In vitro cytotoxicity, pharmacokinetics and tissue distribution in rats of MXN-004, a novel conjugate of polyethylene glycol and SN38

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Abstract
1. MXN-004 is a water-soluble PEGylated 7-ethyl-10-hydroxy-camptothecin (SN38). The aim of this study was to evaluate the in vitro cytotoxicity of MXN-004 and investigate pharmacokinetics and tissue distribution of MXN-004 and its active metabolite SN38 in rats.

2. In vitro cytotoxicity of MXN-004 was tested in A549, HepG2 and Caco-2 cancer cell lines by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and compared with irinotecan. The pharmacokinetics and tissue distribution of MXN-004, irinotecan and their identical active metabolite SN38 were investigated after intravenous administration of MXN-004 and irinotecan at a same dose level of 16 µmol/kg in rats.

3. In vitro cytotoxicity study showed that MXN-004 was more potent in comparison with irinotecan. In rats, MXN-004 exhibited a longer half-life (sixfold) and much greater Vd as compared with irinotecan. The AUC0–∞, T1/2 and Cmax of SN38 after intravenous administration of MXN-004 were higher than those of irinotecan (3.5-, 1.92- and 10.6-fold, respectively). In addition, the concentrations of SN38 released from MXN-004 were significantly higher in all tissues than those from irinotecan, especially in the lung.

4. These results suggested that MXN-004 might be a more potential water-soluble antitumor agent with prolonged half-life of SN38 compared to irinotecan.

Introduction
Camptothecin, a plant alkaloid existing in the bark, stem and fruit of Camptotheca acuminate, is a specific DNA topoisomerase I inhibitor (Ebrahimnejad et al., 2011; Jaxel et al., 1989). DNA topoisomerase I plays an important role in replication and transcription through relaxing the supercoiled DNA (Wang, 1996, 2002). The content of topoisomerase I in tumor cells is dramatically higher than that in normal cells. In addition, the activity of topoisomerase I is significantly increased in the proliferative phase of tumor cells; such drugs as camptothecin targeting on topoisomerase I can specifically inhibit DNA replication of tumor cells to exhibit anticancer effect (Giovanella et al., 1989; Mathijssen et al., 2003; Monnin et al., 1999). Currently, topotecan and irinotecan, two camptothecin analogs approved by FDA for cancer chemotherapy in 1997, have been widely prescribed.

Irinotecan, a semisynthetic water-soluble camptothecin analog, has extensive anticancer activity against a variety of malignancies, including colorectal, small cell lung, gastric, breast, cervical and ovarian cancers; acute leukemia; and acute lymphoblastic leukemia (Midgley et al., 2007; Tsuruo et al., 1988). As a prodrug, irinotecan was hydrolyzed by carboxylesterase II to its active metabolite 7-ethyl-10-hydroxy-camptothecin (SN38) (Ma et al., 2003). However, there are many obstructions to limit the clinical use of irinotecan. First, the metabolic pathways of irinotecan are complicated such as hydrolysis by carboxylesterase II into SN38 and bis-piperidine group, oxidation by CYP3A4 into 7-ethyl-10-(4-N-[5-amino-pentanoic acid]-1-piperidino) carbonyloxyacamptothecin (APC) and 7-ethyl-10-(4-[1-piperidino]-1-amino)-car-bonyloxyacamptothecin (NPC) (Lokiec et al., 1995). Therefore, the bioconversion rate of irinotecan to SN38 relies on the inherited interindividual differences of carboxylesterase activity (Koizumi et al., 2006), and when coadministered with other drugs, irinotecan may induce potential drug–drug interactions due to the participation of CYP enzymes (Ma & McLeod, 2003). Second, the major side effects of irinotecan are delayed diarrhea and neutropenia syndrome, caused by irinotecan and the active metabolite SN38 (Satoh et al., 2013). In addition, it was reported that the bis-piperidine group (inactive metabolite of irinotecan) was also related to the diarrhea (Dodds et al., 2001). Third, the active form of irinotecan with a closed lactone ring can be transformed into inactive carboxylate form through opening.

Keywords
Cytotoxicity, PEGylation, pharmacokinetics, SN38, topoisomerase I inhibitors
the ring (Rivory et al., 1994), and then only a small proportion of irinotecan is transformed into SN38 in humans (Chabot, 1997; Garcia-Carbonero & Supko, 2002). Currently, new irinotecan analogs are sought to alleviate the above-mentioned limitations of irinotecan. Although SN38 exhibits about 100- to 1000-fold more potent \textit{in vitro} cytotoxicity than irinotecan (Mathijssen et al., 2001), it cannot be directly applied to the clinical use because of its poor water solubility (Zhang et al., 2004). It is well known that PEGylation can increase the water solubility of low-molecular-weight drugs, prolong circulation time and change the tissue distribution of parent drug. For example, EZN-2208 (Figure 1), a good water-soluble PEGylated conjugate of SN38, has high drug loading (Zhao et al., 2008) and exhibits higher (10- to 245-fold) cytotoxicity than irinotecan. Compared with irinotecan, EZN-2208 shows higher exposure of SN38 and better therapeutic index in xenograft models of breast, colorectal and pancreatic xenografts (Sapra et al., 2008). At present, EZN-2208 has been evaluated in phase II clinical trial in patients with colorectal cancer and breast cancer and in phase I clinical trial in pediatric patients (Kurzrock et al., 2012; Patnaik et al., 2013). Despite these advantages, EZN-2208 may have the following drawbacks of complicated and high-cost synthesis due to the utilization of multiarm PEG linkers, making industrialized production difficult to achieve.

In our study, MXN-004 (Figure 1) is also a compound of PEGylated SN38 with good water solubility. But unlike EZN-2208, MXN-004 is a small-molecule compound. The objective of this study is to assess the \textit{in vitro} cytotoxicity of MXN-004 using A549, HepG2 and Caco-2 tumor cells by MTT assay, and investigate the pharmacokinetics as well as the tissue distribution of MXN-004 and its active metabolite SN38 in rats. Through these experiments, we want to observe whether MXN-004 exhibits optimized pharmacokinetic behaviors with improved water solubility compared with irinotecan.

**Materials and methods**

**Materials**

MXN-004, irinotecan hydrochloride and SN38 were kindly provided by Nanjing Meixining Medical Technology Co., Ltd. (Nanjing, China). Enalaprilat (purity > 99.0%), as the internal standard (ISTD), was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile of high-performance liquid chromatography grade were purchased from Tedia Company Inc. (Fairfield, OH). All other reagents were of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

Caco-2, A549 and HepG2 cells were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO). Dubelco’s modified
Eagle’s medium (DMEM), fetal bovine serum (FBS), nonessential amino acid, trypsin and penicillin-streptomycin solution (100×) were purchased from HyClone Laboratories, Inc. (Logan, UT).

Animals

Sprague-Dawley rats (male) weighing about 180–220 g, which were purchased from Shanghai SIPPR/BK Experimental Animal Company Ltd. (Shanghai, China), were used for the pharmacokinetic and tissue distribution studies. The rats were kept under customized conditions at a temperature of 25 ± 2 °C and a relative humidity of 50 ± 20% for at least 1 week prior to the study. The animal studies were performed in compliance with the Animal Ethics Committee of China Pharmaceutical University (Nanjing, China).

LC-MS/MS analysis

The plasma and tissue samples were analyzed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system consisted of a liquid chromatography system equipped with a LC-20AD binary pump system, a SIL20AD autosampler, a CTO 20 A oven (Shimadzu, Kyoto, Japan) and a Thermo Scientific TSQ Quantum MS/MS system with electrospray ionization (ESI) source. Data were analyzed by Xcalibur 2.0 software (Thermo Fisher Scientific, Waltham, MA).

The column temperature was kept at 40 °C. The mobile phase consisted of water (containing 0.1% formic acid and 5 mM ammonium acetate, mobile phase A) and acetonitrile (mobile phase B) was pumped at a flow rate of 0.3 mL/min. Samples were chromatographed on Hedera ODS-2 column (2.1 × 150 mm, 5 μm) using a gradient elution. The gradient curve included 0–0.5 min, 20% B; a linear increase to 80% B within 1 min; 80% B for 3 min; a linear decrease to 20% B over 0.5 min; 20% B for 1 min; 60.00 min, stop.

Quantification was performed in selective reaction monitoring mode with a dwell time of 0.2 s for each transition. Positive-ion detection was applied to detecting the transitions m/z 1134.8–392.7 for MXN-004 (collision energy: 60 eV), m/z 587.1–124.0 for irinotecan (collision energy: 34 eV), m/z 392.2–348.3 for SN38 (collision energy: 22 eV) and m/z 377.0–234.0 for enalaprilat (ISTD) (collision energy: 34 eV). High-purity nitrogen was used as nebulizing gas; the parameters of the mass spectrometer were optimized as follows: spray voltage 4500 V; capillary temperature 350 °C; sheath gas 40 Arb; auxiliary gas 15 Arb and collision gas (Ar) 1.5 mTorr.

In vitro cytotoxicity studies

The in vitro cytotoxicity of MXN-004 was assessed in A549, HepG2 and Caco-2 cancer cell lines by MTT assay as compared to irinotecan (Mosmann, 1983). Cell lines were cultured in DMEM containing 10% FBS and penicillin-streptomycin solution (100×), in addition, adding certain amount of nonessential amino acids in Caco-2 medium, and maintained in humidified incubator with 5% CO₂ at 37 °C.

Cell lines were plated in 96-well plates with 5000 per well for 24 h and exposed to a series of concentrations of MXN-004, irinotecan and SN38 for 48 h at 37 °C. After DMEM containing MTT (0.5 mg/mL) was added to incubate for 4 h, the media were removed. The formazan products were solubilized by 200 μL DMSO. After mixing uniformly, the OD₅₇₀nm was read on a Bio-Rad microplate reader. The half maximal inhibitory concentration (IC₅₀) was calculated from triplicate experiments using the modified Karber method.

Pharmacokinetic studies

Eight rats were randomly divided into two groups as subjects. Group 1 was treated with irinotecan solution at the dose of 16 μmol/kg via the tail vein, while an equimolar dose of MXN-004 preparation was administered to Group 2. Blood samples were taken from suborbital vein into heparinized tubes just at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 and 24 h, and then immediately centrifuged at 5000 rpm for 5 min; 50 μL of plasma was quickly transferred to the polyethylene tubes containing 10 μL of 10% glacial acetic acid (Bansal et al., 2008) and quickly stored at −70 °C for analysis.

Tissue distribution studies

Twenty-four rats were randomly divided into two groups (12 rats per group). Group 1 was treated with irinotecan solution at the dose of 16 μmol/kg via the tail vein, while an equimolar dose of MXN-004 preparation was administered to Group 2. The rats were sacrificed in groups at 0.25, 1.0 and 4.0 h (four rats each time point). Blood samples were collected into heparinized tubes and then immediately centrifuged at 5000 rpm for 5 min; 50 μL of plasma was quickly transferred to the polyethylene tubes containing 10 μL of 10% glacial acetic acid and quickly stored at −70 °C until analysis. Meanwhile tissues (heart, liver, spleen, lung, kidney, stomach, brain and colon) were collected and homogenized with 3 mL/g of physiological saline containing 2% glacial acetic acid and then quickly stored at −70 °C for analysis.

Preparation of plasma and tissue samples

Acidified plasma samples were spiked with 50 μL acetonitrile and 100 μL acetonitrile containing ISTD solution (100 ng/mL). The mixtures were vortexed for 5 min and then centrifuged at 16000 rpm for 10 min. The supernatant was collected; 5 μL aliquot of each supernatant was injected into the LC-MS/MS system for analysis. The processing procedure of tissue samples was similar to the above-mentioned handling methods for plasma samples.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were performed by non-compartmental analysis using drug and statistics software (DAS 2.1.1 version, Anhui Provincial Center for Drug Clinical Evaluation, China). Data were presented as mean ± SD; mean values were considered significant when p < 0.05 by using a Student’s t-test.

Results

Method validation

The established method could determine the concentrations of MXN-004, irinotecan and SN38 simultaneously. A 10-point
calibration curve was established within the linearity range from 2 to 2000 ng/mL for MXN-004, irinotecan and SN38. The calibration curves were obtained by plotting the peak area ratios between the analytes and ISTD using weighted ($1/x^2$) least squares linear regression. The variance between theoretical and measured concentration of these three compounds was less than 15%, and the lowest limit of quantification for these three compounds was 2.0 ng/mL in plasma samples, with relative standard deviation (RSD) within 20%. The RSDs of intra- and inter-day precision from the three QC level samples were all within 15%. The values were summarized as follows: 10.58, 6.74, 5.43% for MXN-004; 11.97, 7.28, 6.47% for irinotecan; 9.82, 4.85, 4.33% for SN38. After samples being preacidified, recoveries of these three compounds were kept at 85–115%, and were kept stable up to 4 h at room temperature, 24 h at 4 °C in autosampler and 7 days in −70 °C storage. Meanwhile, the method validation in rat tissue samples was similar to that in rat plasma samples, and the RSDs of intra- and inter-day precision from the three QC level samples were all within 15%.

**In vitro cytotoxicity**

The *in vitro* cytotoxicity of MXN-004 was evaluated individually on Caco-2, A549 and HepG2 cancer cell lines by MTT assay in comparison with irinotecan and their active metabolite SN38. The utilized concentrations were from 0.016 to 50 μM for MXN-004 and SN38, and 1.5625–200 μM for irinotecan. The half maximal inhibitory concentration (IC$_{50}$) values of MXN-004, irinotecan and SN38 for Caco-2, A549 and HepG2 cells were summarized in Table 1. The results showed that MXN-004, irinotecan and SN38 presented dose-dependent cytotoxicity on the three cell lines. MXN-004 consistently showed more potent (11.2- to 26.4-fold) cytotoxicity as compared to irinotecan, but less potent (2.25- to 8-fold) cytotoxicity than SN38 as shown in Table 1.

Table 1. The IC$_{50}$ (μmol/L) of MXN-004, irinotecan and SN-38 to Caco-2, A549 and HepG2 cells (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
<th>MXN-004</th>
<th>Irinotecan</th>
<th>SN-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Caco-2</td>
<td>1.6 ± 0.18</td>
<td>42.3 ± 2.17</td>
<td>0.4 ± 0.08</td>
</tr>
<tr>
<td>Lung</td>
<td>A549</td>
<td>2.4 ± 0.30</td>
<td>26.8 ± 0.68</td>
<td>0.3 ± 0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>HepG2</td>
<td>2.7 ± 0.72</td>
<td>34.8 ± 1.50</td>
<td>1.2 ± 0.47</td>
</tr>
</tbody>
</table>

**Pharmacokinetics**

After intravenous administration of an equimolar dose of MXN-004 and irinotecan (16 μmol/kg) to rats, the plasma concentration–time profiles of MXN-004 and irinotecan were presented in Figure 2, and the respective pharmacokinetic parameters were listed in Table 2. MXN-004 exhibited a rapid distribution phase compared with irinotecan, and the level of irinotecan could not be detected at 24 h after injection. As shown in Table 2, AUC$_{0–\infty}$, $T_{1/2}$, $C_{\text{max}}$ and distribution volume ($V_{ss}$) of MXN-004 were 0.45 ± 0.03 μg/mL, 11.42 ± 3.86 h, 0.15 ± 0.04 μg/mL and 671.74 ± 265.79 L/kg, respectively, while those of irinotecan were 2.19 ± 0.55 μg/mL, 1.87 ± 0.43 h, 1.22 ± 0.13 μg/mL and 12.50 ± 1.46 L/kg, respectively.

The main pharmacokinetic parameters of active metabolite SN38 were summarized in Table 2. Following intravenous administration of MXN-004 and irinotecan, the active metabolite SN38 of MXN-004 and irinotecan occurred immediately. The maximum plasma concentration ($C_{\text{max}}$) and AUC$_{0–\infty}$ of SN38 released from MXN-004 were 1.71 ± 0.57 μg/mL and 1.22 ± 0.39 μg/mL/h, respectively, whereas those bioconverted from irinotecan were 0.16 ± 0.04 μg/mL and 0.35 ± 0.01 μg/mL/h, respectively. Meanwhile, the $T_{1/2}$ of SN38 released from MXN-004 (4.79 ± 2.52 h) was 1.92-fold longer than that from irinotecan (2.49 ± 0.13 h). These results possibly suggested a longer tumor exposure time of SN38 released from MXN-004, which may indicate a potential for increased chemotherapeutic effect. The clearance rate of SN38 released from MXN-004 (5.58 ± 1.90 L/h/kg) was similar to that from irinotecan (8.25 ± 0.13 L/h/kg). The distribution volume ($V_{ss}$) of SN38 released from MXN-004 (34.26 ± 7.61 L/kg) and irinotecan (29.57 ± 1.28 L/kg) was very high.

**Tissue distribution**

The tissue distribution was shown schematically in Figure 4 after intravenous administration of MXN-004 and irinotecan (16 μmol/kg) separately at intervals of 0.25, 1.0 and 4.0 h. Both prodrugs showed rapid distribution to all major tissues.
Table 2. Plasma pharmacokinetic parameters after intravenous administration of MXN-004 and irinotecan at an equimolar dose of 16 μmol/kg to rats (mean ± SD, n = 4).

<table>
<thead>
<tr>
<th></th>
<th>AUC₀→∞ (μg/mL·h)</th>
<th>T₁/₂ (h)</th>
<th>Cmax (μg/mL)</th>
<th>CL (L/h/kg)</th>
<th>Vss (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MXN-004</td>
<td>0.45 ± 0.03</td>
<td>11.42 ± 3.86</td>
<td>0.15 ± 0.04</td>
<td>40.24 ± 3.07</td>
<td>671.74 ± 265.79</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>2.19 ± 0.55</td>
<td>1.87 ± 0.43</td>
<td>1.22 ± 0.13</td>
<td>4.81 ± 1.40</td>
<td>12.50 ± 1.46</td>
</tr>
<tr>
<td>SN38</td>
<td>1.22 ± 0.39**</td>
<td>4.79 ± 2.52</td>
<td>1.71 ± 0.57**</td>
<td>5.58 ± 1.90</td>
<td>34.26 ± 7.61</td>
</tr>
<tr>
<td>SN38b</td>
<td>0.35 ± 0.01</td>
<td>2.49 ± 0.13</td>
<td>0.16 ± 0.04</td>
<td>8.25 ± 0.13</td>
<td>29.57 ± 1.28</td>
</tr>
</tbody>
</table>

aActive metabolite SN38 released from MXN-004.

bActive metabolite SN38 bioconverted from irinotecan.

*p < 0.01 statistical significance compared with SN38b.

Figure 3. Plasma concentration–time profiles of SN38 released from both MXN-004 and irinotecan at an equimolar dose of 16 μmol/kg (A, SN38 released from MXN-004; ▲, SN38 bioconverted from irinotecan; mean ± SD, n = 4).

At 0.25 h post-administration, the average concentration of irinotecan was 21.11 ± 3.72 μg/g in the lung, 20.07 ± 2.05 μg/g in the spleen, 15.26 ± 3.24 μg/g in the kidney and 6.75 ± 0.53 μg/g in the liver. The concentrations were decreased quickly over the time (Figure 4A). By contrast, the average concentration of MXN-004 was highest in the lung (89.86 ± 38.86 μg/g), followed by the spleen (20.89 ± 4.75 μg/g), liver (18.40 ± 5.17 μg/g) and kidney (2.44 ± 0.53 μg/g), and the concentrations were decreased with time except the case in the lung (Figure 4B). The levels of irinotecan and MXN-004 in the brain were lowest of all tissues.

Following intravenous administration, the active metabolite SN38 of MXN-004 and irinotecan could be detected quickly as shown in Figure 5. At 0.25 h post-administration, the highest concentration of SN38 bioconverted from irinotecan was detected in the kidney (0.27 ± 0.11 μg/g), followed by the liver (0.12 ± 0.06 μg/g) (Figure 5A). Compared with irinotecan, the tissue distribution of SN38 released from MXN-004 was dramatically different and was similar to the parent drug, with specific accumulation in the lung (19.98 ± 8.62 μg/g, at 0.25 h). In addition, SN38 concentrations released from MXN-004 in the liver, spleen and kidney were 5.17 ± 1.27 μg/g, 4.87 ± 1.36 μg/g and 4.83 ± 0.51 μg/g at 0.25 h, respectively.

Discussion

Polyethylene glycol of low toxicity has been widely used as injectable formulations since approval by the FDA and EMEA (Salmaso et al., 2010). PEGylation is well known for its ability to increase the water solubility, prolong half-life and improve pharmacokinetic behavior of small-molecule compounds. To overcome the limitation of SN38 in solubility and enhance the anticancer effect compared with irinotecan, MXN-004, as a novel PEGylated 7-ethyl-10-hydroxycamptothecin (SN38), was synthesized. Hence, the aim of the present study was to evaluate and compare the in vitro cytotoxicity and the pharmacokinetic characteristics of MXN-004 and irinotecan in rats.

In vitro, MXN-004 showed more potent cytotoxicity compared with irinotecan on Caco-2, A549 and HepG2 cell lines. Meanwhile, the sensitivity of MXN-004 to these three cell lines was different, with the order of Caco-2 > A549 > HepG2. It was probably caused by the differences in transformation efficiency of SN38, intracellular uptake and transport of SN38 and resistance to SN38 (Sapra et al., 2008). It was reported that P-glycoprotein played an important role in efflux-based transport for both irinotecan and SN-38 (Ramesh et al., 2010). Also, it would be useful to know the interactions (substrate or inhibitor) of MXN-004 with P-glycoprotein as the interactions might impact its ability to penetrate tumor cells. There are no relevant published data at present, albeit, the transporting assay is being conducted in Caco2 cells and P-glycoprotein overexpressed cell line models, and will be displayed in the upcoming paper.

In pharmacokinetic studies, it was found that irinotecan eliminated faster ($T_{1/2} = 1.87 ± 0.43$ h) than MXN-004 ($T_{1/2} = 11.42 ± 3.86$ h). In addition, MXN-004 exhibited a much greater distribution volume ($V_{ss}$) than irinotecan simultaneously. This result suggested that MXN-004 might exhibit specific accumulation in vivo, while irinotecan might mainly distribute in the extracellular fluid. Their identical active metabolite SN38 occurred immediately after the administration, and the pharmacokinetics of SN38 released from MXN-004 was different from that bioconverted from irinotecan as shown in Figure 3. It was reported that the conversion rate of SN38 from irinotecan was about 50% in rodents (Meyer-Losic et al., 2008). Based on the hypothesis that MXN-004 was completely converted to SN38, the pharmacokinetic parameters of SN38 from irinotecan were obtained by considering the transformation efficiency of irinotecan. As shown in Table 2, the maximum plasma concentration ($C_{max}$) of SN38 released from MXN-004 was significantly higher ($p < 0.01$) than that released from irinotecan, and the AUC₀→∞ of SN38 released from MXN-004 was approximately 3.5-fold higher than that
released from irinotecan. SN38 released from MXN-004 exhibited a longer half-life than that bioconverted from irinotecan. It was reported previously that the uptake of liposomes by the reticuloendothelial system (RES) could be prevented by modifying with polyethylene glycol (Allen et al., 1991; Caliceti & Veronese, 2003), resulting in a longer half-life in the plasma of the modified compound. Accordingly, MXN-004 preparation was possible to avoid uptake by RES so that MXN-004 and its active metabolite SN38 kept a longer period of half-life than irinotecan. It was in accordance to Puja Sapra’s study that the plasma half-life of EZN-2208, a PEGylated conjugate of SN38, was extended considerably compared with irinotecan (Sapra et al., 2008). The high transformation rate of MXN-004 to SN38 and longer half-life resulted in the high exposure of active metabolite SN38, which was consistent with the results of in vitro cytotoxicity. The clearance of SN38 from irinotecan in the present experiment was comparable with that reported previously (Guo et al., 2010), which was close to that from MXN-004. The values of high distribution volume of SN38 were in accordance with the distribution of MXN-004 and irinotecan, indicating a significant tissue diffusion of the active metabolite (free SN38). For MXN-004, the PEGylation could probably improve the tendency of tissue targeting, which was more significant by comparison to irinotecan.

Irinotecan was mainly distributed in the rich blood flow organs, such as the liver, spleen, lung and kidney, as shown in Figure 4. The levels of irinotecan in tissues were quickly decreased along with the time. Unlike irinotecan, MXN-004 might have a certain accumulation in the lung, and the concentrations of MXN-004 in the lung were much higher than those of irinotecan (Figure 4). This was similar to the distribution of PEGylated nanostructured lipid carriers loaded with 10-hydroxycamptothecin, PEG-NLCs and PEG-40NLCs significantly improved the HCPT concentration in lung compared with HCPT solution and exhibited pulmonary targeting effect. It could be explained that PEG modification could effectively avoid capture of drugs by RES (liver, spleen), whereas the concentration of drugs in the non-RES, such as the lung, was dramatically increased (Zhang et al., 2008). Indeed, the pulmonary targeting effect of MXN-004 might be associated with the selective absorption of type I alveolar cell (Xiang et al., 2007). The alveolar epithelium is
compared to irinotecan. More potential agent with prolonged half-life of SN38 and pharmacokinetic analysis, MXN-004 was regarded as a smaller than that from MXN-004; the reason might be the bioconverted from irinotecan was not in line with the parent drug; it might be caused by the differences in the distribution of carboxylesterase in tissues. In the kidney, the concentration of irinotecan was much higher than that of MXN-004, but the concentration of SN38 released from MXN-004 was significantly improved. Moreover, MXN-004 actually exhibited improved systemic exposure of SN38, with greater distributing tendencies toward various tissues especially the lung, which was consistent with the parent drug MXN-004, whereas the distribution of SN38 bioconverted from irinotecan was not in line with the parent drug—because of the blood–brain barrier.

Conclusion
In summary, MXN-004 was more cytotoxic compared with irinotecan on Caco-2, A549 and HepG2 cell lines in vitro. In rats, MXN-004 and its active metabolite SN38 exhibited a longer half-life, and the plasma exposure of SN38 released from MXN-004 was significantly improved. Moreover, MXN-004 actually exhibited improved systemic exposure of SN38, with greater distributing tendencies toward various tissues especially the lung by comparison to irinotecan, which was probably ascribed to the raised high water solubility of MXN-004 or natural physicochemical property of the low-molecular-weight PEG. Based on the cytotoxicity and pharmacokinetic analysis, MXN-004 was regarded as a more potential agent with prolonged half-life of SN38 compared to irinotecan.

Acknowledgements
The authors are thankful to Chunfeng Wang, Wei Zhang and Yanli Wen for their help in this study.

Declaration of interest
The authors report no conflicts of interest.

References


