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Development of Monolithic Osmotic Pump Tablet System for Isosorbide-5-Mononitrate Delivery and Evaluation of it In Vitro and In Vivo

Xueyan Duan
Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning, People's Republic of China and Department of Chemistry, Tsinghua University, Beijing, People's Republic of China

Qingfei Liu
Institute of Biomedicine, Tsinghua University, Beijing, People's Republic of China

Yu Zhang
Department of Pharmaceutics, Jiangxi University of Traditional Chinese Medicine, Nanchang, Jiangxi, People's Republic of China

Kaishun Bi
Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning, People's Republic of China

Xi Chen, Yiming Wang and Guoan Luo
Department of Chemistry, Tsinghua University, Beijing, People's Republic of China

The objective of this study is to develop the monolithic osmotic pump tablet system (MOTS) containing isosorbide-5-mononitrate (5-ISMN), and to evaluate its in vitro and in vivo properties. The influences of tablet formulation variables, size and location of the delivery orifice, membrane variables, and pH value of the dissolution medium on 5-ISMN release from MOTS have been investigated. These results demonstrated that the tablet core played an important role in MOTS, and membrane variables could affect the 5-ISMN release rate. The optimal formulation of 5-ISMN MOTS was determined by uniform design. Furthermore, the dog pharmacokinetics and relative bioavailability of the test formulation (5-ISMN MOTS) have been compared with the reference formulation (Imdur®: 60 mg/tablet, a sustained release, SR, tablet system) following an oral single dose of 60 mg given to each of six Beagle dogs. The mean drug fraction absorbed by the dog was calculated by the Wagner–Nelson technique. The results showed that drug concentration in plasma could be maintained more stable and longer after the administration of 5-ISMN MOTS compared with the matrix tablets of Imdur®, and a level A "in vitro–in vivo correlation" was observed between the percentage released in vitro and percentage absorbed in vivo. It is concluded that 5-ISMN MOTS is more feasible for a long-acting preparation than 5-ISMN SR tablet system as once-a-day treatment, and it is very simple in preparation, and can release 5-ISMN at the rate of approximately zero order for the combination of hydroxypropylmethyl cellulose as retarder and NaCl as osmogent.

Keywords isosorbide-5-mononitrate; monolithic osmotic tablet system; in vitro; in vivo; in vivo–in vitro correlation

INTRODUCTION

The osmotic pump system, which has extended the utility of osmotic pressure as the driving force for the controlled release of drugs, has been under development for at least four decades. This system has a large number of advantages, such as drug release at a constant delivery rate over an extended action period, lack of sensitivity to variation of the pH or motility of the gastrointestinal tract, thereby reducing the risk of adverse reactions, and improves patient compliance. On the other hand, the in vivo predictability of release rate of this system can be based on the in vitro data. Various osmotic pumps have been reviewed in US patent literature by Santus and Baker (Santus & Baker, 1995). The first device using osmotic principles to deliver active ingredients was reported in 1950s by Ross and Nelson (Ross & Nelson, 1995). During the past four decades, there has been a

Address correspondence to Guoan Luo, Department of Chemistry, Tsinghua University, Beijing 100084, People's Republic of China. E-mails: dxywfn2008@yahoo.com.cn, liuqf@mail.tsinghua.edu.cn, luoga@mail.tsinghua.edu.cn

growing interest in developing osmotic pump dosage forms for the controlled delivery of drugs (Amidon, Higuchi, & Dressman, 1987; Baker, 1976; Chen & Chou, 1998; Chen, Lee, & Xie, 1998; Cortese, Barclay, & Theeuwes, 1984; Haslam, Merfeld, & Rork, 1989; Higuchi, 1973; Higuchi & Leeper, 1976; Swanson, Barclay, Wong, & Theeuwes, 1987; Theeuwes, 1984; Theeuwes & Higuchi, 1972, 1975; Zentner, Rork, & Himmelstein, 1985), such as the elementary osmotic pump (EOP) (Theeuwes & Higuchi, 1972, 1975), a two-layer osmotic tablet system for the release of water-insoluble drugs (Higuchi 1973; Swanson et al., 1987), a sandwiched osmotic tablet system (Cortese et al., 1984), a monolithic osmotic tablet system (MOTS) (Chen & Chou, 1998; Chen et al., 1998), and so on.

As the model substance, isosorbide-5-mononitrate (5-ISMN) is an organic nitrate vasodilator that acts by relaxing peripheral vascular muscles and reduces systolic blood pressure. After administration to human, 5-ISMN has an elimination half-life of 4–6 h, and shows complete bioavailability after oral administration (Abshagen & Sporl-Radun, 1981; Hutt, Bonn, Fritschi, & Jaeger, 1995). 5-ISMN is cleared almost exclusively from the body by metabolism and the clearance amounts to about 120 mL/min. Only trace amounts of unchanged drug are excreted in the urine. Up to 20% of the administered dose is conjugated to glucuronic acid, whereas the remainder is slowly denitrated to isosorbide, which forms the main metabolic product and is further metabolized to a lesser degree to sorbitol (Kendall, 1990; Schaumann, 1989). A therapeutic window of 5-ISMN plasma concentrations ranging from a threshold of 100 ng/mL to a maximum of about 500 ng/mL is proposed on the basis of pharmacodynamic work in healthy volunteers (Abshagen & Sporl-Radun, 1981; Muck, Bonn, & Rietbrock, 1989). The sigmoid dose–response relationship of 5-ISMN falls in pulmonary arterial pressure and increases the workload in anginal patients who have C50% of 365 ng/mL (Schaumann, 1989). Tolerance is known to develop when drug concentrations are maintained above a certain critical level, and in particular, fluctuation of concentrations occurring with usual therapy is not allowed (Reiniger, Blasini, Brügmann, & Rudolph, 1984). A nitrate-free period of 8–12 h has been advocated (Bogaert, 1991). However, the absence of predose ischaemia rebound in the case of once-a-day sustained release (SR) formulation has been attributed to a nitrate-low period instead of a nitrate-free interval (Olsson, Allgen, Amtorp, Nyberg, & Parker, 1992). It is recognized that when trough concentrations of about 100 ng/mL are exceeded, tolerance starts to develop, and is fully present when trough levels exceed 300 ng/mL (Nordlander, & Walter, 1994; Thadani & Bittar, 1992; Wagner, Heberer, Sprock, Trenk, & Jänschen, 1988). The most common dosing regimen is 20 mg two or three times daily for immediate release tablets, and 40 or 60 mg once daily for SR formulations. Once-daily SR 120 and 240 mg tablets have also shown their long-term efficacy in stable effort angina up to 12 h post-dosage without rebound (zero-hour effect) (Chrysant et al., 1993). The efficacy of multiple dose and once-daily dose is of no difference. However, the latter regimen can provide a better quality of life in large-scale studies (Herrmann, Kuhl, & Maier-Lenz, 1988; Niemeyer et al., 1997). Additionally, the current commercial once-a-day SR tablet,, Imdur® Tablets (AstraZeneca Pharmaceutical Ltd. Co., China; containing 60 mg 5-ISMN, oval tablet with one score major axis = 13 mm, minor axis = 7 mm, thickness = 2 mm, weight of tablet = 310 mg) is larger in size than the tablet we present here. The main disadvantage of SR tablets is that the drug release is often fit to Higuchi equation or first-order equation, not so stable as zero-order release of MOTS, which can lead to a more relatively stable drug plasma concentration after oral administration. Furthermore, it is necessary to compare the in vitro–in vivo correlation (IVIVC) between controlled release delivery system and SR delivery system. It is generally assumed that if the in vivo drug release-controlling mechanism also controls the in vitro drug release, then it would be possible to establish a Level A "IVIVC" (Sinko, Leesman, & Amidon, 1991; USP#13 NF, 2002).

Therefore, the purpose of this article is to develop a monolithic-controlled release tablet system containing 60 mg of 5-ISMN, which is small (diameter: 7 mm, thickness: 0.35 mm, and weight of tablet: 200 mg) and easy to take. The properties, such as drug release in vitro, drug adsorption in vivo, correlation in vitro and in vivo, and so forth, were evaluated. In addition, the dog pharmacokinetics and relative bioavailability of 5-ISMN MOTS were compared with the commercial Imdur® SR tablet following an oral single dose of 60 mg given to each of six Beagle dogs. Consequently, the essential information, the technique for the development of 5-ISMN MOTS, and the basic formulation that exhibited a satisfactory drug release could be provided.

MATERIALS AND METHODS

Materials

The following materials were used: 5-ISMN powder (99.9%, 5-ISMN, WuHan HeZhong Chemical Industrial Co. Ltd., China), 5-ISMN standard (99.8%) and isosorbide-2-mononitrate standard (99.9%) (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China), film-forming polymers cellulose acetate (CA, ShangHai Sinopharm Chemical Reagent Co. Ltd., China), polyoxyethylene glycol 4000 (PEG-4000, ShangHai YunHong Pharmaceutical Excipients and Technology Co. Ltd., China), polyvinylpyrrolidone (PVP-K30, BASF, Germany), hydroxypropylmethyl cellulose "K" 100M (HPMC, ESSINE, Japan), sodium chloride (NaCl, Beijing Chemicals Co. Ltd., China), Methanol [(high performance liquid chromatography) HPLC grade, Merck, Germany], Ethyl acetate (HPLC grade, Tianjin Guangfu Fine Chemical Research Institute, China), and Kalium carbonate (Analysis grade, Tianjin Kernel Chemical Reagents Development Center, China). Acetone (Reagent grade, Beijing Chemical Co. Ltd, China) was used as the coating solvent. Ultra-pure water was prepared from a Milli-Q system (Millipore, Milford, MA, USA). All the other reagents and organic solvents used were of analytical grade.

Animals

Male Beagle dogs (n = 6) (weighing 10–12 kg) were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center, China, and this study was approved by the Ethic Committee for Animal Experiment of the University. The dogs were fed with food and water ad libitum. They were randomly divided into two groups and fasted overnight prior to dosing and until 12 h post-dose. Water was allowed ad libitum 2 h post-dose.

Preparation of Tablets

First, 5-ISMN and several pharmaceutical additives were mixed together by a V-shaped blender (HuiLong Mixtures Equipment Co., Ltd., Jiangsu, China) for 10 min, and then 3% (wt/vol) PVP ethanol solution was added to the mixture and the proper-sized granules were prepared. The dehydrated dry granules Gum Acacia (Wacker, Germany) and magnesium stearate were blended for an additional 50 min. These mixtures were then compressed using a tableting machine (DP/30; Beijing GuoYao LongLi Sci. & Tech. Co., Ltd., China) with a round punch of 7.0 mm in diameter. The coating solution was sprayed onto the tablets utilizing a baffled pan coater (BY300; Shanghai HuangHai Drug Testing Instrument Co., Ltd., Shanghai, China). A spraying rate of 2 mL/min with an atomizing air pressure of 0.6 kg/cm^2 and the inlet air temperature of 40°C were used. The deviation of membrane thickness was controlled less than 5% weight gain of the tablets. The tablets were dried at 50°C for at least 24 h to remove residual solvent after coating. One orifice for drug release was drilled on the surface of each coated tablet using a laser punching machine (Beijing lifelight Sci. & Tech. Co., Ltd., China) before dissolution studies were conducted.

HPLC Analysis for Dissolution In Vitro

The concentrations of 5-ISMN in the in vitro study were determined by the following HPLC method: column, Agilent ODS (250 × 4.6 mm i.d., 5 μm; Agilent Technologies, Inc., Santaclara, CA, USA); mobile phase, methanol–water (70:30, vol/vol); flow rate, 0.8 mL/min; column temperature, 30°C; detection wavelength, 210 nm; and injection volume, 20 μL. The limit of quantitationin this study was 5.0 ng/mL. The accuracy values were <5% at all the investigated concentrations.

Dissolution In Vitro Test

Dissolution in vitro was performed according to the standard of dissolution methodology in US Pharmacopeia (USP) (Apparatus II, rotating paddles, 50 rpm, 37°C, 900 mL of medium). The cumulative release percentage was investigated in three different kinds of dissolution media, that is, deionized water, 0.1 mol/L HCl (pH 1.2), and phosphate buffer solution (pH 6.8). During the release studies, 5 mL samples were withdrawn at 2, 5, 7, 9, 12, 15, and 18 h, and replaced with the equal volume of fresh dissolution medium at the same temperature. The dissolution test was performed in triplicate. All the samples were analyzed by HPLC.

Effect of the Formulation of Tablet Core on Drug Release

To study the effect of osmogent content on the drug release, the 5-ISMN tablets containing various amounts of NaCl, that is, 30, 60, and 120 mg, were prepared, respectively. And the different contents of HPMC (5% wt/wt, 10% wt/wt, and 18% wt/wt) were also compared. The tablet properties and drug release characteristics were evaluated.

Effect of Coating Solution on Drug Release

Based on the basic study, the coating solution was formulated with 3% (wt/vol) CA in acetone–water (97:3, vol/vol) containing different amounts of PEG 4000. The tablet cores were prepared and coated with PEG 4000 coating solution at the levels of 6, 10, and 16% of CA (wt/wt), respectively, and then the properties and drug release characteristics of the coated tablets were compared. Meanwhile, 5-ISMN tablets were prepared and coated with CA to three levels of tablet weight gain, such as 4, 5, and 6% (wt/wt). The coated tablets were then drilled with laser to make a small hole (about 0.5 mm size) in the film at the center of one side of the tablets.

Effect of Orifice on Drug Release

To study the effect of orifice on the drug release, the release of the tablets with different pore size and position were investigated. The drug release of tablets with 0.4, 0.5, and 0.6 mm pore size were compared. Furthermore, the release of the tablets with one or two pores on the same side, and two pores with one on each side were also investigated under three conditions.

Gas Chromatograph–Electron-Capture Detector Method for the Detection of 5-ISMN in Beagle Dog Plasma

A Perkin Elmer (PE) Autosystem XL Gas Chromatograph (GC) (Perkin Elmer, Inc., Shelton, NJ, USA) with a 63 Ni- Electron-Capture Detector (ECD; Perkin Elmer, Inc.) detector, equipped with an autosampler was used, together with a 30 m × 0.32 mm fused silica column coated with cross-linked 35% PH ME siloxane (DB-35) with a film thickness of 0.5 μm (Agilent Technologies, Beijing, China). Nitrogen at a flow rate of 2 mL/min was used as the carrier gas. The inlet temperature was set at 180°C. Splitless injection (1 μL) was used with injection liner, which was replaced after 2 days, and the durability is better than that reported (60 injections) before (Pastera, Vysloužil, & Kŷetina, 2004). The ECD was set at 220°C with a nitrogen make up flow of 60 mL/min. The initial column temperature was 120°C, and the temperature was gradually raised to 150°C at the rate of 20°C/min, then the temperature was elevated to 220°C at a rate of 10°C/min and maintained for 5 min. A Total Chrom Chem-Station (Perkin Elmer, Inc.) was used for controlling the GC apparatus, for acquiring and processing of data.

Pharmacokinetics In Vivo Study

According to a randomized crossover design, six healthy male Beagle dogs received a single dosage of two different formulations: MOTS (60 mg of 5-ISMN) and the marketed SR tablet Imdur® (60 mg of 5-ISMN) with 20 mL water. The washout period was 2 weeks. After administration, the plasma samples were collected into test tubes at pre-dose (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 h post-dose, respectively. The internal standard isosorbide-2-mononitrate was mixed with 0.3 mL plasma sample. Then the plasma samples were treated with 90 μL saturated kalium carbonate and 1.5 mL ethyl acetate. After the plasma samples were mixed for 1 min and centrifuged for 10 min at 3,000 rpm, the upper solution was transferred to another clean glass and evaporated to dryness at 35°C under a gentle stream of nitrogen, the residue was reconstituted in 150 μL ethyl acetate. After being centrifuged for 10 min at 3,000 rpm, the upper layer was transferred into the inner glass sample tube and 1 μL was injected into the GC. Peak area ratios of 5-ISMN/IS were calculated and calibration curves were constructed. The equations of the calibration curves were used to interpolate the concentrations of 5-ISMN in the samples using their peak area ratios.

The pharmacokinetic parameters were determined from the plasma drug concentration–time data. The data were presented as mean values with the standard deviation ($M \pm SD$).

Pharmacokinetic Analysis

Statistical Program for Scientific Studies (SPSS 10.0) package (for a Windows operating system) was used to perform the statistical analysis of data. The analysis of variance (ANOVA) test was performed on the pharmacokinetic parameters C_{max} and area under concentration time curve (AUC), using general linear models procedures, in which the sources of variation were subject, period, and formulation. The two formulations were bioequivalent if their 90% confidence intervals were within the accepted range (0.80–1.25) for log-transformed data. A P-value of less than 0.05 was considered to be statistically significant. Wilcoxon rank sum test was used to evaluate the significance of T_{max}. If the sum was in the range of 28–50, there was no significant difference between two formulations.

Correlation Between In Vitro and In Vivo

An attempt was made to perform a "level A" IVIVC, which showed that the in vivo drug input rate of the dosage form matches 1:1 with the in vitro drug release rate for 5-ISMN MOTS. The IVIVC is thought to be the most useful relationship for predicting in vivo performance from dissolution data in vitro, for establishing the "bioequivalence" of minor formulations and manufacturing site changes without having to do a full-scale human bioequivalence study. The relation between the in vitro dissolution data and in vivo pharmacokinetic data was examined by plotting the percentage drug dissolved in vitro after 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 h versus the percentage absorbed in vivo at equivalent time intervals.

RESULTS AND DISCUSSION

Cumulative Release in Different Dissolution Medium

The release tests of the MOTS were studied in deionized water, 0.1 mol/L HCl (pH 1.2), and phosphate buffer (pH 6.8), respectively. The similar factor f_2 (Shah, TSong, Sathe, & Liu, 1998; Xia & Liu, 2000) was used to evaluate the drug release behavior. They were considered similar if the value of f_2 was above 50 and vice versa. The results of different pH dissolution tests clearly indicated that 5-ISMN release from the MOTS was independent of the pH value of the release medium (f_2: 68.82, 66.54, and 87.77). The reason might be that tablets were made of HPMC. Water coming into the system dissolved these substances and formed an aqueous solution of appropriate viscosity. Thus, it might be expected that the fluids of the gastrointestinal track scarcely affected the 5-ISMN release from MOTS. Hence, water was used as a dissolution medium in the latter study.

Because drug release was independent of release medium, 5-ISMN MOTS might exhibit a comparable in vitro/in vivo release profile.

Effect of the Formulation of Tablet Core on Drug Release

Effective release patterns may be obtained to modulate the proper delivery rate of drug from an osmotic pump. NaCl and HPMC have been used to control the release of 5-ISMN MOTS. To study the influence of the amount of chemicals on 5-ISMN release, MOTS cores with various formulations were prepared and subsequently coated with coating solution, then drilled a circle orifice with a diameter of 0.5 mm. The relationships between the core formulation variables and the cumulative release of 5-ISMN at 18 h were shown in Figures 1 and 2.

The release of tablets containing various osmogent amounts was similar (f_2: 67.68, 76.64, and 62.75). The drug release profile (Figure 1) showed that NaCl played an important role during the late period. The reason might be because

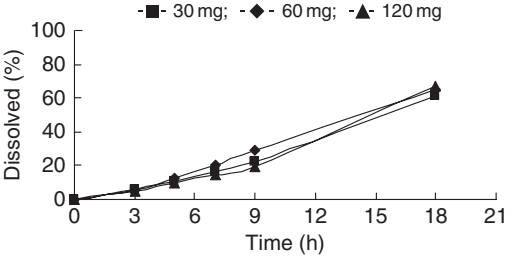

FIGURE 1. Dissolution profiles of 5-ISMN from osmotic pump systems containing various amounts of NaCl (n = 6).

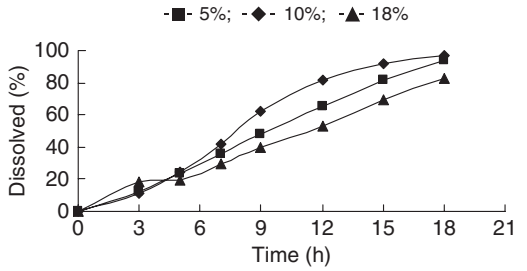

FIGURE 2. Dissolution profiles of 5-ISMN from osmotic pump systems containing various amounts of HPMC (n = 6).

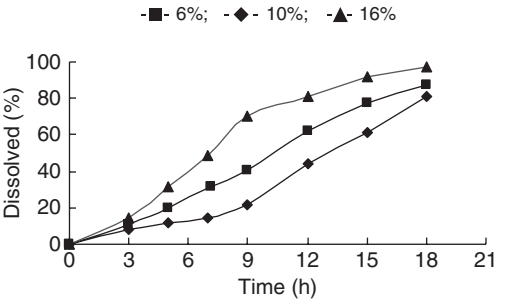

FIGURE 3. Dissolution profiles of 5-ISMN from osmotic pump systems at different levels of coating solution containing various amounts of PEG (n = 6).

of the interaction between 5-ISMN and the osmogent. The drug has a high solubility in water (209.1 mg/mL, 37°C), but has little solubility in saturated NaCl solution (31.9 mg/mL, 37°C); hence, the drug itself could create a proper saturated pressure as the driving force at the beginning of drug release profile, whereas the osmogent could produce a marked effect on the late period of drug dissolution profile. This phenomenon was similar to that discussed earlier (Mohanmmadi, Adrangui, & Siahi, 2000).

As 5-ISMN has a high solubility in water, the release rate was too fast even without osmogent, it is necessary to reduce the drug dissolution in vitro. Lu (Lu & Jiang, 2000) prepared the naproxen delay-released tablets with HPMC as retarder. And PVP was also used as retardant agent to prepare atenolol MOTS (Liu & Wang, 2008). In this study, HPMC was also used to reduce the drug release. The results indicated that HPMC could reduce the drug release rate (f_2: 48.94, 40.91, and 41.33), and the drug release rate decreased with the increase of HPMC content. This was because of the higher viscosity of the HPMC K100M polymer.

Effect of Coating Solution on Drug Release

CA is widely used to control the release of water-soluble molecules. Water is imbibed into the tablet core through the semipermeable coating; hence, the release kinetics is limited by the membrane formulation variables. In this study, the permeability of the coating to water could be adjusted by controlling the membrane structure in order to control 5-ISMN release kinetics. The effect of PEG on drug release was mainly investigated. Figure 3 showed that the increment of PEG 4000 level led to an increase of drug release correspondingly. Because PEG 4000 was a hydrophilic plasticizer, it could easily leave the CA membrane and enter into the aqueous environment, and as a consequence, the PEG left behind a porous structure, thereby increasing the permeability of the CA membrane and the drug release rate of MOTS.

The similarity factor f_2 was used to evaluate the influence of PEG 4000 on drug release profile. The f_2 values obtained were 45.88, 31.55, and 41.94, which indicated that PEG 4000 played an important role in drug dissolution.

Influences of the Membrane Thickness

To study the effect of membrane thickness on the kinetics of drug release from the system, tablets were coated with a membrane made of CA and PEG 4000 (10%) with the coating weight at different levels (3, 4, and 5% of the core weight). The orifice diameter was 0.5 mm. As shown in Figure 4, the membrane thickness played an important role in drug dissolution (f_2: 43.65, 30.71, and 42.06), the increase of thickness resulted in an increase of the resistance of the membrane to water diffusion, or a decrease of the rate of water imbibing. As a result, the 5-ISMN release rate decreased. Hence, the proper membrane thickness was necessary to ensure that 5-ISMN release from the MOTS was according to zero-order kinetics.

Influences of the Delivery Orifice

Once the core formulation and the membrane variables were decided, the 5-ISMN release rate from the MOTS would be affected by the orifice size. It was reported that there should be an appropriate range for the EOP. It must be smaller than the maximum limit to minimize the contribution to the delivery rate made by diffusion through the orifice. Also it must be larger than the minimum limit to minimize the influence of hydrostatic pressure inside the system (Theeuwes, 1975; Theeuwes & Higuchi, 1975).

The coated tablets were drilled on the surface with a round orifice of various sizes. The results were in accordance with

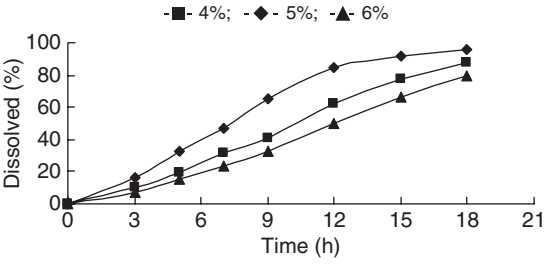

FIGURE 4. Dissolution profiles of 5-ISMN from osmotic pump systems with the different membrane thickness (n = 6).

those of EOP. It was clear that no significant difference existed in the release profiles for orifice diameters ranging from 0.4 to 0.6 mm (f_2: 83.84, 89.00, and 90.54). It could be observed that the 5-ISMN MOTS was disrupted with an orifice diameter of 0 mm (without orifice) in the late experiment period. The continuous water influxing into the MOTS without an orifice resulted in an increase of the volume of tablet core, and led to an increase of the hydrostatic pressure inside the system. This hydrostatic pressure formed would cause membrane disruption and crack the formation on the CA membrane. Subsequently, the 5-ISMN release was initiated via the crack. As the forming moment and the size of the crack could not be controlled or predicted, the system without the orifice would be uncontrollable.

In this study, it could also be found that the number and the position of orifice have made no difference on 5-ISMN release. Based on the results mentioned above, an orifice diameter of 0.5 mm was used in the following studies.

Optimal Core Formulation and Preparation

Uniform design was used to optimize the formulation of 5-ISMN MOTS. According to the above study, the amount of HPMC, coating membrane thickness, and PEG played important roles in drug release. Hence, the three factors were studied and a feasible wide range of experimental conditions was chosen for each factor.

The scales of factors were as follows: HPMC, 2–17% (wt/wt); PEG, 6–16% (wt/wt); membrane film weight increase, 4–6% weight of the core tablet. According to the uniform design formation table, the experimental scheme is shown in Table 1.

In this study, the quadratic polynomial step by step regression method was used to simulate the experimental results. The following model was recommended when the F-test was significant and $\alpha = 0.1$: $Y = 0.09A^2 + 1.96C^2 + 0.68AC + 1.81B - 54.66$, where A was HPMC, B was PEG, C the film weight increased, and Y the similar factor f_2, correlation coefficient $R = .9990$, test value $F = 122.88$, which revealed that these three parameters had relatively significant effect on the drug release. The optimal combination of factors (A, 16%; B, 15%; and C, 5%) was obtained by uniform design. Three batches of 5-ISMN MOTS of the optimized formulation were prepared and one orifice (0.5 mm) was drilled on each tablet. The results indicated that the optimized formulation was stable, reproducible, and robust.

TABLE 1
Factors and Levels of the Uniform Design Experiment

No.	A (%)	B (%)	C (%)	f_2
1	2	14	6	50.07
2	8	16	5	55.79
3	14	8	4	46.61
4	17	12	4	70.31
5	5	6	6	49.01
6	11	10	5	55.92

A represents HPMC; B represents PEG; C represents coating weight addition.

Pharmacokinetics In Vivo Study

The correlation coefficient of the calibration curves was 0.9931. The precision and accuracy of the method were satisfactory in the concentration range of 10–800 ng/mL. The lowest limit of detection was 5 ng/mL. The recovery of extraction method at three levels of 20, 100, and 400 ng/mL were above 90%, and the overall coefficients of variation were less than 10% at all the levels. The method was robust to detect the concentration of 5-ISMN in dog plasma, which was improved on the basis of the original approach (Skutta, Böttcher, & Brandt, 1989).

The plasma concentration–time profile of 5-ISMN MOTS is shown in Figure 5. It indicated that the test formulation had a lagged T_{max} when compared with the reference formulation. With regard to C_{max}, the reference formulation showed a higher value. 5-ISMN MOTS had a longer steady efficient plasma concentration platform than the reference in vivo. The sustained plasma level was because of the constant release pattern of the osmotic tablets in vivo. This was a preliminary indication that true zero-order release was obtained for 5-ISMN MOTS under the in vivo environment. The pharmacokinetic parameters of 5-ISMN following administration of the two formulations in healthy dogs are shown in Table 2.

Bioequivalence Evaluation

The relative bioavailability of 5-ISMN was 106.9%. It could be predicted that 5-ISMN MOTS was bioequivalent to Imdur®. The statistical results of ANOVA are shown in Tables 3 and 4.

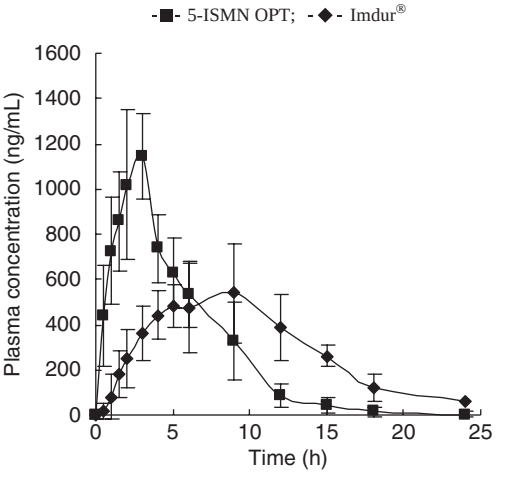

FIGURE 5. Mean plasma concentration–time profiles for 5-ISMN MOTS and Imdur® containing 60 mg 5-ISMN ($n = 6$).

TABLE 2
Pharmacokinetic Parameters (n = 6) Following Oral Administration of 5-ISMN MOTS and Imdur® at Single Dose of 60 mg in Dogs, Respectively

Parameter	$M \pm SD$ (OPT)	$M \pm SD$ (SR)
$t_{1/2}$ (h)	4.52 ± 1.14	2.16 ± 0.75
C_{max} (mg/L)	631.85 ± 194.94	1,234.73 ± 178.42
$AUC_{0\sim24}$ (mg/L h)	7,254.68 ± 1,819.55	6,787.58 ± 769.28
$AUC_{0-\infty}$ (mg/L h)	8,034.55 ± 1,910.76	6,868.00 ± 813.22
MRT_{0-24} (h)	9.48 ± 0.67	1.82 ± 1.01
$MRT_{0-\infty}$ (h)	12.08 ± 3.14	5.02 ± 1.17
T_{max} (h)	6.50 ± 1.97	2.67 ± 0.52
CL (L/h)	3.34 ± 3.00	4.17 ± 3.34
Vd (L)	23.97 ± 23.48	14.60 ± 14.32

TABLE 3
ANOVA Analysis for ln $AUC_{0\sim24}$ of 5-ISMN MOTS

Source of variation	F	$\alpha = 0.05$	Significance
Subject	1.19	$F(5,4) = 6.26$	$P > 0.05$
Period	2.92	$F(1,4) = 7.71$	$P > 0.05$
Formulation	0.23	$F(1,4) = 7.71$	$P > 0.05$

TABLE 4
ANOVA Analysis for ln C_{max} of 5-ISMN MOTS

Source of variation	F	$\alpha = 0.05$	Significance
Subject	1.83	$F(5,4) = 6.26$	$P > 0.05$
Period	2.13	$F(1,4) = 7.71$	$P > 0.05$
Formulation	35.44	$F(1,4) = 7.71$	$P < 0.05$

AUC had no significant deviation between formulation, individual, and period, respectively. From the two one-side F tests and 90% confidence interval analysis of log-transformed data, the interval for AUC_{0-24} was found to be 1.062–1.109, which was within the commonly accepted bioequivalence range of 0.80–1.25. C_{max} was different between the two formulations, which showed that C_{max} was bioinequivalent. From Wilcoxon rank sum test, it was found that T_{max} had significant difference between the two formulations (Sum 1 = 21, Sum 2 = 57). T_{max} of MOTS was greater than that of Imdur®. All these results demonstrated that AUC was bioequivalent, T_{max} was increased, but C_{max} was decreased compared with Imdur® SR tablets. Therefore, 5-ISMN MOTS had a typical advantage and property of a controlled release formulation, which makes it more feasible for a long-acting preparation as a once-daily treatment. It has been well known that the difference of T_{max} and C_{max} between the two formulations may lead to the different efficacy in clinical treatment. Bioequivalence from pharmacokinetics just provides a relative reference. The pharmacodynamic bioequivalence of 5-ISMN MOTS will be clinically studied in the future when compared with Imdur®.

Correlation Between In Vitro and In Vivo

The fraction-absorbed calculation employed the Wagner–Nelson method (Wagner et al., 1964), and was applied to the mean 5-ISMN plasma concentration–time data. Figure 6 showed the percentage absorbed in vivo versus the time taken for test. From the figure, it was apparent that an initial burst commonly associated with MOTS resulted in an initial rapid absorption, and a prolonged and slow absorption was consistent with actual in vitro release in the dissolution profiles.

Level A "IVIVC" with relatively high correlation coefficient was obtained as shown in Figure 7. The regression equation of the curve and the correlation coefficients (r) were calculated as follows: $Y = 1.024\ X + 8.878$, $r = 0.9762$ ($r > r_{5,0.001} = 0.9507$), which indicated that there was a good correlation between in vitro and in vivo study of 5-ISMN MOTS. Therefore, the quality of MOTS containing 5-ISMN could be easily controlled by drug release in vitro.

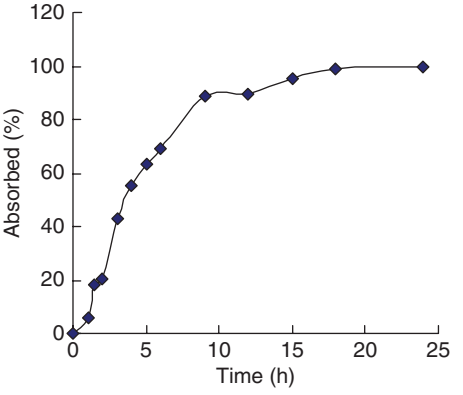

FIGURE 6. Percentage in vivo absorbed versus time for 5-ISMN MOTS after deconvolution of plasma concentration–time profiles according to the Wagner–Nelson method.

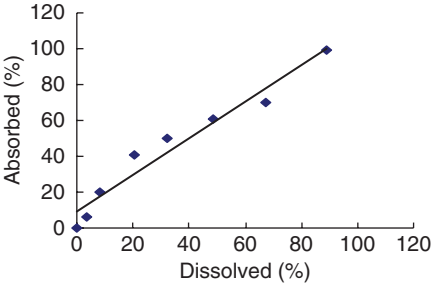

FIGURE 7. Level A IVIVC for 5-ISMN MOTS.

The development of successful IVIVCs have been reported for a number of formulations, including niacin, 6-*N*-cyclohexyl-2′-*O*-methyladenosine (Li, Pan, Nie, & Wu, 2004; Royce, Li, Weaver, & Shah, 2004; Turner, Federici, Hite, & Fassihi, 2004), and so forth. This observation further proved that the dissolution profiles and release kinetics could be used as a means to predict the absorption kinetics and overall plasma concentration–time profiles for the formulations.

CONCLUSION

In this study, the MOTS containing 5-ISMN was successfully developed with a simple and conventional technology. The method had better reproducibility, practicability, and commercial value than that of some literatures reporting on osmotic pump system. The tablet core was prepared at the proper proportion of NaCl and HPMC to ensure that 5-ISMN was released at zero order and at a constant rate up to 24 h, 5-ISMN and NaCl as the osmogent, and HPMC as the retarder. The mechanism of drug release was somewhat different from that of the literatures (Lu & Jiang, 2000; Sui & Li, 2003) where NaCl as the osmogent and HPMC as the retarder. 5-ISMN MOTS was smaller and easier to take than the commercial SR tablet. 5-ISMN MOTS had sufficiently high plasma levels during most part of the day followed by a lower concentration phase in order to prevent nitrate tolerance. This study further confirmed that the general use of in vitro dissolution data could predict in vivo disposition for highly soluble drugs. Furthermore, the developed 5-ISMN MOTS demonstrated a bioavailability comparable to that of a marketed SR formulation. In conclusion, 5-ISMN MOTS is more feasible for a long-acting preparation than the currently marketed SR tablet Imdur® as a once-daily treatment. It is believed that 5-ISMN MOTS prepared with a simple and conventional technology has an excellent commercialization potential.

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