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Evaluation of Sustained-Release Capsules of Molsidomine (SIN-10) in Dogs and Monkeys[1)]

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The bioavailability and pharmacokinetics of molsidomine (SIN-10) from a capsule containing wax-coated beads, were compared with those from a conventional tablet in dogs and monkeys during the formulation study of a sustained release dosage form of SIN-10. Sustained-release capsules (SR capsules) A, B and C contained coated beads with different amounts of wax, and SR capsule D contained 20% immediate release beads and 80% wax-coated beads. In dogs, SR capsules A and B did not satisfactorily prolong the effective plasma concentration. With capsule C, although satisfactory maintenance of effective plasma concentration was obtained, the bioavailability was only to half of that after administration of a conventional tablet. On the other hand, in monkeys, reasonable prolongation of effective plasma concentration (depending upon the amount of wax coating) with satisfactory bioavailability was obtained for each SR capsule. With an SR capsule D, more than 12 h duration of effective plasma concentration of molsidomine and control of blood pressure from immediately after administration of the capsule were achieved in monkeys.

Keywords——molsidomine; sustained release; wax-coated beads; beagle dog; cynomolgus monkey; bioavailability; blood pressure

Molsidomine (SIN-10), a vasodilator, has been used in the prophylaxis and treatment of angina pectoris as an immediate release tablet[2–5)] (Morial tablets, Takeda Chemical Industries Ltd.). As a next step, the development of a sustained-release preparation was expected to result in prolonged maintenance of the drug concentration within the therapeutic range for prophylaxis, and to avoid a rapid increase immediately after administration and hence prevent side effects such as headache or vertigo.

Several types of oral sustained release preparations have been reported; barrier-coated tablets,[6)] hydrophilic matrix tablets,[7,8)] wax matrix tablets,[9–11)] and capsules containing wax-coated beads[12)] or polymer-resin-coated beads.[11,13)] In this study, the capsule containing wax-coated beads was chosen, since drug release and transit time in the gastrointestinal tract (GI tract) of a single-unit type sustained release preparation may remarkably vary markedly between patients and be influenced by food intake[14)] as compared with a multiple-unit type preparation.

As previously reported,[15)] SIN-10 is absorbed from all parts of the rat small intestine, and the absorption site of SIN-10 is broad enough to justify the use of sustained release preparations of SIN-10.

In this work, the relationship of the amount of wax coating with the duration of effective plasma level of SIN-10 and its metabolite (SIN-1C in Chart 1)[16)] was studied in dogs and monkeys.

Experimental

Preparation of Sustained Release Capsules (SR Capsules)——Nonparails were coated with SIN-10 and powdered

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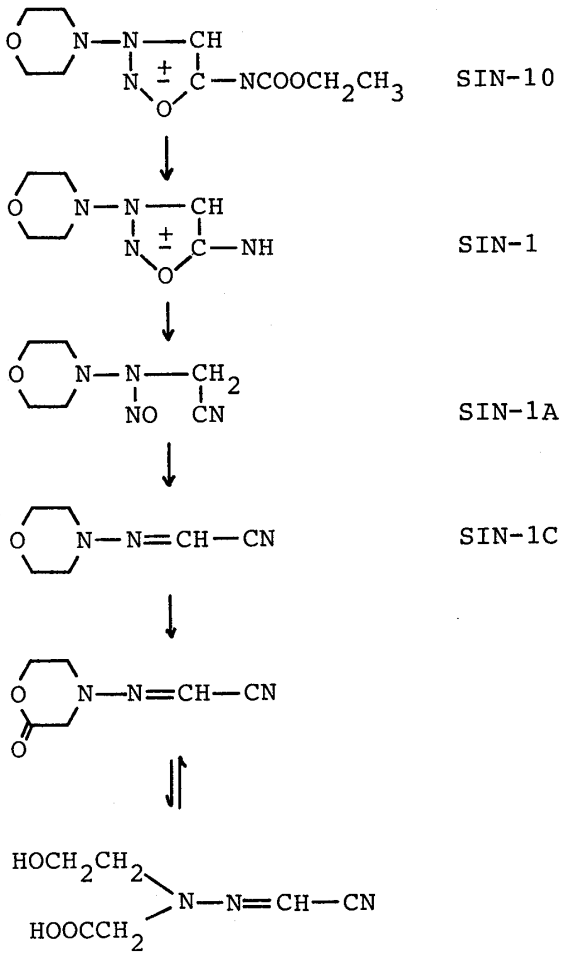

Chart 1. Possible Metabolic Pathway of SIN-10 in Rats

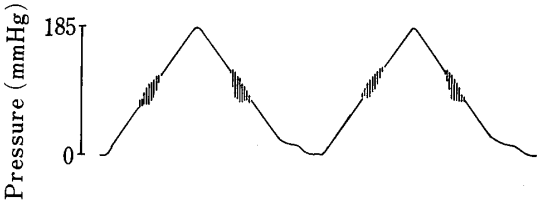

Fig. 1. The Pulses from the Paw Artery of Monkeys Recorded on a Polygraph

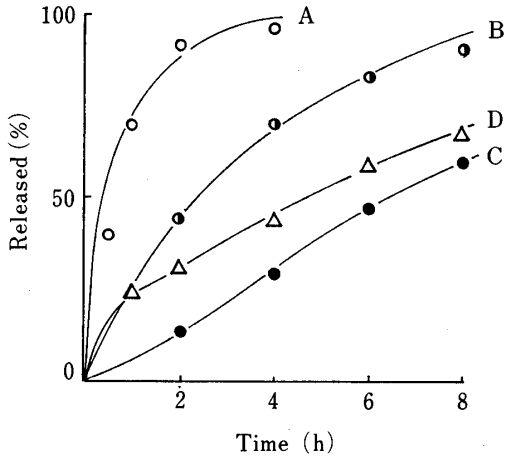

Fig. 2. *In Vitro* Releases of SIN-10 from SR Capsules A (○), B (◑), C (●), and D (△) as Determined by the Rotating Basket Method

sugar under a water spray, and dried to make drug-coated beads. The drug-coated beads were then coated with wax, which mainly consisted of paraffin, in a centrifugal fluidizing granulator (CF).[17] The capsules were filled with wax-coated beads, A, B, and C, each with different amounts of wax; they are termed SR capsules A, B, and C, respectively. An additional SR capsule, which was termed SR capsule D, containing 20% uncoated immediate release beads and 80% wax-coated beads C, was also prepared.

***In Vitro* Release Studies**——The release of SIN-10 from these SR capsules was measured by the JP X rotatory basket method. A capsule was placed in a basket with a 36 mesh screen. The basket was rotated at 100 rpm in 500 ml of pH 2.2, 5.0, or 8.0 phosphate buffer solutions at 37 °C. The fluid was sampled at suitable intervals and the concentration of SIN-10 was measured by ultraviolet (UV) spectrophotometry at 310 nm to obtain cumulative percent dissolved-time profiles.

Beagle Dogs——Three male beagle dogs weighing 10.9, 11.1, and 14.1 kg were fasted for 24 h; they were allowed to take water *ad libitum*. An SR capsule containing 4 mg of SIN-10 was administered orally to each dog with 50 ml of water. Blood samples were collected into heparinized syringes at suitable intervals and plasma SIN-10 concentrations were determined by the method of Koyama *et al.*[18] Two conventional tablets each containing 2 mg of SIN-10 were administered orally as a standard preparation to compare the time course of plasma concentrations with those obtained from the SR capsules.

Cynomolgus Monkeys——Because of their wild nature, the cynomolgus monkeys used in this study exhibited remarkable intersubject variation in absorption characteristics. As a preliminary study, twelve females were given a conventional tablet after fasting for 24 h and the time courses of plasma concentration were obtained. Then six monkeys, which exhibited particularly rapid absorption, weighing 2.51, 2.69, 2.97, 2.62, 2.34, and 2.83 kg, were selected out of the twelve and randomly divided into two groups. One group was used for the bioavailability study, and the other for the pharmacodynamic study. In the bioavailability study, each monkey, after fasting for 24 h, was given orally either an SR capsule containing 2 mg of SIN-10 or a tablet with 10 ml of water. Blood samples were collected at suitable intervals and plasma SIN-10 concentrations were determined by the same method as was used in

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Table I. *In Vitro* Release of SIN-10 from SR Capsule B in McIlvaine Buffer Solutions

Time (h)	pH		
	2.2	5.0	8.0
2	35.8	35.4	38.6
4	64.2	63.6	69.3
6	79.2	76.8	85.1
8	88.7	86.9	93.1

% released by rotatory basket method (JP X, 100 rpm).

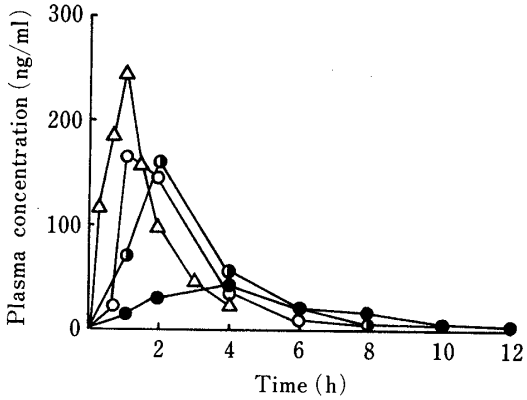

Fig. 3. Plasma SIN-10 Concentrations after Oral Administration of Tablets (△), and SR Capsules A (○), B (◑), and C (●) in Dogs

Table II. Mean(±SEM) Plasma Concentrations of SIN-10 in Dogs after Oral Administration of Tablets and SR Capsules

Time (h)	Tablet	SR capsule		
		A	B	C
0.33	115.5±82.1			
0.5		21.2±12.0		
0.66	183.0±72.2			
1	244.9±38.0	166.9±68.1	68.6±31.4	15.1±4.8
1.5	156.1±26.3			
2	101.7±15.8	146.7±40.5	161.8±40.6	30.4±7.8
3	43.1±10.0			
4	22.0± 6.7	35.6±18.5	53.4±16.0	37.5±7.4
6		10.5± 4.6	17.7± 9.7	20.3±2.8
8		3.7± 3.5	3.9± 1.5	12.9±2.2
10			1.3± 1.3	6.8±1.8
12			0.2± 0.2	5.3±0.8

ng/ml.

the dogs. In the pharmacodynamic study, and SR capsule D containing 3 mg of SIN-10, or a conventional tablet, was administered to each monkey, and the plasma concentrations of SIN-10 and SIN-1C were determined. The urinary recoveries of SIN-10 and SIN-1C in 24 h were also determined in the same way as described for the plasma concentrations. Pulses from the paw artery were measured as blood pressure (BP) readings[19] using a cuff with a microphone and an electronic sphygmomanometer (NARUCO, PE-300), when the blood samples were collected. The pulses were recorded using a polygraph (Nihon Koden). Systolic BP was determined as the upper end and diastolic BP as the lower end of the pulses, as shown in Fig. 1.

Results

In Vitro Release of SIN-10

Figure 2 shows the release of SIN-10 from SR capsules A, B, and C in a pH 5 phosphate buffer solution. The release was retarded by an increase in the amount of wax coating, and the times necessary for 50% release from SR capsules A, B, and C were 0.4, 2.2, and 6.4 h, respectively. The release was not influenced by the pH of the medium in the pH range of 2.2—8.0, as summarized in Table I.

Beagle Dogs

The time courses of plasma concentration after administration the tablets or the SR

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TABLE III. Pharmacokinetic Parameters in Dogs

	Tablet	SR capsule		
		A	B	C
C_{max} (ng/ml)	244.9	166.9	161.8	37.5
t_{max} (h)	1	1	2	4
AUC (ng·h/ml)	410.7	455.4	464.4	210.0
k_a (h^{-1})	2.07	1.83	0.71	0.32
k_e (h^{-1})	1.04	0.76	0.53	0.57

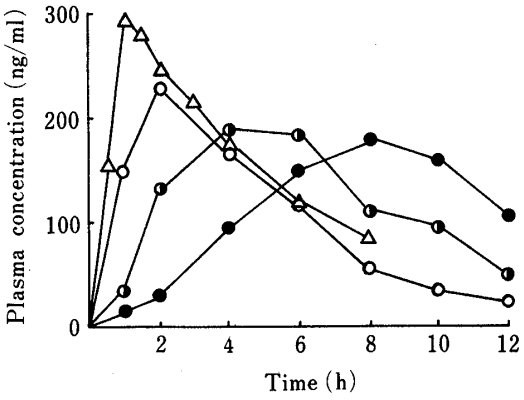

Fig. 4. Plasma SIN-10 Concentrations after Oral Administration of Tablets (△), and SR Capsules A (○), B (◑), and C (●) in Monkeys

TABLE IV. Mean(±SEM) Plasma Concentrations of SIN-10 in Monkeys after Oral Administration of Tablets and SR Capsules

Time (h)	Tablet	SR capsule		
		A	B	C
0.5	152.6± 80.5			
1	292.2±128.9	148.6±72.1	37.0±12.2	16.1± 9.5
1.5	279.5± 81.5			
2	242.8± 60.1	231.6±72.3	135.6±80.0	32.4±12.5
3	213.0± 36.7			
4	175.8± 26.6	163.5± 2.4	190.4±38.8	96.6±34.4
6	121.8± 8.1	118.9±18.8	187.3±58.8	152.8± 8.7
8	86.2± 23.0	54.3± 9.4	110.6±38.2	179.7±12.8
10		34.3± 7.7	92.7±35.9	158.4±16.2
12		24.3± 8.6	50.1±21.9	106.4±18.7

ng/ml.

capsules are shown in Fig. 3 and Table II. After the tablets were administered, the plasma concentration rose rapidly and reached the maximum within 1 h. Absorption of SIN-10 from the SR capsules was slower than that from tablets. The times required to reach the maximum concentration (T_{max}) after the administration of SR capsules A, B, and C were 1, 2, and 4 h, respectively. T_{max} was delayed and the maximum plasma concentrations (C_{max}) were lowered as the amount of wax coating increased. In particular, in the case of SR capsule C, C_{max} and area under the blood concentration curve (AUC) were lowered to 15.3% and 51.5% of those of the tablets, respectively, whereas the AUCs for SR capsules A and B did not differ from that of the tablets. Pharmacokinetic parameters calculated on the basis of a single compartment open model are summarized in Table III.

Cynomolgus Monkeys

Bioavailability Study——The time courses of plasma concentration after administration of a tablet and SR capsules A, B, and C are shown in Fig. 4 and Table IV. Plasma concentration after SR capsules were administered rose gradually as the amount of wax coating increased; T_{max} were 2, 6, and 8 h, and C_{max} were 231.6, 190.4, and 179.7 ng/ml, respectively. AUCs were observed to be constant irrespective of the amount of wax coating. The apparent absorption rate constant (k_a) and the elimination rate constant (k_e) were

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TABLE V. Pharmacokinetic Parameters in Monkeys

	Tablet	SR capsule		
		A	B	C
C_{max} (ng/ml)	292.2	231.6	190.4	179.7
t_{max} (h)	1	2	4	8
AUC (ng·h/ml)	1355.8	1262.4	1452.6	1346.2
k_a (h^{-1})	6.01	1.38	0.42	0.11
k_e (h^{-1})	0.19	0.25	0.23	0.14

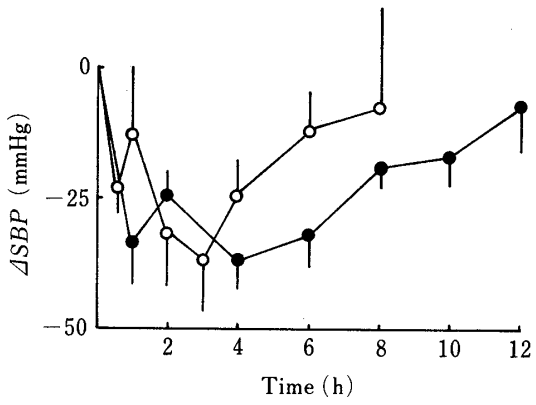

Fig. 5. The Reductions of Systolic Blood Pressure (ΔSBP) after Oral Administration of the Tablet (○) and SR Capsule D (●) in Monkeys

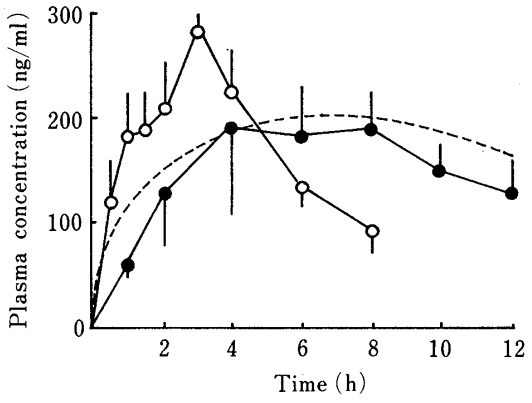

Fig. 6. Plasma SIN-10 Concentrations after Oral Administration of the Tablet (○) and SR Capsule D (●) in Monkeys

The broken line shows estimated plasma concentration after the administration of SR capsule D.

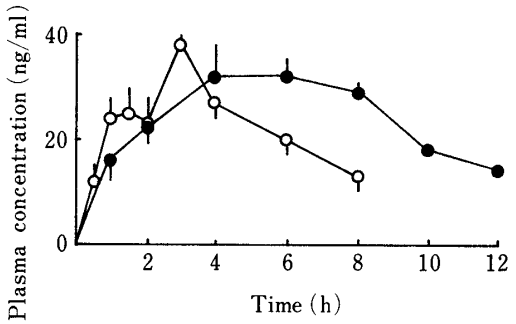

Fig. 7. Plasma SIN-1C Concentrations after Oral Administration of the Tablet (○) and SR Capsule D (●) in Monkeys

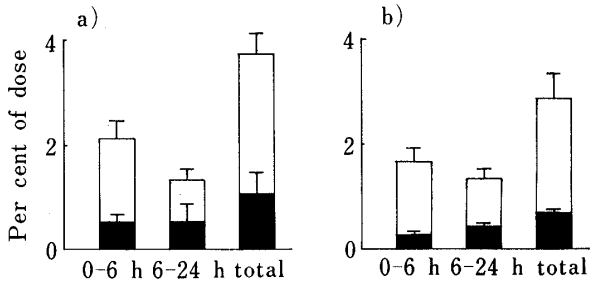

Fig. 8. Urinary Recovery of SIN-10 (■) and SIN-1C (□) after Oral Administration of the Tablet (a) and SR Capsule D (b) in Monkeys

calculated on the basis of a single compartment open model and are summarized in Table V. The k_a value was dependent on the amount of wax coating, but the k_e value was independent of it and was about $0.2\,h^{-1}$ for all preparations.

Pharmacodynamic Study——The vasodilative effect of SIN-10 is mainly due to the active metabolites, SIN-1 and SIN-1A,[19] in Chart 1. Unfortunately, the metabolic rates of SIN-10 to SIN-1A and SIN-1A to inactive SIN-1C are so fast that only SIN-10 and SIN-1C among the metabolites shown in Chart 1 could be assayed. Therefore, in this study, BP, instead of the active metabolites, was measured as an indicator of therapeutic effect. The time course of BP drop after administration of a tablet or an SR capsule D is shown in Fig. 5. Since similar time courses of drop in systolic and diastoric blood pressures were observed, only systolic blood pressure drop (ΔSBP) is shown as a therapeutic indicator in this paper. Blood pressure fell

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immediately after the administration, and ΔSBP was maintained at -30 mmHg for 6 h, but then gradually recovered. Similar time courses of plasma SIN-10 and SIN-1C concentration were observed after the administration (Figs. 6 and 7), but a higher plasma concentration of SIN-10 than of SIN-1C was maintained for 12 h. As shown in Figs. 5, 6, and 7, both ΔSBP and an effective plasma concentration were maintained for more than 12 h; remarkably prolonged action was seen as compared with the tablet. The values of per cent of the dose of SIN-10 and SIN-1C recovered in the urine within 24 h were 1.04 and 2.68 for the tablet, and 0.64 and 2.20 for the SR capsule (Fig. 8), respectively. No difference between the tablet and SR capsule with respect to the urinary excretion of SIN-10 and SIN-1C was observed. More than 95% of the administered SIN-10 was excreted as further metabolites, as shown in Chart 1.

Discussion

Before the *in vivo* studies were conducted, it was confirmed that the release rates of SIN-10 from SR capsules *in vitro* were not influenced by change in the pH of the medium (Table I). This finding indicates that the preparations should release the drug evenly in the GI tract during transit. In the *in vivo* studies, evaluation of the bioavailability of the SR capsules was carried out in two kinds of experimental animals, beagle dogs and cynomolgus monkeys. In dogs (Fig. 3), satisfactory prolongation of an effective plasma concentration was not observed; in particular, the AUC after administration of SR capsule C was only about a half of that in the case of SR capsule A or B, or the tablets (Table II). Two reasons for the decrease of AUC in the case of SR capsule C can be considered. First, the SR beads dispersed from the capsule may have passed through the absorption site (small intestine) before the release of SIN-10 was completed. Second, the elimination rate constant of SIN-10 in dogs may be so large and the absorption rate constant (or release rate) so small that the plasma concentration can not be maintained at an effective level.

In monkeys, it was observed that T_{max} was delayed with increase in the amount of wax coating. Plasma concentration was satisfactorily prolonged and maintained for 12 h, and no decrease in AUC was found even after SR capsule C was administered.

Since, in this study, a remarkable difference in the duration of effective plasma concentrations was found between dogs and monkeys, especially in the case of SR capsule C administration, the authors reviewed published comparisons of this biological parameter in dogs, monkeys, and humans. Many workers[20-23] have reported recently on GI motility in dogs, monkeys and humans. However, most of these studies used saline, water or test meals, and only a few used a solid dosage form. The reported values for gastric emptying time (TG_{50}) after saline or water administration in the fasted state were used as a measure of GI motility. The TG_{50} values in dogs and monkeys were 2—5[20] and 15[21]—30[22] min, respectively. Malagelada *et al.*[23] reported that TG_{50} after saline administration to human beings was 12—24 min. These studies indicate that GI motility in monkeys and human is slower than that in dogs.

In the present study, the values of elimination rate constant of SIN-10 in dogs, monkeys, and humans after tablet administration were 1.04, 0.19, and 0.36 h^{-1},[24] respectively. Since both the GI motility and pharmacokinetic behavior of SIN-10 in humans resemble those in monkeys more than those in dogs, monkeys seem to be preferable to dogs for estimating the behavior of SIN-10 sustained release preparations in human. Based on the results of the bioavailability studies, the preferred dosage form for clinical use should be an SR capsule containing a mixture of immediate release and SR beads to provide a reasonable plasma concentration and a therapeutic effect immediately after administration. Lee and Robinson[25] reported in their review that the optimum ratio of sustaining dose to immediate release dose was 2.77—4.16 if the duration of release was intended to be 8—12 h. We chose 4.0

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as the ratio, and the SR capsule containing a mixture of 20% noncoated immediate release beads and 80% SR beads C (SR capsule D) was selected as a dosage form for clinical use. The estimated plasma SIN-10 concentration after SR capsule D administration was calculated from the plasma concentration after the administration of a tablet (as immediate release beads) and SR capsule C in Fig. 4, and is shown in Fig. 6 as a broken line. After SR capsule D was administered, ΔSBP and an effective plasma concentration were sustained for 12 h as shown in Figs. 5, 6, and 7, and the plasma concentration coincided well with that estimated.

In this study, a remarkable inter-species variation of plasma concentration between dogs and monkeys was observed. At present, the inter-species differences between humans and these two animals are not clear. Forthcoming clinical studies should clarify the situation.

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