Preparation of Controlled Release Tablets of TA-5707F with Wax Matrix Type and Their in Vivo Evaluation in Beagle Dogs

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Studies on controlled release dosage forms were conducted by using waxy materials for a newly developed anti-allergy drug, 6-methyl-N-(1H-tetrazol-5-yl)-2-pyridinecarboxamide (TA-5707F), which is not maintained at an effective level in blood for a long duration. Four kinds of tablets were prepared by changing the amount of hydrogenated oil (K3 wax) and polyethylene glycol-6000 in the tablets. Then, they were administered orally to beagle dogs, and the TA-5707F concentration in the plasma was determined by a HPLC method. The pharmacokinetic parameters were estimated and compared with the results of the in vitro dissolution test determined by the JP paddle method and by the disintegration method. The linearity between the in vivo mean dissolution time (MDT) and in vitro MDT was good in both in vitro dissolution methods. However, the MDTs obtained by the disintegration method were one-third of the in vivo MDT, while those obtained by the paddle methods were 3 times higher. This suggests that both the diffusion of TA-5707F through the waxy matrix and the erosion of the wax matrix caused by the gastrointestinal (GI) tract mobility contributed to the in vivo dissolution mechanism.

The blood levels were very low when the tablet was administered after giving food. The prolongation of resident time in the stomach and the low solubility of TA-5707F in an acidic medium seemed to be related to the phenomena. By the depression of GI motility using propantheline bromide, the blood levels could be markedly prolonged and the area under the plasma concentration-time curve (AUC) increased 1.3 times.

Key words wax matrix; bioavailability; food; dissolution

TA-5707F,1) 6-methyl-N-(1H-tetrazol-5-yl)-2-pyridinecarboxamide (Fig. 1), was newly synthesized by Tanabe Seiyaku Co., Ltd., and has been developed as an anti-allergy drug.2) The Phase I study revealed that the elimination half-life in humans was short, as it was in the experimental animals.3) Thus, the tablets had to be taken more than five times a day to sustain the efficacy with a plain tablet, and this seemed to be a definite drawback for clinical compliance. Therefore, we carried out formulation studies of sustained release tablets in an attempt to decrease the dosing frequency.

The mechanisms of release from oral formulations are classified into diffusion, erosion, ion exchange, complex dissociation and osmotic pressure.4) The methodology of the release control by using waxy materials has been applied to Herbersses5) tablets in our laboratory, and the dissolution mechanism has been considered to be based on both the diffusion of drugs through the waxy materials and erosion of the wax matrix. This method has many advantages, such as feasible mass production, low cost and good stability of dissolution rate during the shelf life. Thus, we first applied the wax matrix method to prepare TA-5707F controlled release tablets. In the preparation of the tablets, TA-5707F, K3 wax, polyethylene glycol-6000 (PEG6000) and other excipients were used. The studies of Herbersses6) tablets suggested that the amount of wax and PEG markedly affects the dissolution rate. Thus, five different kinds of releasing rate tablets were prepared by modification of the components of the tablets. An erosion of the wax matrix tablets in the gastrointestinal tract might occur as the result of mechanical agitating stress. Therefore, the in vitro dissolution of these tablets was evaluated by the JP paddle method and by the JP disintegration method. At the same time, these tablets were administered to beagle dogs and their blood levels were determined. We then examined the correlation between the in vivo and the in vitro findings. The effects of food and an anticolinergic agent on the bioavailability of the tablets were studied to evaluate the applicability of this controlled release methodology to a clinical study in humans.

MATERIALS AND METHODS

Materials TA-5707F was synthesized by Tanabe Co., Ltd., Osaka and passed in-house specifications. K3 wax and PEG6000 were purchased from Kawaken Co., Ltd., Tokyo and Sanyo Kasei Co., Ltd., Kyoto, respectively. All excipients were sieved through a 24 mesh screen before use. All other chemicals used were of analytical grade available from commercial suppliers.

Measurement of Physical Properties Solubility: 0.5—2.5 g of TA-5707F powder was added to 100 ml of buffer solution (pH 1—10, ionic strength 0.5), and the TA-5707F in the buffer solution and the pH of the solutions were determined after mixing for 24 h at 25 °C.

Apparent Partition Coefficient: To 5 ml of TA-5707F buffer solution (2 μg/ml) was added an equal volume of n-octanol, and after mixture for 1 h at 25 °C, the amount of TA-5707F in the aqeous solution was determined.

\[
\text{apparent partition coefficient} = \frac{B - A}{A}
\]

\[A: \text{final TA-5707F concentration in buffer solution}\]
\[B: \text{initial TA-5707F concentration in buffer solution}\]

Fig. 1. Chemical Structure of TA-5707F

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Table 1. Formulations and Properties of Plain and Wax Matrix Tablets

<table>
<thead>
<tr>
<th></th>
<th>Plain</th>
<th>W-1</th>
<th>W-2</th>
<th>W-3</th>
<th>W-4</th>
<th>W-5</th>
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<tr>
<td>TA-5707F</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>100</td>
</tr>
<tr>
<td>Lactose</td>
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<td>63</td>
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<td>2</td>
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<td>2</td>
<td>2</td>
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<td>23</td>
<td>23</td>
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</tr>
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<td>CMC-Ca</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>9</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Corn starch</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mg stearate</td>
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<td>2</td>
<td>2</td>
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<td>Hardness (kg)</td>
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<td>8.1</td>
<td>8.2</td>
<td>8.8</td>
<td>8.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>190.8</td>
<td>192.0</td>
<td>192.3</td>
<td>191.4</td>
<td>191.0</td>
<td>192.5</td>
</tr>
</tbody>
</table>

CMC-Ca, carboxymethylcellulose Ca; PVP, polyvinyl pyrrolidone; HSD, hydrogenated silicon dioxide.

**Preparation of Wax Matrix Tablets** Six kinds of tablets (including plain tablets) in Table 1 were prepared. The powders of TA-5707F, lactose, K3 wax and PEG6000 were mixed and sieved through a 24-mesh screen. The granules for tabletting were prepared by heating the mixture and melting K3 wax and PEG6000 in a heated planetary mixer at 95°C. After being cooled, the granules were screened through a 24 mesh sieve to remove any oversized caked material. Magnesium stearate was added to the granules, which were then compressed to make the tablets (8 mm diameter). The compression pressure was 0.51 per punch.

**Measurement of Tablet Hardness** The crushing strength of a tablet was determined by the Tablet Tester 60 (Schleuniger Co., Germany).

**Dissolution Studies** The paddle method (100 rpm) and the disintegration method (30 strokes/min, no disk) of the Japanese Pharmacopoeia (JP XII) were applied using a dissolution medium consisting of 900 ml of the first fluid (pH 1.2) and the second fluid (pH 6.8), and at a temperature of 37±0.5°C. The dissolution percentages were determined by monitoring the TA-5707F in the test medium by a UV-240 spectrophotometer (Shimadzu Co., Kyoto, Japan) at 280 nm.

**Animal Experiments** Male beagle dogs, weighing 11—15 kg, were fasted overnight and were used except in the studies to determine the food effect.

**In Situ Study:** The dog was anesthetized with sodium pentobarbital. The absorption for 5 h from the stomach, the duodenum, the jejenum, the ileum and the colon were evaluated by injecting the TA-5707F solution (100 mg/30 ml) into each loop (30 cm in length). The stomach loop was made by ligating the pylorus, the duodenum loop by ligating the distal part from the pylorus, the jejenum loop by ligating the distal part from the duodenum, the ileum loop by ligating the proximal part from the cecum and the colon loop by ligating the distal part from the cecum. After injection of TA-5707F solution into each loop, the plasma concentration of TA-5707F was determined and the AUC was compared with that obtained after intravenous (i.v.) administration.

**In Vivo Study:** TA-5707F aqueous solution or tablet was administered orally with 50 ml of water, or 10 mg of sodium TA-5707F dissolved in 2 ml of 0.9% saline was injected. In the study of the effect of food, the tablet was administered immediately after the ingestion of 150 g of dog food. As for the studies on the effect of the suppression of small intestinal motility, an anticholinergic drug, propantheline bromide (30 mg), was administered orally at 30 min prior to the administration of the tablets. Blood samples were taken with heparinized syringes at appropriate intervals and were immediately centrifuged for 10 min at 3000 rpm to obtain plasma samples. The plasma samples were kept frozen until the HPLC assay.

**Assay of TA-5707F in Plasma** The plasma concentration of TA-5707F was determined by HPLC. To 0.5 ml of plasma were added 1 ml of 1 M potassium dihydrogen phosphate (pH 3.0) and 10 ml of chloroform. After 10 min of shaking and centrifugation at 2000 rpm for 5 min, 8 ml of chloroform was transferred to a glass tube containing 1 ml of 0.01 M potassium dihydrogen phosphate. After shaking and centrifugation again, 0.5 ml of aqueous layer was mixed with 0.02 ml of enzamide solution (100 µg/ml, internal standard) and 75 µl of mixture was injected into the HPLC. The HPLC was done with a Hitachi L6200 pump and detector equipped with an L-4000 UV monitor (Hitachi, Ltd., Tokyo, Japan) using a Hypersil ODS-5µ (250 mm x 4 mm i.d., Chemco Ltd., Osaka, Japan) column with 0.05 M potassium dihydrogen phosphate (pH 3.0)–acetonitrile (75:25) as the mobile phase at a flow rate of 1.0 ml/min with detection at 280 nm. In this assay method, the recovery of TA-5707F from plasma was 90.5% with 1.5% (C.V.) at 2 µg/ml and the detection limit was 0.02 µg/ml.

**Pharmacokinetic Analysis** The maximum plasma concentration (Cmax) and the time to reach the Cmax (Tmax) were determined from the observed plasma concentrations. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The elimination rate constant after oral administration was determined from the slope of the linear phase of log-transformed plasma concentrations. The concentration–time data after i.v. administration were fitted to a two-compartment model by nonlinear least squares regression analysis (MULT8) to obtain the rate constants. The mean dissolution time (MDT) in vivo was calculated from the difference of mean residence time (MRT) after oral administration of the tablet and the aqueous solution. The amount absorbed by time t was calculated according to Eq. 1 assuming that the plasma concentration at any time after oral administration was in the postdistribution phase.

\[ A_t = \frac{C_i}{(1+k_i)} \int_0^t C(t) dt \times V_d \text{area} \]

\[ V_d \text{area} = \text{dose} \times f/k_t/AUC \]

- A_t: the amount of TA-5707F absorbed by time t
- C_i: plasma concentration at time t
- k_t: elimination rate constant
- f: fraction absorbed (f=1)
- V_d \text{area}: volume of distribution according to area after oral administration of aqueous solution
RESULTS AND DISCUSSION

Physical and Biological Properties  TA-5707F dissolved very slightly in the acidic range of pH 1—5, and its solubility increased sharply from pH 4 and reached more than 20 mg/ml at pH 6. The logarithm of apparent partition coefficient (log P') to n-octanol was more than 0 at less than pH 4 and was the highest at pH 3. This means that TA-5707F was lipophilic in the acidic region. It decreased to less than 0 in the neutral pH region (Fig. 2) and showed hydrophobicity.

The plasma concentration after i.v. administration declined in a biphasic elimination manner. The β elimination half life was about 0.6 h. The plasma concentration rapidly increased after the oral administration of aqueous solution, reaching a maximum 15 min after the administration and declining at the same rate with i.v. administration (Fig. 3). The absolute bioavailability after oral administration of the aqueous solution was almost 100% and the mean absorption time calculated from the moment analysis was considerably fast (0.2 h). These findings suggest that the membrane permeability of TA-5707F is not a serious problem and that the release from the preparations in the intestine is the key for maintaining high bioavailability. The absolute bioavailability of TA-5707F evaluated after injection into the loop of the stomach, the duodenum, the jejunum, the ileum and the colon, was approximately 50, 90, 100, 100 and 40%, respectively. Therefore, the maintenance of a sufficient release rate in the colon seemed to be important for the development of a long-acting TA-5707F preparation.

Dissolution Study  Figure 4 shows the release rates of TA-5707F in the first and the second fluids from the wax matrix tablets prepared with various amounts of K3 wax and PEG6000 (Table 1). The comparisons among W-1—W-4 showed that the release rate decreased with an increase the content of K3 wax, and the comparison between W-2 and W-5 showed that PEG6000 increased the release rate. Generally, the release rate in the second fluid was faster than that in the first fluid. The difference in the release rate among the tablets was more obvious with the disintegration method than the paddle method. The reason the former method gave a faster dissolution rate could be that TA-5707F dissolved in the disintegration method in addition to diffusing through the waxy matrix and to the erosion of the waxy materials since the agitation power is stronger than the paddle method. On the other hand, the effect of agitation power with the paddle method was studied by increasing the paddle revolution rate from 50 to 200 rpm. However, the difference in the releasing rate among the preparations was less clear at 50 rpm than at 100 rpm, and the intra-deviation of the determination was greater at 200 rpm, as the tablets were apt to float and rotate, following the dissolution medium flow. TA-5707F seemed to be released in the paddle method mainly by diffusion and not much by erosion, due to the method's weaker agitation power. The comparison of the two methods revealed that the destruction power of the disintegration method was much stronger than that of the paddle method. The disintegration method better reflects the physiological state of the gastrointestinal (GI) tract than the paddle method regarding these TA-5707F tablets.

Plasma Concentration of Wax Matrix Tablets  Figure 5 shows the plasma concentration of TA-5707F after oral administration of the plain tablets and the four kinds of wax matrix tablets to dogs, and Table 2 shows the pharmacokinetic parameters. The T_max was delayed and the C_max was decreased with an increase in K3 wax content in the tablets. The MDT after oral administration to the dogs seemed to correlate well with the MDT obtained by the paddle and disintegration methods, but in the paddle method, the difference of in vivo MDT between W-3 and W-4 was not clear (Fig.6). These findings suggested that even in the slower release tablets, as W-4, erosion of the tablets occurred as a result of the strong stirring strength in the dog intestine. Consequently, the MDT determined by the paddle method did not correspond with that
obtained in the in vivo experiment due to the weak stirring strength of the paddle method in generating erosion. Comparison of the MDT with that obtained in the dogs by the disintegration method suggested that the release rate in the dog intestine was one-third of that obtained by the disintegration method. Figure 7 shows the relationship between the time required for 50% of TA-5707F to be released from the tablets by the disintegration method ($T_{50}$) and the pharmacokinetic parameters. The $T_{\text{max}}$ increased linearly with $T_{50}$, but the AUC and $C_{\text{max}}$ declined in a biphasic manner. The AUCs of plain, W-1 and W-2 tablets were similar, regardless of $T_{50}$, but the AUC decreased significantly in the W-2 to W-4 tablets with an increase of $T_{50}$; the $C_{\text{max}}$ declined sharply in the W-2 tablet and the slope became moderate in the W-2 to W-4 tablets. Figure 8 shows the time course of percent absorption in dogs calculated by the Nelson Wagner method using the individual parameters for each dog. The percent absorption reached 100% 1 h after administration of the plain tablet. The percent absorption of the wax matrix tablets decreased with an increase in $T_{50}$ and the slope of the percent absorption tended to be moderate at 4 or 5 h in the W-3 and W-4 tablets. As the tablets reached the large intestine at these time points, the low absorption of the large intestine was considered to be the cause of the biphasic behavior of AUC and $C_{\text{max}}$. The bending point shown in Fig. 7 might reflect the situation of tablets when they reached the large intestine, namely, the tablets after the disintegration test at about 0.7 h resemble those reaching the large intestine.

**Effect of Food** The W-2 tablet, which had an equivalent AUC with a plain tablet and showed comparatively good plasma concentration profiles, was administered to dogs under the fed condition (Fig. 9). The $C_{\text{max}}$ decreased to one-eighth of that administered under a fasted condition and the plasma concentration was sustained at an extremely low level from 30 min to 7 h after oral administration. Miwa reported that the circadian rhythm

![Figure 4](image-url)

**Table 2. Pharmacokinetic Parameters of TA-5707F after Oral Administration in Dogs (100 mg)**

<table>
<thead>
<tr>
<th></th>
<th>$AUC_{0}$ (µg h/ml)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain</td>
<td>$13.4 \pm 0.5$</td>
<td>$9.2 \pm 0.5$</td>
<td>$0.9 \pm 0.1$</td>
</tr>
<tr>
<td>W-1</td>
<td>$13.4 \pm 0.5$</td>
<td>$7.0 \pm 1.0$</td>
<td>$1.7 \pm 1.1$</td>
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<tr>
<td>W-2</td>
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<td>$4.0 \pm 0.7$</td>
<td>$3.0 \pm 0.4$</td>
</tr>
<tr>
<td>W-3</td>
<td>$8.5 \pm 0.9$</td>
<td>$2.6 \pm 0.2$</td>
<td>$2.9 \pm 0.8$</td>
</tr>
<tr>
<td>W-4</td>
<td>$5.2 \pm 0.8$</td>
<td>$1.6 \pm 0.3$</td>
<td>$4.0 \pm 1.0$</td>
</tr>
</tbody>
</table>

Each value shows the mean ± S.E. of four dogs.

![Figure 5](image-url)
Fig. 6. Correlation of in Vivo and in Vitro Mean Dissolution Time of the Wax Matrix Tablet

In vitro mean dissolution was determined in JP 2nd fluid by the disintegration test method (30 strokes/min) and paddle method (100 rpm).

Fig. 7. Correlation between the Pharmacokinetic Parameters and the Time Required for 50% of TA-5707F to be Released from Wax Matrix Tablets by Disintegration Test Method

Pharmacokinetic parameters were calculated using the plasma concentrations after oral administration of the wax matrix tablet (100 mg). Each value is expressed as the mean ± S.E. of four dogs.

Fig. 8. Time Courses of Percent Absorption in Dogs after Oral Administration of Wax Matrix Tablet (100 mg)

(○) plain tablet; (●) W-1; (□) W-2; (■) W-3; (□) W-4. Each value is expressed as the mean ± S.E. of four dogs.

of gastric motility in dogs fed once a day showed instinct characteristics. That is, the content of the stomach was emptied into the small intestine by vigorous hunger constriction under the fasted condition, while the hunger constriction stopped and weak motility was retained for more than 10 h immediately after the food was ingested. Therefore, under a fasted condition, the tablet was emptied into the intestine easily after administration, but it stayed in the stomach for more than 10 h under the fed condition. These findings suggested that TA-5707F was released out very slowly, due to its low solubility in an acidic medium, from the tablets retained in the stomach for a long time and that a low plasma concentration was sustained under the fed condition. 

Comparing the above result with that of Herbeser®, which contains diltiazem hydrochloride (Dil·HCl) and is prepared by a similar wax matrix method, the effect of food was greatly different. The bioavailability of Herbeser® was not affected much by food.5) This could be because the releasing rate of Dil·HCl in the stomach from Herbeser® was not decreased even if the tablets were retained in the stomach under the fed condition. The in vitro releasing rate in the JP first fluid was not decreased in Herbeser®, while it greatly decreased in the TA-5707F tablets. The difference in the solubilities in the JP first fluid and water (Dil·HCl: more than 50%, TA-5707F: less than 0.05%) would
concentration increased gradually and the level at the 7th hour after administration was higher than that in the untreated dogs. This was considered to have been caused by the prolongation of the small intestinal transit time by the suppression of GI motility. Consequently, the absorption from the W-3 tablet in humans was suggested not to be reduced and the plasma concentration would be sustained since the small intestinal transit time is longer in humans than in beagle dogs.  

The present findings suggest the usefulness of the wax matrix method in the development of controlled release dosage forms of TA-5707F to decrease the dosing frequency for clinical use. However, some problems, such as a presumable fluctuation in plasma levels due to the inter- and intra-variations of gastric pH and GI motility, remain, and should be clarified by a clinical test since the dissolution of TA-5707F from the wax matrix tablets was affected by the stirring strength and pH. These fluctuations might be partly caused by the physicochemical properties of pH dependency of solubility or partly by the difference in the absorption rate at each part of the GI tract. To overcome such defects and develop a more efficient formulation for clinical use, further studies are underway in our laboratory.

### REFERENCES