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In vivo performance of time-controlled explosion system (TES) in GI physiology regulated dogs

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Abstract

In vivo oral absorption study of time-controlled explosion system (TES), using gastrointestinal (GI) physiology regulated dogs, was carried out to predict the feasibility in humans. TES is characterized by rapid drug release with a pre-programmed lag time, which can provide a programmed release system synchronized with circadian rhythm (e.g. asthma attack in the morning), a colon targeting system and a sustained release system with different lag times. In this study, TES containing diclofenac sodium with different lag times of 3 and 6 h (TES-3h and TES-6h) were prepared. TES-3h exhibited good performance in all six GI physiology regulated dogs without remarkable reduction of AUC. In the case of TES-6h, drug absorption was observed $\sim$6 h after administration in four of six dogs, but plasma level was low. Further, the location of the dosage forms after oral administration was estimated from the gastric emptying time (GET) and the small intestinal transit time (SITT) using a double marker method. As a result, in vivo performance of TES correlated with the intestinal location. It was concluded that TES-3h would perform well in humans and that the environmental water content in the GI tract affected the in vivo dissolution profile of TES when the drug release was initiated after entering the colon. © 1998 Elsevier Science B.V.

Keywords: Time-controlled explosion system (TES); Diclofenac; Gastrointestinal transit

1. Introduction

For most oral pharmaceutical products, the bioavailability study, using dogs, has been carried out to predict the human absorption. In studying the sustained drug release dosage forms, however,

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such a dog study cannot predict the human absorption behavior due to many physiological differences between humans and dogs, e.g., gastric pH, the length of intestine and aqueous moieties in colon (Aoyagi et al., 1982; Ogata et al., 1982; Dressman, 1986). In order to overcome such problems, Sagara et al. (1992) has developed the GI physiology regulated dog, whose gastric pH, gastric emptying time (GET) and small intestine transit time (SITT) behaves almost identically to those of the human.

Recently, we have been developing a novel controlled drug release system named time-controlled explosion system (TES) (Ueda et al., 1994a). TES consists of four separate layers of seed, drug layer, swelling agent layer and water insoluble membrane. In this system, a rapid drug release is initiated by destruction of outer membrane. The lag time is precisely programmed by changing the outer membrane thickness. As the destruction of the outer membrane is caused by the water uptake of the swelling agent, the lag time is independent of the physicochemical properties of the encapsulated drug.

Previous dog studies (Ueda et al., 1994b) demonstrated that the TES containing metoprolol performed well in vivo in all regions from the small intestine to the colon. In vivo drug release profile was evaluated directly from the percent recovery in the dog intestine. However, a bioavailability study exhibited the reduction of absorption with longer lag time. Because TES is a multi particle system, the membrane destruction time showed wider distribution according to the longer lag time (Ueda et al., 1994a). As a result, the apparent drug dissolution rate was reduced and the non-linear first pass effect would be magnified.

The aim of this study was to predict the feasibility of TES inside human alimentary tract. For this purpose, TES containing diclofenac sodium (diclofenac TES) was produced. Diclofenac sodium was used as a model drug, since it is known to be absorbed constantly from the upper to the lower intestine (Gleiten et al., 1985) with a linear hepatic first pass effect (Tsunoo et al., 1989). Also, lag times of diclofenac TES were adjusted to be 3 and 6 h. This is because the combined formulation of TES-3h, with a rapid release formulation, can easily provide twice-a-day dosage form for the drugs which need q.i.d. administration. In the meantime, TES-6h provides the colon targeting system, since the colon arrival time of the dosage forms after oral administration was reported around 4–5 h (Davis et al., 1986). After oral administration of diclofenac TES to the GI physiology regulated dogs, the drug absorption profiles were compared with that of the conventional tablet. In addition, the relationship between the performance of dosage form and the location of TES in the GI tract was investigated by measuring GET and SITT using the conventional double marker method (Mizuta et al., 1990).

2. Materials and methods

2.1. Materials

Diclofenac sodium was purchased from Yonezawa-Hamari Chem (Japan). Conventional preparation containing 25 mg of diclofenac sodium (Voltaren® tablet) was purchased from Japan Ciba-Geigy (Hyogo, Japan) for the reference. Nonpareil® 103 (Freund Industrial, Japan) was used as a seed particle onto which drug was adhered. Low-substituted hydroxypropylcellulose (L-HPC®, grade LH-31, Shin-etsu Chemicals, Japan) was used as a swelling agent for TES. Ethylcellulose (Etocel® 10cps, Dow Chem, Japan) was coated as a water insoluble polymer membrane. Hydroxypropylmethylcellulose (TC-SRW®, Shin-etsu Chemicals) was used as a binding agent. Pentagastrin and atropine sulfate were purchased from Sigma (St. Louis, MO) for the GI physiology regulation. Other chemicals were of analytical grade and used without further purification.

2.2. Preparation of diclofenac TES

A total of 500 g of nonpareil® 103 was placed into the coating pan of a centrifugal granulator (CF-360, Freund Industrial, Japan) rotating at 100 rpm. A total of 1000 g of diclofenac sodium powder was continuously added with spraying of

5% hydroxypropylmethylcellulose (HPMC) binding solution A (a mixture of ethylalcohol and water, 4/1 v/v). Next, 500 g of these drug loaded seeds were placed into the coating pan rotating at 100 rpm. Also, 1000 g of presieved (< 500 μm) L-HPC® (grade LH-31) was continuously added with spraying of 5% (w/v) HPMC binding solution B (a mixture of ethylalcohol and dichloromethane, 4/1 v/v), to obtain the core particles of TES. Finally, using a fluid bed granulator (Flow-Coaler Mini, Freund Industries, Japan), 6% ethylcellulose (EC)/talc suspension was sprayed onto these core particles of TES to make the water insoluble polymer membrane, where EC was dissolved in a mixture of ethylalcohol and dichloromethane (4/1, v/v) and talc was added at a weight ratio 1/1 to EC. At each process, the solvent was evaporated to dryness for 24 h in a vacuum oven, heated at 40°C.

2.3. In vitro drug dissolution study

The diclofenac release profile from diclofenac TES was investigated according to the paddle method of the Japan Pharmacopoeia (JP) XIII. Diclofenac TES (185 mg, c.a. 200 particles), which contained 25 mg of diclofenac sodium, was filled in JP No. 2 gelatin capsule. Nine hundred ml of distilled water was maintained at 37°C and stirred at 100 rpm. Dissolved didofenac was monitored by an automatic dissolution test apparatus equipped with an UV diode array spectrophotometer (Hewlett Packard, Model 8451A) at a wavelength of 275 nm. All the experiments were carried out in triplicate and the mean value was plotted as a function of time.

2.4. In vivo drug absorption study in dog

Six male beagle dogs weighing between 9 and 13 kg were used. Each dog was fasted overnight prior to and 8 h after taking a dosage form. Water was given ad libitium. The GI physiology was regulated with a combined treatment of intramuscular injection of pentagastrin (10 mg/kg $\times$ 2, with a 45 min interval) with intravenous injection of atropine sulfate (0.02 mg/kg) according to Sagara's method. Conventional preparation (Voltaren® tablet) and diclofenac TES (No.2 capsule) were orally administered to the normal (non-treated) dogs and to the GI physiology regulated dogs, with coadministration of 40 ml water.

Blood samples were withdrawn at 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 h after oral administration for the tablet, at 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 14 h for TES-3h and at 5, 5.5, 6, 6.5, 7, 8, 10, 12 and 14 h for TES-6h. They were centrifuged at 14 000 rpm for 5 min to separate the plasma. The plasma samples were kept frozen at -20°C until assay.

2.5. Assay for the plasma concentration of diclofenac

A total of 50 μl of plasma sample was vortexed with 200 μl of methanol and then cooled at -20°C for 30 min. The sample was centrifuged at 14 000 rpm for 5 min. Then, 20 μl of the supernatant was injected into the HPLC system. The HPLC system was equipped with a Capcell Pak C18 SG120 column (4.6×150 mm, Shiseido, Japan) and a Waters model 468 UV spectrophotometer set at 275 nm. The mobile phase consisted of a mixture of 0.1 M ammonium acetate (pH 4.5) and acetonitrile (2/3, v/v). The flow rate was maintained at 1.0 ml/min. The detection limit of assay was 0.02 μg/ml.

2.6. Measurement of GET and SITT in dog

GET and SITT were measured by a double marker method (Mizuta et al., 1989), using acetoaminophen (AAP) and salicylazosulfapyridine (SASP) as marker compounds. This measurement was conducted separately from the absorption study, since the day-to-day variation in GET and SITT of beagle dogs was small. A total of 20 ml of aqueous solution, in which 200 mg of AAP was dissolved and 250 mg of SASP was suspended, was administered to dogs with a zonde cannula Consecutively, another 20 ml of water was administered using the same method. Blood samples were withdrawn at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 h after administration. Mean absorption time of AAP was defined as the GET, the first appearance of sulfapyridine (SP) in plasma was defined as a colon

arrival time (CAT), and SITT was obtained by subtraction GET from CAT.

3. Results

3.1. In vitro dissolution study of diclofenac TES

The structure of TES-3h and TES-6h is illustrated in Fig. 1, and their characteristics are summarized in Table 1. The lag time is controlled by the EC/talc membrane thickness. In vitro drug release profiles of each sample at 37°C are shown in Fig. 2. The lag time of TES was tentatively defined as the time when 5% of the diclofenac had dissolved. The lag time of TES-3h and TES-6h were 3.3 and 6.1 h, respectively. Complete dissolution was observed at $\sim$2.5 h after the lag time for TES-3h and about 3.5 h for TES-6h.

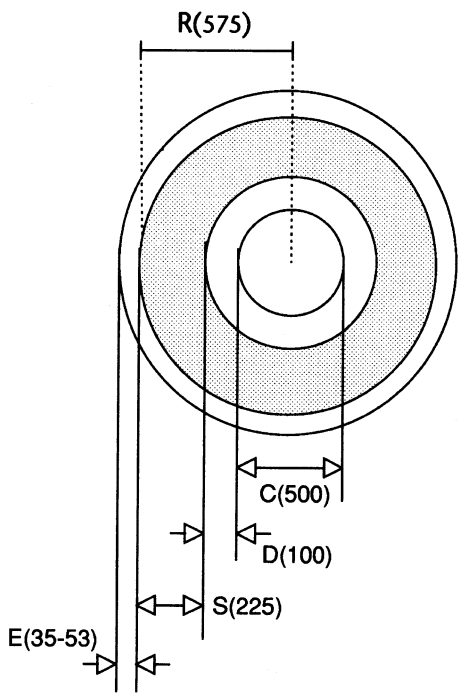

Fig. 1. Schematic view of structure and composition of TES preparation. (C, core; D, drug; S, swelling agent; E, EC membrane).

Table 1
Characteristics of diclofenac-TES

Component	Total diameter (μm)	Layer thickness (μm)
Core (nonpareil 103)	500	—
Drug layer (diclofenac Na)	700	100
Swelling layer (L-HPC)	1150	225
EC/talc membrane		
(TES-3h)	1219	35
(TES-6h)	1255	53

3.2. In vivo drug absorption study using normal dogs

Fig. 3 shows the time courses of mean and individual plasma diclofenac concentration after oral administration of each preparation to normal (nontreated) dogs. Pharmacokinetic (PK) parameters are summarized in Table 2. In the case of TES-3h, good performance was observed in two out of six dogs. Diclofenac absorption started about 3 h after administration, similarly to the in vitro dissolution pattern and no reduction was observed in the bioavailability (BA). However, poor absorption was observed for TES-6h

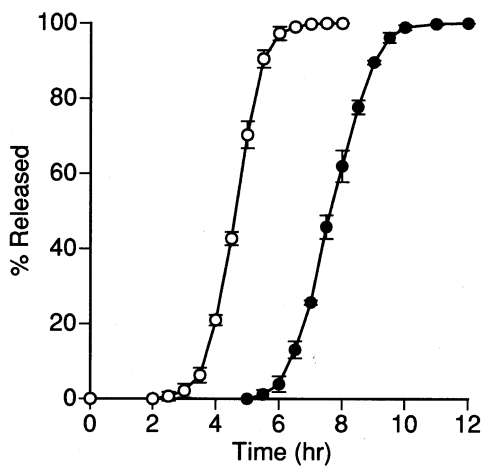

Fig. 2. In vitro drug release profile of TES-3h (○) and TES-6h (●) containing 25 mg of diclofenac sodium in distilled water at 37°C. Each study was carried out in triplicate and each point represents mean ± S.D.

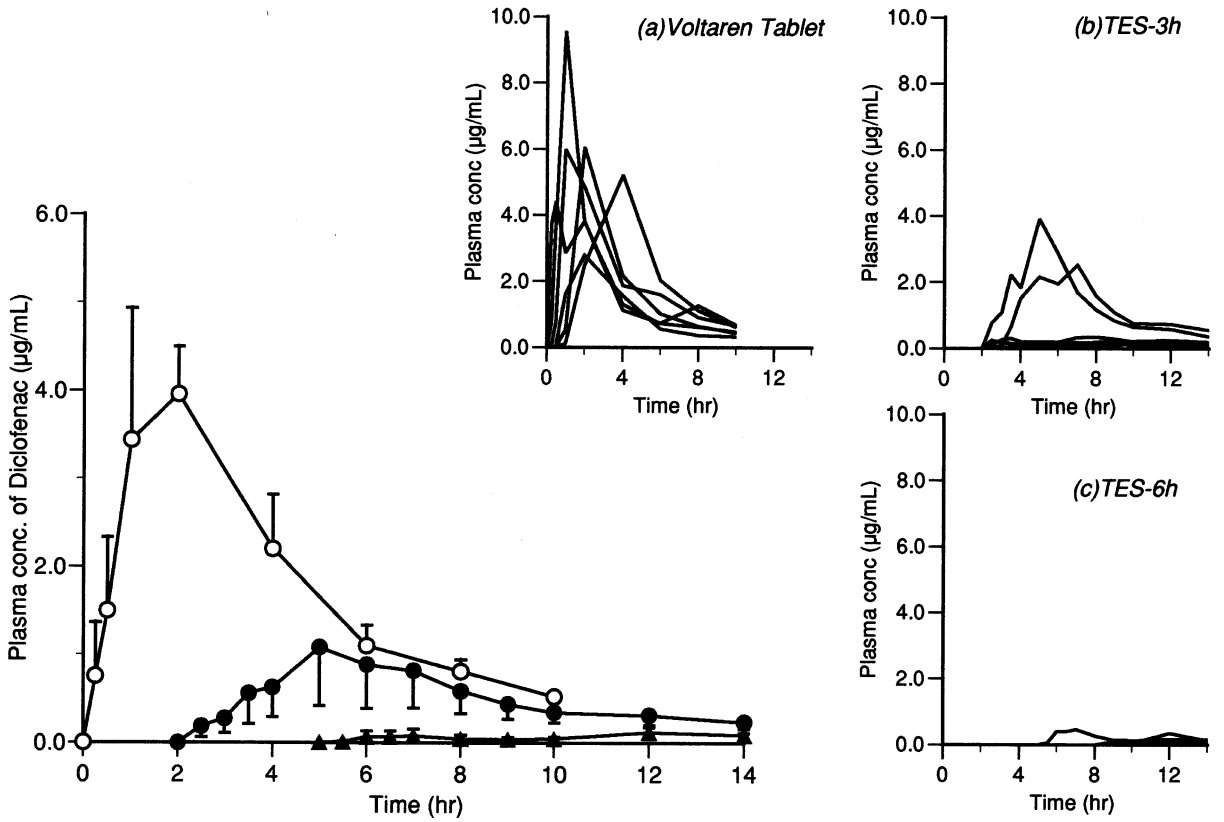

Fig. 3. Time course of mean and individual plasma concentration of diclofenac after oral administration of Voltaren tablet (○, a), TES-3h (●, b) and TES-6h (▲, c) to normal dogs at a dose of 25 mg/body.

3.3. In vivo drug absorption study using GI physiology regulated dogs

Fig. 4 shows the time courses of plasma diclofenac concentration in GI physiology regulated dogs. PK parameters are summarized in Table 3. TES-3h exhibited a good absorption performance in all dogs. The relative BA was 80.1% as compared with the absorption of the conventional tablet. The mean C_{max} was also about 80% as compared with the tablet. In the case of TES-6h, drug absorption was observed around 6 h after administration in four out of six dogs, but the plasma concentration was extremely low and the relative BA was also small as compared with the tablet.

3.4. GET and SITT of dogs

The GET and the SITT of dogs measured by double maker method were listed in Table 4. The mean GET of non-treated and GI physiology regulated dogs were 0.27 and 0.50 h, respectively. The mean SITTs were 3.4 h (ranged from 1.8 to 5.4) and 4.5 h (ranged from 4.0 to 5.7) for non-treated and GI physiology regulated dogs, respectively. These results indicated that the SITT was well regulated using the combined treatment of

Table 2
Pharmacokinetic parameters after oral administration of diclofenac preparations to normal dogs

Preparation	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0\text{-}n}$ (μg/ml·h)	MRT (h)	BA_{rel} (%)
Voltaren tablet	5.66 ± 0.92	1.8 ± 0.5	18.00 ± 1.64	3.5 ± 0.3	100
TES-3h	1.23 ± 0.65	7.4 ± 1.4	6.16 ± 2.86	8.1 ± 0.5	31.5
TES-6h	0.18 ± 0.08	—	0.60 ± 0.28	—	3.0

Mean $\pm$ S.D., $n = 6$.

pentagastrin with atropine. These values were similar to previously published data (Mizuta et al., 1989; Sagara et al., 1992).

4. Discussion

In this study, TES-3h and TES-6h were first administered to the normal (non-treated) dogs. Previous dog studies (Ueda et al., 1994b) using the normal dogs demonstrated that the TES containing metoprolol performed its drug release function in all regions from the small intestine to the colon. Also, diclofenac sodium is known to be absorbed constantly from the upper to the lower intestine (Gleiten et al., 1985) and does not exhibit a non-linear hepatic first pass using this study dose (Tsunoo et al., 1989). However, the results show that diclofenac TES did not perform well in vivo concerning the oral absorption, in comparison with that of the conventional tablet. Since the destruction of the outer membrane is independent of the physicochemical properties of the encapsulated drug, the difference between the two experiments is due to the solubility and/or the dissolution rate of the two drugs, i.e. > 100 mg/ml for metoprolol tartarate and 1.2 mg/ml for diclofenac sodium in JP 2nd fluid, respectively. For the less soluble drugs, the drug release rate may be governed by the water content in the GI tract. The liquid flow in duodenum, jejunum, ileum and colon of humans were reported as 3–5, 1.5–2, 0.7–1.2 and 0.1 l/day (Hirtz, 1985), respectively. For this reason, the location of the dosage form in GI tract must be critical to predict the performance of the dosage form. In fact, the measurement of GET and SITT supported the TES-3h results, in which the system performed well only in the non-treated dogs with a longer SITT (Dogs 2 and 4).

When using the GI physiology regulation, which prolonged the SITT beyond 4.0 h in all dogs, TES-3h exhibited good performance in all dogs without remarkable reduction of AUC. Drug absorption started about 3 h after administration and exhibited the same C_{max} and AUC in comparison with the tablet. This suggested that the SITT strongly affected the performance of TES in the GI tract. In the case of TES-6h, drug absorption started around 6 h after administration in four out of six dogs, but the plasma concentration was low. This implied that the destruction of outer membrane occurred in all dogs but the drug dissolution was not completed.

These results, together with the previous metoprolol study, suggested that the location of the dosage form, where drug release was initiated, was critical in the case of drugs with low solubility. This is because the in vivo drug dissolution may be limited by small amounts of water around the dosage forms when the outer membrane is destroyed after entering the colon. Narisawa et al. (1995) also demonstrated that in the case of controlled release preparations, the drug solubility strongly affected the dissolution rate in the lower site of canine GI tract. From the physiological point of view, this dog study could not predict the whole feasibility of TES-6h in humans, since the water content in the colon is relatively rich in humans as compared to dogs, especially in ascending colon (hashed) and transit colon (semi solid).

5. Conclusion

From this study using GI physiology regulated dogs, it was suggested that TES-3h containing

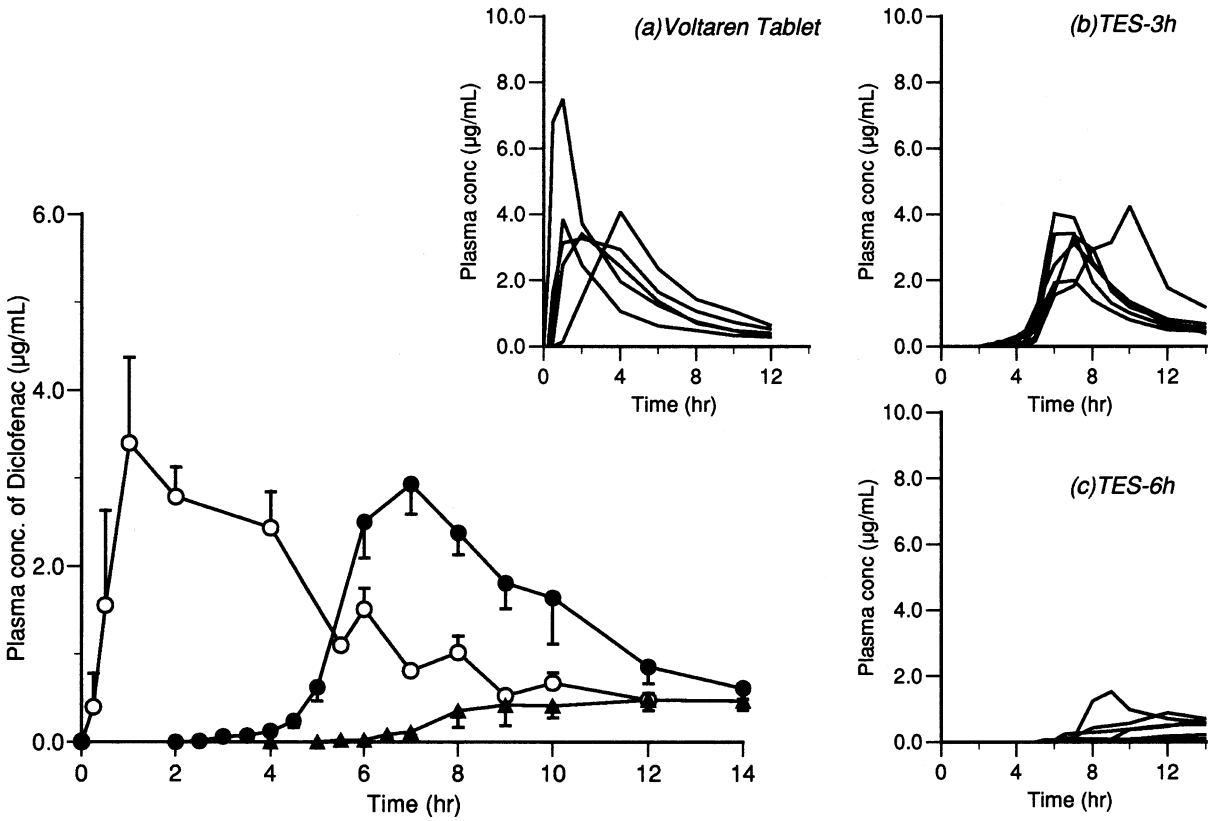

Fig. 4. Time course of mean and individual plasma concentration of diclofenac after oral administration of Voltaren tablet (○, a), TES-3h (●, b) and TES-6h (▲, c) to GI physiology regulated dogs at a dose of 25 mg/body.

diclofenac sodium would function well in humans without any reduction of bioavailability. Thus the combination of this system with rapid release formulation can provide twice-a-day dosage form with drugs which need q.i.d. administration.

Further, this study demonstrated that TES reproduced the in vitro lag time in vivo for 6 h, even though the drug dissolution was not complete. For the poorly water soluble drugs, the location of TES, where drug release was initiated, was found to be an important factor which determines the drug release rate, mainly due to the small volume of water content in the GI lumen. Therefore, the solubility of drug must be taken into account for the design of colon targeting system.

Table 3
Pharmacokinetic parameters after oral administration of diclofenac preparations to GI physiology regulated dogs

Preparation	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0\text{-}n}$ (μg/ml·h)	MRT (h)	BA_{rel} (%)
Voltaren tablet	4.23 ± 0.66	1.8 ± 0.5	19.14 ± 1.62	4.4 ± 0.4	100
TES-3h	3.29 ± 0.33	7.5 ± 0.6	15.11 ± 1.83	8.4 ± 0.2	80.1
TES-6h	0.66 ± 0.21	—	2.96 ± 0.92	—	15.6

Mean $\pm$ S.D., $n = 6$.

Table 4
The GET and the SITT of normal and GI physiology regulated dogs

	GET (h)		SITT (h)	
Dog no.	Normal	Regulated	Normal	Regulated
1	0.15	0.17	2.85	4.33
2	0.57	0.86	5.43	4.14
3	0.36	0.57	3.14	4.43
4	0.17	0.96	4.83	4.04
5	0.17	0.28	1.83	5.72
6	0.19	0.17	2.31	4.33
Mean	0.27 ± 0.07	0.50 ± 0.14	3.40 ± 0.58	4.50 ± 0.25

Mean $\pm$ S.D., $n = 6$.

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