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Preparation and Bioavailability of Sustained-Release Doxofylline Pellets in Beagle Dogs

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The objective of this study was to develop doxofylline-loaded sustained-release pellets coated with Eudragit® NE30D alone (F1) or blend of Eudragit® RL30D/RS30D (F2) and further evaluate their in vitro release and in vivo absorption in beagle dogs. Doxofylline-loaded cores with a drug loading of 70% (w/w) were prepared by layering drug-MCC powder onto seed cores in a centrifugal granulator and then coating them with different kinds of polymethacrylates in a bottom-spray fluidized bed coater. Dissolution behaviour of these formulations was studied in vitro under various pH conditions (from pH 1.2 to pH 7.4) to evaluate the effect of pH on drug release profiles. It was found that F2 produced a better release profile than F1 did and two different release mechanisms were assumed for F1 and F2, respectively. The relative bioavailability of the sustained-release pellets was studied in six beagle dogs after oral administration in a fast state using a commercially available immediate release tablet as a reference. Coated with Eudragit® NE30D and a blend of Eudragit® RL30D/RS30D (1:12), at 5% and 8% coating level, respectively, the pellets acquired perfect sustained-release properties and good relative bioavailability, with small fluctuation of drug concentration in plasma. But combined use of mixed Eudragit® RL30D/RS30D polymers with proper features as coating materials produced a longer T_{max}, a lower C_{max} and a little higher bioavailability compared to F1 (coated with Eudragit® NE30D alone). The C_{max}, T_{max} and relative bioavailability of F1 and F2 coated pellets were 15.16 μg/ml, 4.17 h, 97.69% and 11.41 μg/ml, 5 h, 101.59%, respectively. Also a good linear correlation between in vivo absorption and in vitro release was established for F1 and F2, so from the dissolution test, formulations in vivo absorption can be properly predicted.

Keywords doxofylline; sustained-release pellets; centrifugal granulation; bioavailability

INTRODUCTION

Doxofylline (DOX) is a novel bronchodilator xanthine drug and its bronchodilator activities have been demonstrated in animal studies and in clinical trials involving patients with either bronchial asthma or chronic obstructive pulmonary disease (COPD) (Franzone, Cirillo, & Barone, 1998; Villani et al., 1997). It was shown that doxofylline has less extra-respiratory effects than theophylline. Furthermore, unlike theophylline, doxofylline did not antagonize calcium channel blocker receptor, nor did it interfere with the influx of calcium into the cells (Goldstein & Chervinsky, 2002). Since doxofylline was found to relieve airway obstruction similar to theophylline but with less adverse events, it has a better perspective in clinical application. In the present study, two kinds of sustained-release pellets of DOX were prepared and their bioavailabilities were compared with commercial tablets, in order to provide patients with other better choices of good compliance.

It is generally known that multiple-unit sustained-release dosage forms, such as pellets, present some biopharmaceutical advantages in comparison with the single-unit dosage forms, particularly regarding the duration and the individual reproducibility of gastric emptying and intestine transit time (Zhang et al., 2005). Multiple-unit dosage forms can distribute in the gastrointestinal tract (GI tract) homogeneously, thus, maximizing drug absorption and reducing peak plasma fluctuations. This minimizes the risk of local GI tract irritation and dose dumping, decreases dosing frequency, increases patient compliance, and improves the safety and efficacy of the active ingredient (Hu, Liu, Tang, Zhang, 2006). Moreover, unlike simple tablets, the multiple units pellets contain many individual units, the release failures of which hardly affect the total release behavior.

Centrifugal granulation, an advanced method for manufacturing multiple-unit, sustained-release and drug-loaded pellets for oral administration, has numerous advantages, such as flexibility in operation, ease of automation compared with other pelletization techniques, and lower manufacturing costs. In this study, the seed cores of doxofylline pellets were prepared first by centrifugal granulation in a laboratory-scale centrifugal granulator. And then coating procedures were performed in a bottom-spray fluidized bed coater, which is also preferably used for coating matrix pellets/core pellets or drug-layered pellets (Rahman, 2005; Yu, Chen, & Gau, 2006).

It was shown in our previous study that the absorption of DOX occurred in all intestinal tracts, and had no significant

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differences in various intestine segments. Eudragit® NE30D, Eudragit® RS30D, and Eudragit® RL30D were used for coating to produce doxofylline sustained-release pellets. Different dissolution media were used in in vitro dissolution test to study the release properties of pellets coated with Eudragit® NE30D or the mixture of Eudragit® RS30D and Eudragit® RL30D in a proper ratio. In a pharmacokinetics study, a commercial immediate-release DOX tablet was used as a reference and was compared with two sustained-release DOX pellet formulations (referred to F1 and F2). The results showed that the sustained-release profile could be obtained by using different formulations.

MATERIALS AND METHODS

Materials

DOX was purchased from Hei Longjiang Fuhe Huaxing Pharmaceutical Co. Ltd. (Hei Longjiang, China). Methacrylic acid copolymers (Eudragit® NE30D, Eudragit® RS30D and Eudragit® RL30D) were supplied by Römh GmbH Chemische Fabrik, Darmatadt, Germany. Hydroxypropylmethylcellulose (HPMC) (Methocel E5) was gifted by Coloron (Shanghai, China). Talc was obtained from Yulin Talc Factory (Shannxi, China). Microcrystalline cellulose (MCC, Avicel pH101) was provided by Changshu Pharm. Adjunct Co. (Changshu, China). Other excipients used to prepare the pellets were standard pharmaceutical grade. Acetonitrile was HPLC grade and other reagents were analytical reagent. Doxofylline standard substance (99.9%) was provided by Chinese National Institute for the Control of Pharmaceutical and Biological Products. Commercially available immediate release DOX tablet (200 mg, Hei Longjiang Fuhe Huaxing Pharmaceutical Co. Ltd., China) was chosen as the reference in bioavailability study. The hard gelatine capsules used in this study were supplied by Suzhou Capsule Co. (Suzhou, China).

Male beagle dogs from Laboratory Animal Center of Shenyang Pharmaceutical University were used. Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in Shenyang Pharmaceutical University.

Preparation of Drug-Loaded Pellets

A laboratory-scale centrifugal granulator (Model BZJ-360M, Beijing Tianmin High Technology Development Co., China) was used for preparing both MCC seed cores and drug-layered pellets. In each experiment, 400 g microcrystalline cellulose powder was loaded into the processing chamber and moistened by continuously spraying with distilled water. After 10 min of wetting, the MCC powder was added to the above wetted mass via a hopper, keeping all the process parameters constant as follows: rotational speed of plate: 200 rpm; blower rate: 8 × 20 L/min; air flow rate: 20 L/min; spray air pressure: 0.5 MPa; rotating rate of powder feeder: 10 rpm. The final product was discharged from the processing chamber and dried at 60°C in an oven for 2 h. After sieving, the resultant seed cores were approximately 0.4–0.5 mm in diameter.

The powder mixture of DOX and MCC (120 mesh) with the ratio of 9:1 was layered onto the previously prepared MCC seed cores by spraying the binder solution (3% w/w aqueous solution of HPMC) using the same process parameters as those described above.

Preparation of Coated Pellets

Three types of acrylic-based aqueous polymeric dispersions, Eudragit® NE30D, Eudragit® RS30D, and Eudragit® RL30D, were used to prepare sustained-release DOX coated pellets and the formulation design was listed as follows:

Formulation 1 (F1): coated with Eudragit® NE30D, resulting in 5% coat loading.

Formulation 2 (F2): coated with Eudragit® RL30D: Eudragit® RS30D (1:12), resulting in 8% coat loading.

The coating suspensions were prepared as follows: Talc as an anti-adherent and sodium dodecyl sulfate (SDS) as an anti-static agent, were added to purified water and homogenized for 10 min in a homogenizer. The desired amount of mixed Eudragit® RS30D and Eudragit® RL30D or Eudragit® NE30D was added to the talc dispersion under constant stirring and adjusted to the final volume with purified water. The polymer solid concentration of these coating suspensions was 15% w/w. Triethyl citrate (TEC) was added as a plasticizer in the coating suspension of mixed Eudragit® RS30D and Eudragit® RL30D, since both Eudragit® RS30D and Eudragit® RL30D exhibited a high minimum film-forming temperature.

Pellets of 500 g were coated in the fluidized-bed coating machine (GPCG 1, Glatt GmbH, Binzen, Germany) by spraying the coating dispersion from bottom onto the pellet surface. The resultant pellets were dried in an oven at 40°C for 24 h, resulting in pellet sizes ranging approximately from 0.9 to 1.4 mm in diameter. Coated pellets were finally filled into the 0 size hard gelatine capsules, which can accommodate about 300 mg of the drug in the form of pellets for in vitro and in vivo study.

Determination of Drug Content

The samples were determined by a UV spectroscopic method according to the Chinese Pharmacopoeia 2005 (CHP 2005). From each batch of the coated pellets, 10 g of them was ground to fine powder. Then a certain amount (approximately equivalent to 25 mg DOX) of powders were accurately weighed and then added to a 100 ml volumetric flask containing 70 ml of distilled water. After ultrasonic extraction for 30 min, the solution was diluted with water to 100 ml and then filtered through a 0.45 μm membrane. Precisely 2 ml of this solution was transferred to a 100 ml volumetric flask and water was added up to 100 ml. The absorption of sample solution was

measured spectrophotometrically at 273 nm. The intra-day accuracy of this method ranged from 98.5% to 102.1%, while the intra-day precision ranged from 0.7% to 2.4%. The inter-day accuracy ranged from 96.8% to 103.6%. The precision and accuracy of the method were both well consistent with analysis requirement and no absorption of the physical mixture of the excipients existed at 273 nm.

In Vitro Tests

In vitro drug release was determined using the CHP 2005 Type 2 dissolution testing apparatus (paddle method). In this test, 900 ml dissolution medium was kept at 37 ± 0.5°C and the rotating speed was 50 rpm. Water of different pH values was used as different dissolution media.

The drug pellets equivalent to 300 mg DOX were used in all dissolution studies. 4 ml samples were withdrawn and replaced with an equal volume of the same fresh medium by an auto-sampler at 1, 2, 4, 6, 8, 12, 14, and 16 h. The sample solutions were diluted and spectrophotometry was carried out at a wavelength of 273 nm.

Dogs Bioavailability Tests

The bioavailability study was in accordance with GLP standards. Six healthy male beagle dogs weighing 15 ± 2.5 kg were used in this study. The study was conducted according to a single-dose, randomized, three crossover design, and the washout period was 1 week between treatments. In this design, the beagle dogs were randomly selected to receive a single-dose, two 0-sized gelatin capsules containing coated pellets equivalent to 600 mg DOX (300 mg/capsule) or three commercial immediate-release tablets (200 mg/tablet) with an overnight fasting but free access to water for 12 h before pellets administration. Blood samples (2 ml) were collected into centrifuge tubes (containing sodium heparin as an anticoagulant) at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 36 h after dosing. An indwelling cannula placed in the forearm was used for drawing blood during the 36 h. Following centrifugation, the plasma was then pipetted into polypropylene tubes and frozen immediately, stored at –20°C until analysis.

A solution of caffeine (81.60 μg/ml) was used as the internal standard. 20 μl of this solution was pipetted into a 15 ml tube and evaporated to dryness at 60°C under nitrogen flow. A 400 μl aliquot of dog plasma was added and the samples were vortexed for 3 min. 200 μl of perchloric acid (12%) was added and the sample was vortexed for another 3 min. The mixture was centrifuged at 4000 rpm for 10 min. And the remaining supernatant was injected into HPLC with a quantum of 20 μl.

Doxofylline plasma concentrations were determined using a validated high-performance liquid chromatography (HPLC), which was equipped with a Hitachi L-7100 pump, a Hitachi UV-VIS L-7420 detector, a Hitachi L-7200 autosampler, and a Hitachi D-7000 HSM administration. The HPLC system consisted of a Hypersil C18 column (5 μm, 4.6 mm × 250 mm) with a mobile phase containing acetonitrile /water (18/12, v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 273 nm. Calibration curves were prepared by adding 20 μl doxofylline standard solution with different concentrations to blank plasma in order to obtain eight plasma concentrations ranging from 0.16 to 40.00 μg/ml. Calibration curves were linear with a correlation coefficient of 0.9994. Limit of detection and limit of quantitation were 0.02 and 0.1 μg/ml, respectively, calculated from the mean linear regression equation.

Data Analysis

The pharmacokinetic parameters were calculated using non-compartmental model. The area under the plasma concentration-time curve from 0 h to time t h ($AUC_{0\text{-}t}$) was calculated using the trapezoidal method. The peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration-time profile. The area from 0 time point to infinity was calculated by: $AUC_{0\text{-}\infty} = AUC_{0\text{-}t} + C_t/K_e$, where C_t is the plasma DOX concentration observed at time t, K_e is the apparent DOX elimination rate constant obtained from the terminal slope of the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability (F) was calculated by: $F = AUC_T/AUC_R$. The percentage absorption in vivo was calculated by:

$$F_a = \frac{c_t + k_e AUC_{0\to t}}{k_e AUC_{0\to\infty}} \times 100\%$$

Differences of AUC, C_{max}, and T_{max} among the formulations (F1 and IR, F2 and IR, F1 and F2) was analyzed with the paired t-test and a 95% confidence interval was used to measure the statistical differences; and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characteristics of the Pellets

The doxofylline-loaded pellets coated with 5% (w/w) Eudragit® NE 30D or 8% (w/w) Eudragit® RL30D/ Eudragit® RS30D (1:12) were spherical and smooth. Homogenous and uniform polymer films were observed. A good agreement between the actual and the theoretical weight of the coated pellets was obtained, indicating that there was no significant loss of coating material during the coating process. These findings reflected a high coating efficiency (88.7%, w/w) similar to that reported by Toru Maejima (Maejima & McGinity, 2001). Complete film formation could be assumed, since the coated pellets were subject to heating in an oven dryer at 40°C. For 24 h and 0.5% (w/w) Aerosil® 200 was added into the coated pellets to prevent them from agglomerating during the curing process.

In Vitro Release

Dissolution testing in distilled water is useful only for initial development of dosage forms. Polymeric coatings with pH-independent permeability may give rise to pH-dependent drug release profile due to the difference in drug solubility at various pH values. Therefore, for the variable environment of gastrointestinal tract, the drug release characteristics were evaluated at different pH. The profiles of DOX released from sustained-release pellets with different coating formulations in dissolution media of different pH are shown in Figure 1.

It was found that there were no significant differences in the release profiles of DOX either from F1 or F2 in media with different pH. When comparisons were made between these two formulations, the release rate from F1 was slightly faster than that of F2. The accumulated release percentages of DOX of F1 and F2 were 59.90% and 43.90% by 4 h and 72.89% and 58.89% after 6 h, respectively, in pH 1.2. In pH 6.8 phosphate buffer, the release rates of DOX from F1 and F2 were 58.64% and 40.64% by 4 h and 75.11% and 62.11% by 6 h, respectively.

In this study, Eudragit® NE30D, Eudragit® RL30D, and RS30D were chosen and two different coating types were used to investigate coating types' effect on DOX release characters.

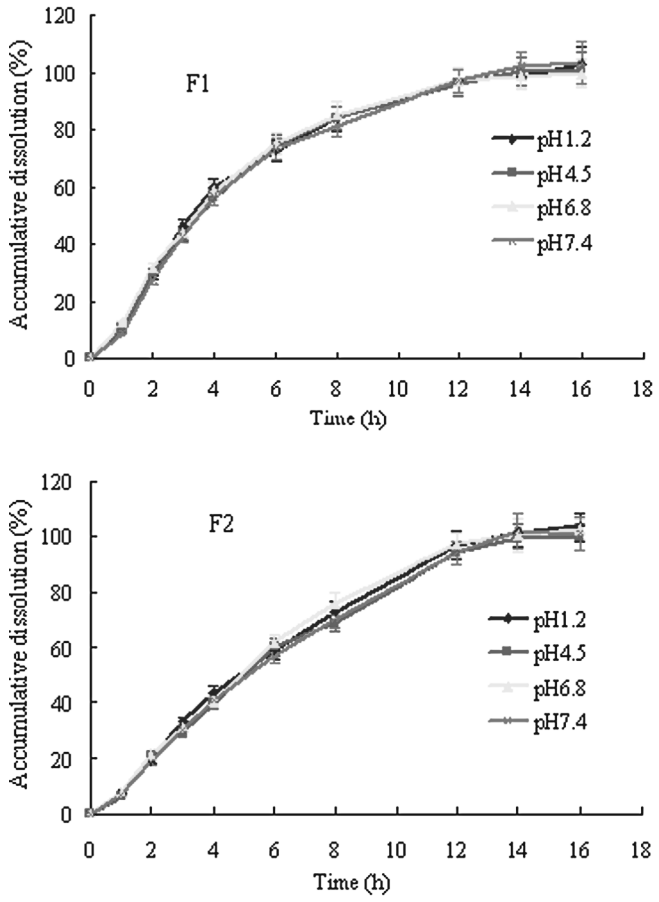

FIGURE 1. Comparison of doxofylline dissolution profiles for pellets coated with two formulations (F1, F2) in media with different pH.

It was demonstrated that the release rates of DOX from F1 (i.e., the pellets coated with 5% Eudragit® NE30D) and F2 (i.e., pellets coated with a 8% loading of the mixture of Eudragit® RL30D and Eudragit® RS30D), are not significantly affected by the pH of the dissolution media used. Although these three coating materials were both pH-independent and permeable when they got in touch to aqueous medium, the result was that F2 did a better performance, and probably this was due to coating materials' permeability and different drug release mechanism of two formations.

Different dissolution results were due to the different nature of these coating materials. Eudragit® NE30D, a polymer composed of methyl methacrylate and ethyl acrylate monomers in a ratio of 2:1 and containing 30% solid content in aqueous dispersion, has molecular weight of about 800,000. Due to its low glass transition temperature (Tg) at −8°C and a minimum film formation temperature at 5°C, soft and flexible films can be prepared at room temperature without addition of any plasticizer (Zheng & James, 2003). The tackiness of various polymers films were investigated and was in the order of Eudragit® NE30D > RS 30D > RL 30D > Aquacoat (Wesseling, Kuppler, & Bodmeier, 1999). Because the permeability of the film is pH-independent, Eudragit® NE30D has been used widely in sustained-release film coatings (Bodmeier & Paeratakul, 1989; Watano et al., 1997), granulations (Niskanen, Yliruusi, Niskanen, & Kontro, 1990), and as a component of transdermal films (Valenta & Biebel, 1998).

The coating formulation of F1 consisted of an aqueous dispersion of Eudragit® NE 30D, talc as the antiadherent agent, and sodium dodecyl sulfate as the antistatic agent. The coating layer of Eudragit® NE 30D was moderately permeable to water molecules, which are capable of penetrating into the pellet core. Thus, a drug concentration gradient was formed within the Eudragit® NE 30D coating layer, providing the primary driving force for drug release. So drug diffusion across coating membrane was presumed. Provided the coating layer was a homogenous uninterrupted membrane, the additives were evenly distributed, and there were many interstices of the correct molecular size in the membrane (Sun, Shi, Zhang, & Gao, 2005). As medium around the pellets penetrated into the coating film, drug molecules initially dissolved in the core, then distributed and diffused with the concentration gradient through these microgaps, and finally released into the outer medium. Thus mechanisms of release behavior could be explained by Fick's diffusion equation:

$$dm/dt = -DKA\,{}^{\Delta}C/h \qquad (1)$$

where dm/dt is the diffusion rate, D the diffusion coefficient, K the distribution coefficient of the drug between the polymer membrane and fluid in the core, A is the surface area of membrane, $^{\Delta}C$ is the concentration difference across membrane, and h is the thickness of membrane. Since D and A were given for DOX and coating membrane, and $^{\Delta}C$ was constant in the initial

release phase and decreased exponentially in the later phase, dm/dt was inversely related to h, as confirmed in dissolution test (Figure not shown), the thicker the coating film, the slower the drug release rate. The decrease in release rate with increasing coating load may be attributed to the increased diffusion path length with an increase in the thickness of the coating membrane (Kim et al., 2005).

There is generally a lag time in dissolution test when Eudragit® NE was used for coating (Rahman, 2005). In order to avoid this phenomenon and achieve better release profile during 12 h, coating level is low (5%). But, there was also a slight restricted release during first two h, as shown in Figure 1 (F1).

As for F2, the sustained-release layer was formed by Eudragit® RL30D and RS30D, which are cationic copolymers of ethyl acrylate, methyl methacrylate, and trimethylammonioethylmethacrylate chloride. Both of them were pH-independent and permeable and had the same chemical structure except that Eudragit® RL has double the number of hydrophilic quaternary ammonium groups than Eudragit® RS. When Eudragit® RL30D/RS30D was used for coating, triethyl citrate (TEC) was added as a plasticizer. The film did not dissolve but swelled to form micropores with the diameter of 1˜5 μm and 0.1˜0.6 μm. Since the release of most drugs from Eudragit® RL is faster than that from Eudragit® RS, dosage forms can be coated with different combinations of the two polymers to provide various degrees of sustained-release of the drug. In this study, the combination of the two polymers was 1:12 in the coating formulation. Microporous membrane diffusion across coating membrane was presumed and the release rate can also be described by Equation 2 when the microporous membrane diffusion was operating.

$$dm/dt = -D_p A \Delta C / h \text{(Jennifer et al.,1994)} \qquad (2)$$

Where D_p is the aqueous diffusivity of the drug and other parameters were the same as Equation 1. Two driving forces were assumed to account for drug release from the coated pellets. The first is drug concentration differences between the outside and inside of coating film. In Figure 1 (F2), during the first 8 h, drug release profiles were near a straight line, that is, linearity between accumulative dissolution and time. Thus, there also may be the possibility of release being driven by an osmotic pressure difference between the core materials and the release environment. Because of high loading of DOX in the core and its higher solubility, when the pellets came into contact with an aqueous environment, water is imbibed through coating, creating a solution in the core. When the excipients and drug dissolved in the imbibed water, generating the interior osmotic pressure, the osmotic pressure difference between the core and external medium then performed as the driving force for solute efflux through micropores in the coating like a small osmotic pump, which released drug by zero-order kinetics as illustrated in Figure 1 (F2).

In addition, MFT(minimum film formation temperature), as an important parameter of coating materials that affects drug release, should be seriously considered during formulation preparation process. In contrast to Eudragit® NE30D, the MFT of Eudragit® RS30D/RL30D were 40–50°C and unable to form coating films alone. In F2, 20% triethyl citrate (TEC) was added and lowered the MFT below 20°C. Additionally, pellets coated with acrylic dispersions containing a high concentration of 10% or 20% TEC showed good mechanical strength against physical stress and stabilized drug release profiles during storage (Maejima et al., 2001).

Bioavailability

The in vivo pharmacokinetic behavior of pellets coated with different formulations was investigated. Figure 2 shows mean plasma concentration-time curves after administration of two types of sustained-release DOX pellets and IR DOX tablets. Their bioavailability parameters are listed in Table 1. The differences in bioavailability were not very significant.

The plasma level of IR DOX tablets increased quickly and the maximum concentration (33.60 μg/ml) was reached at 1 h after administration. And there was a dramatic decrease in plasma concentration between 1 and 10 h. For pellets coated with F1, the maximum concentration (15.16 μg/ml) was reached at 4 h after administration; it was lower than that of the IR DOX tablets and slightly higher than those of pellets coated with F2, showing comparative absorption occurred in vivo. For pellets coated with F2, maximum concentration (11.41 μg/ml) was reached at 5 h after administration, while the decrease of drug concentration occurred at a lower rate than that of both IR tablets and F1. Opposing to the almost undetectable drug concentration in plasma of IR DOX tablets at 16 h after administration, the drug concentration of F1 and F2 fell slowly even at 24 h

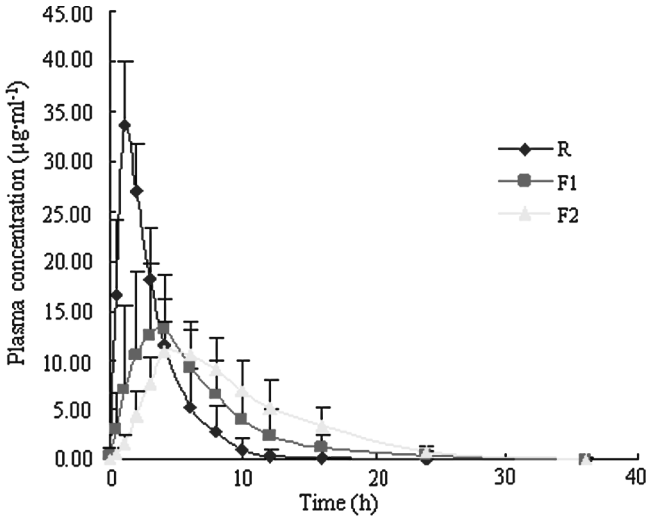

FIGURE 2. Mean plasma doxofylline concentration-time profiles of two sustained DOX pellets and IR DOX tablets.

TABLE 1
The Pharmacokinetic Parameters of Different MH Formulations (n = 6)

Parameters	IR (Reference)	F1	F2
C_{max} (μg/ml)	33.60 ± 6.47	15.16 ± 6.62	11.41 ± 2.98
T_{max} (h)	1.08 ± 0.20	4.17 ± 1.47	5.00 ± 1.67
Ke (h^{-1})	0.34 ± 0.17	0.19 ± 0.10	0.16 ± 0.07
t1/2 (h)	2.55 ± 1.45	4.16 ± 1.54	5.09 ± 1.99
AUC_{0-36} (μg ·h/ml)	116.28 ± 35.85	113.59 ± 26.26	118.13 ± 33.26
$AUC_{0-\infty}$ (μg ·h/ml)	116.44 ± 36.04	114.09 ± 25.14	119.02 ± 31.90
MRT (h)	3.75 ± 1.13	7.56 ± 1.12	9.33 ± 0.93
F (%)	—	97.69 ± 13.89	101.59 ± 14.15

IR: IR DOX tablets; F1, F2: pellets coated with Formulation 1, Formulation 2, respectively.

after administration, when the drug concentration was 0.43 μg/ml and 0.75 μg/ml, respectively. The blood sampling and drug detection were lasted until 36 h, showing that DOX also was absorbed at the final segment of intestinal tract, such as ileum, cecum, and colon. Also, the AUC_{0-36} of F1 was 113.59 ± 26.26 μg ·h/ml, which was similar to that of F2 (118.13 ± 33.26 μg ·h/ml). Compared with IR DOX tablets, the relative bioavailability judged from the AUC_{0-36} were 97.69% and 101.59%, respectively, for pellets coated with F1 and F2. Hence, the two test formulations and reference were statistically bioequivalent ($P < 0.05$).

In Vitro-In Vivo Relationship

In vitro dissolution tests were performed in different dissolution media to study the release behaviors of different formulations and concomitantly establish a correlation between in vitro release and in vivo absorption for the sustained-release pellets.

The release profiles of pellets F1 and F2 in pH 6.8 phosphate buffer, which were of the same batch, are illustrated in Figure 3; the percentage absorption in vivo of the two formulations was assessed by the Wagner-Nelson method. The percentage absorption F(t) exhibited a good linear relationship with the cumulative percentage release f(t), r being 0.9614 and 0.9705 for the formulation F1 and F2, respectively. Figure 4 demonstrated a good correlation between the in vitro release and in vivo absorption processes for F1 and F2, validating the in vitro dissolution conditions and verifying the utility of the sustained-release dosage forms. In addition, both the slope and intercept of F2 correlation equations were just a little higher than those of F1, indicating that the percentage absorption of F1 is slightly lower than that of the F2 when the percentage release is the same.

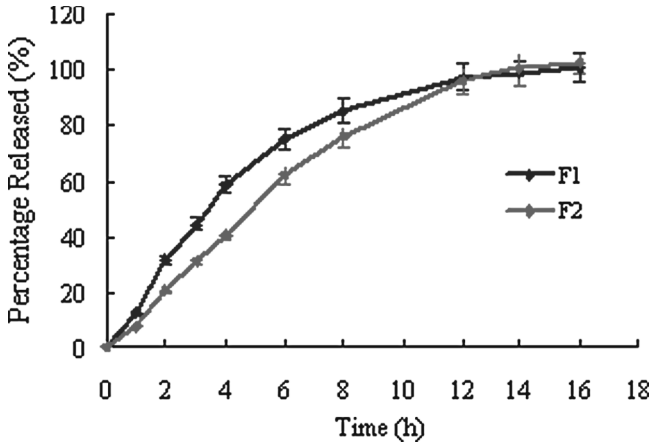

FIGURE 3. In vitro DOX release versus time from F1 and F2 of the same batch in pH 6.8 dissolution media.

Two aspects need to be carefully considered when evaluating the in vivo behavior of the drug, that is, the factors influencing its in vivo absorption and the formulation factors influencing its in vitro release. The in vitro release behavior and the transfer mechanism of pellets are important for developing a sustained-release dosage form for such kinds of drug. Therefore, it is concluded that the absorption mechanism of the sustained-release pellets combined the two aspects above.

In general, synchronizing drug release and pellets across the absorption sites may improve drug bioavailability and reduce side effects (Giacomo et al., 2005). The in situ intestinal absorption kinetics of DOX in rats has been studied in our previous research. It showed that DOX exhibited no site specificity absorption in the intestine, because there were no significant differences among the absorption rate of various segments in intestine tract. The bioavailability of sustained-release preparations of DOX (F1 & F2) were equivalent with that of the commercial immediate-release tablets probably due to the good absorption of DOX in whole intestine tract. Combining the in

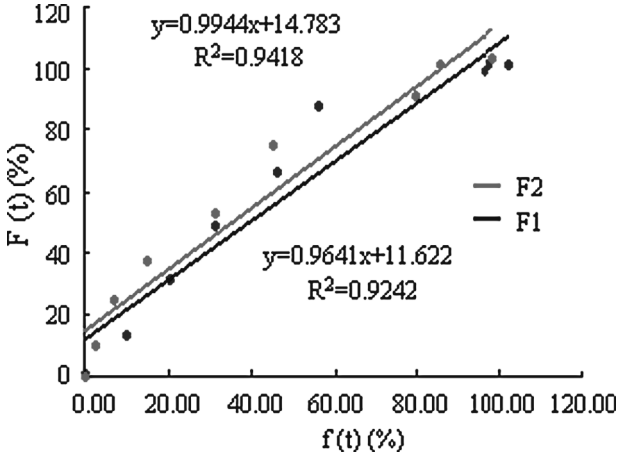

FIGURE 4. Correlation of self-made sustained-release pellets (F1, F2) in vivo and in vitro.

vitro dissolution tests with the in vivo experiment, it was hypothesized that passive diffusion of doxofylline's absorption in the intestine tract occurred and there was no saturable phenomenon during the absorption process. This was in line with the absorption characteristics described by Franzone (Franzone, Cravanzola, & Masera, 1981). From this point of view, proper coating of pH-independent polymer Eudragit® NE30D or Eudragit® RL30D/RS30D with other excipient is a better choice.

CONCLUSION

Two kinds of sustained-release pellets were obtained in this study using Eudragit® NE30D alone (F1) and a blend of Eudragit® RL30D/RS 30D (1:12) (F2) as coating materials. In dissolution tests, F2 did performed better than F1, and different release mechanisms were guessed, which were due to the final permeability of the coating formulation. It can be said the drug's release mechanism was film permeability-dependent, that is, as for DOX, when film permeability reached a critical point, DOX's release character of sustained-release pellets changed from diffusion across membrane to microporous membrane diffusion. Of course, this dependent relationship has to be further researched.

The in vivo bioavailability study of both formulations showed expected sustained-release characteristics of the coated pellets compared with IR DOX tablets. But F2, coated with blend of Eudragit® RL30D/RS30D, exhibited more excellent sustained-release effects and higher relative bioavailability than F1 and this due to different release characters in dissolution tests. The in vitro-in vivo relationship study assured this and it seemed from the in vitro test, the in vivo result could be predicted.

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