rpm. 2.5 ml of sample was drawn at different time intervals and replaced with an equivalent volume of fresh dissolution medium. The amount of drug in the medium was assayed spectrophotometrically (UV detection at 267 nm).

2.4. In vivo absorption study

The granules and powder were administered orally by force to 21 female white leghorn chickens (1.24–1.48 kg body wt). Five kinds of granules consisting of SMX and EC (SMX/EC = 1:1 and 1:2) or SMX, EC and HPC. In the case of the latter granules, 2.5, 5 or 10% of HPC was added to the mixture of SMX and EC. The granules were pushed into the beak which was then shut, forcing the chicken to swallow. Finally, granules were washed down with approx. 2 ml of water. Nine chickens were examined in the case of SMX powder and EC granules and another nine for EC + HPC granules according to a crossover fashion at 2 week intervals using three chickens as one group. Another three chickens were used for studying granules containing 10% HPC.

The blood samples were taken from the subwing vein at different time intervals until 96 h after administration. The heparinized blood was centrifuged at 3000 rpm and the separated plasma samples were stored at −20°C.

2.5. Plasma assay

The method for assay of SMX and N_4-acetyl metabolite in plasma samples was reported earlier (Astbury and Dixon, 1987; Van der Steuijt and Sonneveld, 1987; Erdmann and Canafax, 1988). In the present study, however, we developed a more simple HPLC method and use for the assay of SMX in plasma (Scheme 1). A standard curve with excellent linearity ($r = 0.9999$) and high recovery rate (97.3–106.5%) was obtained. The intra- and inter-day coefficient of variation was 0.4–3.9 and 1.6–9.2%, respectively. The detection limit was 1–5 ng. N_4-Acetyl SMX was included in the SMX plasma concentration due to its rather low level. N_1-Acetyl metabolite was not detected in plasma.

2.6. Statistical analysis

Analysis of variance was used for the detection of significant differences between the pharmacokinetic parameters. The least significant difference method was used for the detection of a significant difference between each value. The T_{max} and C_{max} were the observed values. The AUC_{0-96} was calculated according to the trapezoidal rule and $AUC_{0-\infty}$ was evaluated using the program MULTI (Yamaoka et al., 1981). The MRT was obtained by moment analysis (Yamaoka et al., 1978).

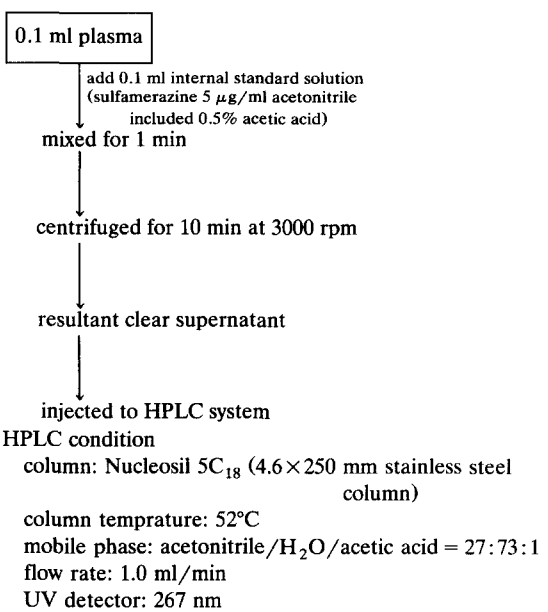
3. Results

3.1. Transit of metal balls

Fig. 2 shows the results from experiments on the transit of the metal balls in the alimentary tract of chicken under fasting conditions. The metal balls were not crushed and remained in the gizzard over 30 h under both the fasting and non-fasting conditions.

3.2. In vitro dissolution of SMX from the granules

The granules in the present study were prepared using a water insoluble polymer (EC) and



Scheme 1. Procedure and HPLC condition for the determination of sulfamethoxazole and *N*-acetylsulfamethoxazole in chicken plasma.

the drug release seemed to progress by grinding in the gizzard of chicken except for drug existing on the surface of granules. However, drug release from the granules was too low for obtaining dissolution profiles by the usual dissolution test method. Consequently, we applied the bead method to the rotatory basket method of the JP ('Beads in Basket' method, BIB method). Initially, the condition of the BIB method was examined by changing the number of polymer beads

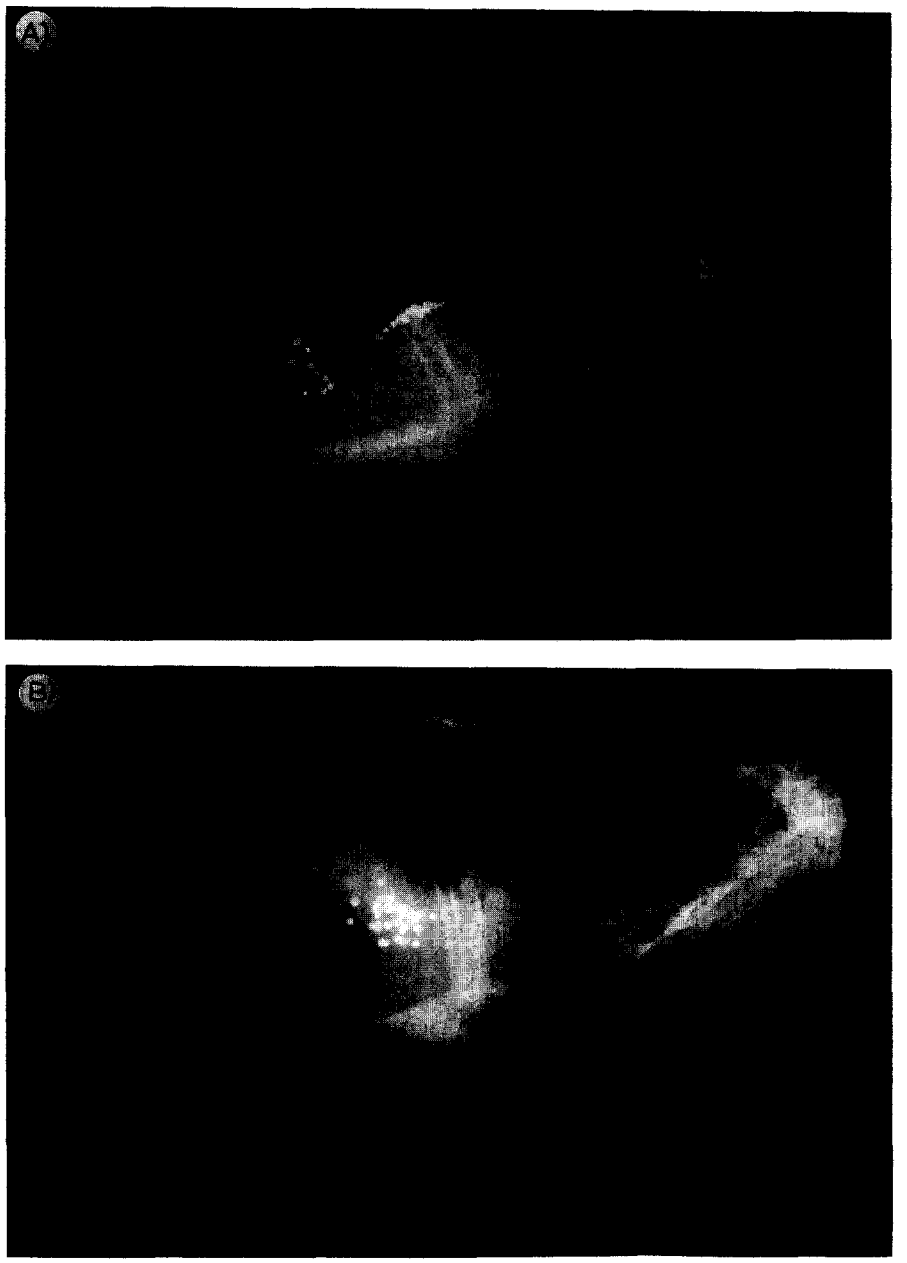


Fig. 2. Typical roentgenogram in chicken after oral administration of 30 metal balls (2–4 mm in diameter) under fasting conditions. (A) Immediately after administration; (B) 30 h after administration.

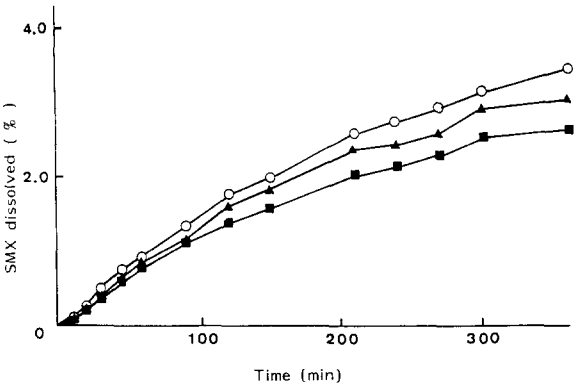


Fig. 3. Dissolution profiles of sulfamethoxazole from SMX granules (SMX/ethylcellulose/HPC = 50:47.5:2.5) obtained by the beads in basket method in pH 3.6 phosphate buffer at 37°C, 200 rpm. Each point represents the mean of three experiments. (■) 10 beads, (▲) 20 beads, (○) 30 beads.

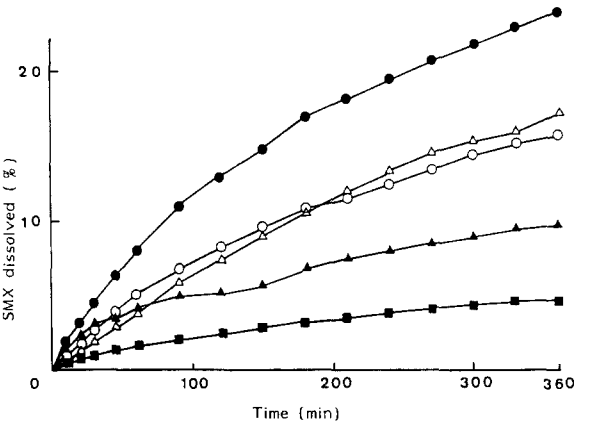


Fig. 4. Dissolution profiles of sulfamethoxazole from SMX granules (25 mg as SMX) obtained by the beads in basket method in pH 3.6 phosphate buffer for 30 beads, at 37°C, 200 rpm. Each point represents the mean of three experiments. (▲) SMX/EC = 1:1, (■) SMX/EC = 1:2, (○) SMX/EC/HPC = 50:47.5:2.5, (●) SMX/EC/HPC = 50:45:5, (△) SMX/EC/HPC = 50:40:10.

(10, 20 or 30 beads) or rotation speed (100 or 200 rpm). Fig. 3 shows the dissolution profiles of SMX from the granules consisting of SMX, EC and HPC (50:47.5:2.5) obtained at 200 rpm and for different numbers of beads. The external appearance of granules on changing the number of polymer beads did not demonstrate any marked change. As a result, the condition was fixed at 30 beads and 200 rpm since these parameters led to the highest dissolution rate under the conditions tested.

The in vitro dissolution behavior of five different granules is summarized in Fig. 4. SMX granules consisting of EC alone showed slow dissolution. On the other hand, granules prepared using a mixture of EC and HPC displayed greater dissolution. The addition of HPC should render some brittleness to granules so that dissolution of SMX from granules increases. However, the greatest dissolution was not observed in granules containing 10% HPC but in those containing 5% HPC.

3.3. Absorption of SMX after administration to chickens

3.3.1. SMX powder and SMX / EC granules

The plasma concentration-time profiles and pharmacokinetic parameters after administration

Table 1
Pharmacokinetic parameters after oral administration of SMX powder and SMX-EC granules (100 mg SMX/kg) to nine chickens under non-fasting conditions

Dosage form	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-96} (μg h ml^{-1})	$AUC_{0-\infty}$ (μg h ml^{-1})	MRT_{0-96} (h)	$MRT_{0-\infty}$ (h)
(1) Powder	106.37 ± 64.33	1.67 ± 0.71	1363.4 ± 592.2	1363.4 ± 592.2	12.7 ± 4.5	12.7 ± 4.5
(2) SMX/EC granules (1:1)	4.84 ± 2.07	27.50 ± 17.46	266.21 ± 123.66	439.5 ± 316.0	46.80 ± 5.03	98.5 ± 37.6
(3) SMX/EC granules (1:2)	4.07 ± 1.79	41.22 ± 33.07	215.12 ± 91.59	394.7 ± 251.3	48.00 ± 4.78	117.7 ± 62.0
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
Multiple range test [a]	1) > 2) > 3)	3) > 2) > 1)	1) > 2) > 3)	1) > 2) > 3)	3) > 2) > 1)	2) > 3) > 1)

[a] Combinations underlined did not differ significantly ($p > 0.05$).

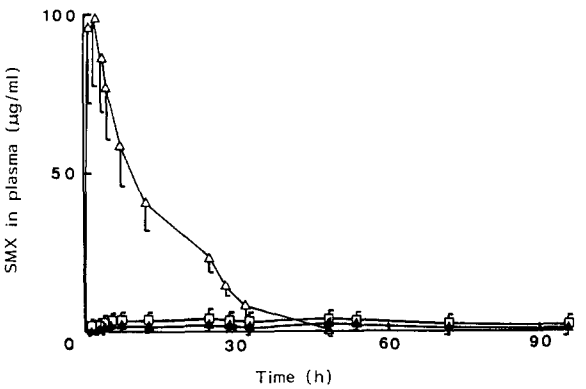


Fig. 5. Plasma concentration-time profiles of sulfamethoxazole after oral administration of SMX as powder or granules (100 mg SMX/kg) to nine chickens under non-fasting conditions. Each point represents the mean ± S.E. (△) Powder, (□) granules (SMX/EC = 1:1), (▲) granules (SMX/EC = 1:2).

of SMX as powder or two kinds of granules consisting of SMX and EC alone (100 mg SMX/kg) to nine chickens are shown in Fig. 5 and Table 1, respectively. The plasma concentration in the case of SMX powder was high and rapidly reached C_{max} (106.4 μg/ml) at 1.7 h after administration, and the elimination half-life was 7.6 h. On the other hand, the plasma concentration of SMX/EC granules was very low and the C_{max} was only 1/20 of that of the powder. The AUC value after administration of the SMX/EC granules was also lower than that of powder. However, the granules demonstrated prolonged release of SMX until the endpoint of measurement, 96 h after administration, indicating the possibility of a sustained release preparation utilizing the frictional power of the gizzard.

3.3.2. SMX / EC / HPC granules

In order to improve the dissolution of SMX from the granules, HPC was added as an adjuvant. The plasma concentration-time profiles and the pharmacokinetic parameters after administration of SMX/EC/HPC granules (100 or 300 mg/kg) to 12 chickens are shown in Fig. 6 and Table 2, respectively. Dissolution of SMX from granules was increased by the addition of HPC. The C_{max} values increased with increasing HPC concentration and the granules containing 10% HPC showed the highest C_{max}. In the dissolution study, the granules containing 10% HPC did not show the highest dissolution rate. The difference between the results in vitro and in vivo appeared to be due to different strength of friction in the basket and in the gizzard. The latter might be stronger than in vitro and the addition of HPC at a higher concentration could affect directly the dissolution in vivo. However, addition of 10% HPC was undesirable for the purpose of prolonging the release time as the values of T_{max} and MRT decreased. On administration of 300 mg SMX/kg as granules containing 2.5% HPC to chickens, the C_{max} of SMX reached 60.2 μg/ml

Table 2
Pharmacokinetic parameters after oral administration of SMX granules to nine chickens under non-fasting conditions

Granules	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-96} (μg h ml^{-1})	$AUC_{0-\infty}$ (μg h ml^{-1})	MRT_{0-96} (h)	$MRT_{0-\infty}$ (h)
(1) SMX/EC/HPC (50:47.5:2.5) (100 mg/kg)	11.90 ± 4.98	28.44 ± 12.72	522.2 ± 219.9	540.6 ± 220.3	34.07 ± 5.34	37.94 ± 6.53
(2) SMX/EC/HPC (50:45:5) (100 mg/kg)	13.13 ± 5.87	23.83 ± 14.00	631.3 ± 237.4	810.9 ± 523.4	36.78 ± 6.50	60.92 ± 49.89
(3) SMX/EC/HPC (50:47.5:2.5) (300 mg/kg)	60.21 ± 15.79	30.22 ± 14.30	2795.6 ± 861.3	2954.9 ± 876.1	36.28 ± 6.83	41.72 ± 11.04
	$p < 0.01$	N.S.	$p < 0.01$	$p < 0.01$	N.S.	N.S.
Multiple range test [a]	3) > 2) > 1)		3) > 2) > 1)	3) > 2) > 1)		

N.S., not significant ($p > 0.05$).
[a] Combinations underlined did not differ significantly ($p > 0.05$).

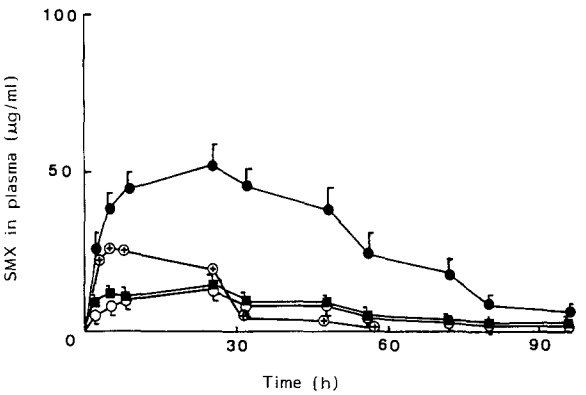


Fig. 6. Plasma concentration-time profiles of sulfamethoxazole after oral administration of SMX granules (100 or 300 mg SMX/kg) to nine chickens under non-fasting conditions. Each point represents the mean ± S.E. (○) SMX/EC/HPC = 50:47.5:2.5 (100 mg SMX/kg), (■) SMX/EC/HPC = 50:45:5 (100 mg SMX/kg), (●) SMX/EC/HPC = 50:47.5:2.5 (300 mg SMX/kg), (⊕) SMX/EC/HPC = 50:40:10 (100 mg SMX/kg).

at 30 h and the effective plasma concentration (5 μg/ml) was maintained for 4 days.

4. Discussion

Usually, the dosage form for chickens is powders, and thus the duration of its effect is limited by the elimination half-life and the drug tends to scatter from the food box. In order to maintain an effective plasma concentration for a prolonged time and to administer a certain quantity of drug to chickens, we designed sustained release granules which release drug with the aid of the crushing power of the gizzard.

SMX, the model drug in this study, is used as an antibacterial agent for many kinds of domestic animals. The effective plasma concentration is over 5 μg/ml (Heinze and Wachtel, 1992) and the elimination half-life is 8.77 h in chickens (Reddy et al., 1988). The maximum plasma concentration and the biological half-life of SMX obtained in this study by administration of 100 mg/kg SMX powder, which was 5-fold the usual preventive dose, were 106.4 μg/ml and 7.6 h, respectively. The C_{max} value after administration of SMX granules was lower than that of the powder but the MRT value was statistically greater vs that of the powder ($p < 0.05$). From the study using roentgenography, we ascertained that the metal balls (2–4 mm in diameter) remained for a long time in the gizzard. These results suggest that the hard granules can remain in the gizzard before moving into the small intestine as an effective position for absorption. EC and HPC are used as pharmaceutical excipients for humans and are known as safe materials. In this study we used EC as a vehicle to make the granules hard. Although the granules prepared with EC alone gave a prolonged MRT, an effective plasma concentration was not achieved. Therefore, we mixed 2.5–10% of HPC with granules in order to confer brittleness on the granules and to increase the release of SMX.

We applied the bead method to the rotatory basket method of the JP to test drug release from the hard granules. This beads in basket method was confirmed as being superior to the ordinary rotatory basket method for testing the new type of granules in this study.

The results of the dissolution test showed that drug release from the granules prepared with EC and HPC was better than that for EC alone and that release increased with increasing amount of HPC. The results of in vitro dissolution tests showed a similar tendency to the in vivo study except for the granules containing 10% HPC which gave the highest C_{max} value in the granules tested. Therefore, it seemed that the crushing power of the gizzard and the frictional power of polymer beads in the basket differed in degree and in manner of action. The plasma concentration profile of SMX in the granules containing 10% HPC also showed that the dissolution of granules in vivo can be increased by the addition of HPC but the duration of release might be shortened. Hence, we chose granules containing 2.5% HPC expecting longer release in vivo and 300 mg SMX/kg was administered to chickens. As a result, the effective plasma concentration of SMX was maintained for approx. 4 days, which is 8-fold longer compared with the ordinary administration of SMX powder. The preventive plasma concentration was maintained over 4 days. The AUC value in the case of administration of 300

mg SMX/kg as the granules containing 2.5% HPC was more than 3-fold that in the administration of 100 mg SMX/kg as the same granules. This result suggests that friction between granules in the gizzard was increased by the administration of the granules in 3-fold greater amount.

In this study, the plasma level of SMX in chickens at 9 a.m. tended to be higher than at 5 p.m. Usually, chickens are fed at 8–9 a.m. and sleep at 5–6 p.m. Movement of the gizzard becomes active with feeding (Akahori et al., 1968), therefore, the plasma level of SMX in chickens to which granules are administered at 9 a.m. becomes higher than that at 5 p.m., obviously because of the active movement of the gizzard.

Many kinds of devices which release a drug continuously in the rumen have been developed for usage in cows (Blodinger, 1983; Brown, 1990), however, a sustained release dosage form for chickens has not yet been developed. The results in this study suggest the possibility of development of sustained release dosage forms utilizing the frictional power of the gizzard of chickens and other domestic poultry having a similar digestive organ.

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