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RESEARCH PAPER

Docetaxel-Loaded Thermosensitive and Bioadhesive Nanomicelles as a Rectal Drug Delivery System for Enhanced Chemotherapeutic Effect

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ABSTRACT

Purpose To investigate the potential of thermosensitive and biadhesive nanomicelles in improving the bioavailability of docetaxel (DCT) and its chemotherapeutic effect.

Method DCT-loaded nanomicelles were prepared by emulsufication and characterized in terms of physico-chemical and visco-elastic parameters. The optimzed formulation was evaluated for *in vivo* localization, pharmacokinetic and anti-tumor efficacy.

Results The hydrodynamic size of DCT-loaded nanomicelles was approximately 13 nm and the nanomicelles exhibited a sufficient gelation strength (9250 mPa·s) and bioadhesive force (2100 dyn/cm^2) to be retained in the upper part of rectum. We observed a high rectal bioavailability of 29% DCT compared to that following oral administration in rats, as it successfully evaded the multidrug efflux transporters and hepatic first-pass metabolism. Plasma concentration around ~50 ng/mL was maintained throughout the study period (12 h) while Taxotere® attained subtherapeutic range within 4 h of drug administration. Results also revealed that the rectally administered DCT-loaded nanomicelles exhibited a significant anti-tumor effect (200 mm^3) with a reduced toxicity profile when compared to orally administered DCT (950 mm^3). Furthermore, histological study showed that the rectal mucosa was completely intact with no signs of irritation upon treatment with DCT-loaded nanomicelles.

Conclusions Taken together, our novel thermosensitive and biadhesive nanomicelles demonstrated the ability to improve the bioavailability and chemotherapeutic potential of DCT *in vivo*. To the best of our knowledge, this is the first report describing the rectal delivery of DCT-loaded nanomicelles.

KEY WORDS anti-cancer effect · docetaxel · liquid suppository · rectal delivery · thermosensitive and bioadhesive nanomicelles

INTRODUCTION

Docetaxel (DCT), an anti-mitotic taxane drug, is a widely used antineoplastic agent. DCT has shown clinical efficacy against many cancers, including breast, ovarian, and non-small lung cancers (1). However, its clinical use causes several side effects including musculoskeletal toxicity,

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neutropenia, neuropathy, and hypersensitivity reactions (2,3). Recently, a commercial injectable product, Taxotere®, has been shown to exhibit therapeutic efficacy, but it suffers from severe side effects both from drug and from the solvent system containing polysorbate 80 and ethanol, which are used to enhance the drug solubility (4,5).

To avoid these disadvantages, many researchers have studied the possibility of oral administration of DCT as an alternative dosage form. However, this dosage form resulted in poor oral bioavailability (less than 5%) in animals (1,6). Such a low oral bioavailability of DCT was attributed to its poor water solubility of about 5 μg/mL (1), multidrug efflux mediated by P-glycoprotein (7,8) and high hepatic first-pass metabolism (9). To overcome these problems, various oral formulations of DCT such as SMEDDS (6–8,10), prodrug (11,12), microcapsules including liposomes (13), solid dispersions (14) and nanoparticles (15) have been attempted to achieve therapeutically relevant DCT concentrations in plasma. Although the above formulations have their own advantages, their limitations far outweigh the benefits, and more importantly, none improved the oral bioavailability beyond 10%. In addition, our research group and others have used P-glycoprotein inhibitors such as curcumin, cyclosporine A, ketoconazole and interferon-alpha to improve the oral bioavailability of DCT (6,16–18). Although the oral bioavailability of DCT increased in some cases, pretreatment with P-glycoprotein inhibitors may lead to patient non-compliance and more side effects. Therefore, further efforts should be made to improve the bioavailability and to minimize the toxicity profile of DCT to enhance the therapeutic action.

In this regard, rectal administration of drug via suppository may be a viable alternative to oral administration to effectively counter bioavailability problems and to increase the therapeutic potential of any given drug. However, a conventional solid suppository will melt or soften in the rectum, and such suppositories will be uncomfortable for patients (19). Therefore, a thermosensitive liquid suppository that will gel (Sol → Gel) instantly at physiological body temperature and at the same time be bioadhesive to rectal tissues is an attractive prospect (20,21). Towards this goal, a novel DCT-loaded liquid suppository with easy administration, improved antitumor efficacy and less toxicity using thermosensitive and bioadhesive nanomicelles has been developed. The aim of this study was to investigate the potential of thermosensitive and bioadhesive nanomicelles and rectal administration to improve the bioavailability of DCT and, more importantly, the anti-tumor efficacy and toxicity profile of DCT. To the best of our knowledge, no report on rectal delivery of DCT-loaded nanomicelles exists in the literature to date.

MATERIALS AND METHODS

Materials

Docetaxel was obtained from Taihua Co (Xi'an, China). Poloxamer 188 (P188) and poloxamer 407 (P407) were procured from BASF (Ludwigshafen, Germany). Taxotere® was purchased from Sanofi-Aventis Korea (Seoul, South Korea). Tween 80 (polysorbate 80) was purchased from DC Chemicals (Seoul, South Korea). All other chemicals were of reagent grade and were used as supplied.

Preparation of Docetaxel-Loaded Liquid Suppository

Surfactant phase was prepared by dissolving a weighed quantity of docetaxel (0.25 g) into Tween 80 (10 g). The aqueous phase was prepared by dissolving P 188 (15 g) and P 407 (11 g) in ultra pure water (63.75 g) at 4°C. The surfactant phase containing docetaxel was then gently poured into the poloxamer solution with continuous agitation. The whole solution was kept at 4°C until the solution became clear.

Dynamic Light Scattering Measurements

Hydrodynamic size, size distribution and ζ-surface charge of the nanomicelles were determined at 25°C by dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments, U.K.) equipped with a He–Ne laser that operated at a wavelength of 635 nm. Measurements were performed at a fixed scattering angle of 90°. Software (version 6.34) that employs cumulants analysis was provided by the manufacturer and used to analyze the size, polydispersity index (PDI), and ζ-surface charge.

Transmission Electron Microscopy (TEM)

The morphology of the liquid suppository was examined using a transmission electron microscope (TEM, Hitachi H-7600, Tokyo, Japan) at an accelerating voltage of 100 kV. Briefly, a drop of micellar preparation was applied to a carbon-coated copper grid and the particles were allowed to adhere to the carbon substrate. This was followed by the addition of 2% phosphotungstic acid (PTA) solution for negative staining.

Gelation Temperature

Gelation temperature was measured as reported previously (22). Briefly, 2 g of liquid suppository was placed in a 10 mL transparent glass vial along with a magnetic bar (10 × 3 mm). The glass vial was placed in a low-temperature water bath and a digital thermometer (SK-1250MCII, Sato, Japan) was immersed in the liquid suppository. The liquid suppository

was gradually heated at constant rate of 1°C/min with a constant stirring of 50–80 rpm. The temperature was raised from 20°C to 40°C. The point at which the magnetic bar stopped rotating was noted as the gelation temperature.

Gelation Time and Gel Strength

The gel strength is the viscosity of the liquid suppository at physiological body temperature. A modern rheometer MCR 301 (Physica, Germany) was employed to perform rheological studies on the DCT-loaded liquid suppository. The experiment was conducted at two different temperatures: 25°C and 36.5°C. A circulating water bath (TC10, Germany) was used to control the temperature and software (UDS 200) provided by the manufacturer was employed to calculate the rheological parameters. The equipment was set up as reported previously (23).

Bioadhesive Force

Female New Zealand rabbit weighing about 2 kg was fasted for 24 h before the experiments but allowed free access to water. This rabbit was sacrificed and its rectum was sectioned. The bioadhesive force of the liquid suppository was examined as reported previously (21). Briefly, a tissue section was cut from the fundus of the rabbit rectum and placed in a transparent glass vial. The glass vial with the tissue section was stored at 37±0.5°C for 10 min. A modified balance was connected to one end of the tissue in the glass vial while the other end of the tissue was connected to a vial that was placed in an adjustable pan. The liquid suppository was placed on the rectal tissue in the vial. The weight at the other end of the balance was consistently increased until both vials detached. This detachment force (dyne/cm^2) or bioadhesive force was calculated from the minimal weights that detached the two vials.

Pharmacokinetic Study

Animals

The experimental protocols for the animal study were approved by the ethics committee of Seoul National University. Male Sprague–Dawley rats (approximately 250 ±20 g body weight, Orient Bio. Inc.; Seungnam, South Korea) were selected and fasted for 24 h before the commencement of experiments. Each rat was administered intravenously or orally with a docetaxel solution, or rectally with liquid suppositories.

Administration and Blood Collection

The rats were divided into three groups and fasted for 24 h prior to the experiments. The rats were anesthetized with diethylether and polyethylene tubing was inserted into the surgically bisected right femoral artery of the rat. The rats in first group were rectally administered with a DCT-loaded liquid suppository [(DCT/P407/P188/Tween 80 (0.25/11/15/10%)] at a dose of 2 mL/kg (5 mg/kg as docetaxel). Liquid suppository was administered into the rectum of rats at 4 cm above the anus with a 45° slope using a stomach sonde needle (22). For comparison of bioavailability, the rats in the other groups received DCT solution orally at a dose of 30 mg/kg as DCT (six-fold higher concentration than the liquid suppository) or intravenously at the same dose of 5 mg/kg as DCT. The DCT solution was prepared by diluting the commercial injectable product (Taxotere®) with 13% ethanol in water prior to injection. Serial blood samples (0.3 mL) were collected from the right femoral artery at specified intervals. The blood samples were centrifuged at 3,000 g for 10 min and then plasma was separated.

Blood Sample Analysis

Plasma (150 μL) were mixed with acetonitrile (1.5 mL) and 50 μL of propyl paraben (5 μg/ml) were added as an internal standard. The mixture was centrifuged at 3000 g for 10 min and the supernatant was separated and subjected to centrifugal evaporation (EYELA CVE-200D, Tokyo, Japan) at 40°C. The residue obtained after the evaporation was reconstituted with 100 μL of mobile phase and quantified by HPLC (Hitachi, Tokyo, Japan). The column was Intensil C8 (GL science, 3.5 μm, 15 cm×0.46 cm) with a UV/Vis detector (Model L-2420) set at a wavelength of 232 nm with a flow rate of 1.0 mL/min. Acetonitrile and phosphate buffer (pH 5) at a volume ratio of 49/51 was used as the mobile phase (24).

In Vivo Anti-Tumor Efficacy

Female Cg-Foxnl-nu/CrljBgi nude mice (Orient Bio. Inc.; Seungnam, South Korea) weighing 15±1 g were separately housed in the cages at a temperature of 20–24°C and a relative humidity of 55±10% for 1 week prior to the experiments. Each mouse was administered intravenously or orally with a docetaxel solution, or rectally with liquid suppositories. The experimental protocols for the animal study were approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

The antitumor effects of oral/IV docetaxel solution and DCT-loaded liquid suppository were studied in 5-week old female Cg-Foxnl-nu/CrljBgi nude mice (Orient Bio. Inc., Seungnam, South Korea). For tumor induction, 1×10^6 KB cells in 0.1 mL of PBS were subcutaneously injected into the right flank (7). This day was designated as day 0 and the treatment of nude mice started when the tumor volume reached 100–150 mm^3 (day 7). The mice were divided into nine groups with five mice in each group. The first three groups

respectively received phosphate-buffered saline solution (PBS, pH 7.4), suppository base, and DCT-loaded liquid suppository via rectal route at a dose of 5 mg/kg as DCT. Similarly, the remaining six groups received PBS, respective solution base, and DCT solution orally or intravenously through the tail vein. In this study, DCT suspension via rectal route was not considered as an additional control since the present work aimed at comparing the bioavailability from oral and rectal routes and viz-a-viz its anti-tumor potentials. When the tumor volume grew approximately 100–150 mm^3, above formulations were administered to the 9 groups of mice on days 7, 10, and 13. The length and width of the tumor in each mouse were measured using calipers. The anti-tumor effect of the DCT-loaded liquid suppository was compared to that of orally and intravenously administered DCT solution in the tumor-bearing mice by observing tumor volume reduction and measuring total body weight.

Identification of In Vivo Localization

DCT-loaded liquid suppository was mixed with 0.1% blue lake dye and administered to the rectum of rats at a dose of 1.5 g/kg. The localization of DCT-liquid suppository in the rectum was identified by the presence of the blue dye at 5 min and 12 h after administration (22).

Histological Analysis

The DCT-loaded liquid suppository was administered into the rectum of rats on days 0, 2, and 4 as mentioned earlier. On days 1, 3, and 5, the rectum was isolated, washed, fixed with 10% neutral carbonate-buffered formaldehyde solution and embedded in paraffin. The paraffin was sliced, and the sections were stained with Haematoxylin and Eosin dye for 15 min (25). The quantum of stain was observed under a light microscope (Nikkon E400; Tokyo, Japan). The drug treated rectal tissues were compared with fresh rectal epithelium.

Statistical Analysis

All data were expressed as mean ± S.D. Data were statistically analyzed by ANOVA with Student-Newman-Keuls post-hoc test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Physicochemical Characterization of DCT-Loaded Liquid Suppository

The DCT-loaded thermosensitive liquid suppository was successfully prepared with 0.25% DCT, 15% P188, 11% P407 and 10% Tween 80 after optimizing several key process and formulation variables (22,26). The rate and extent of permeability of particles across the mucosa depends on their size range. The DLS analysis showed that the micelles were around 13.1 ± 1.4 nm with a polydispersity index of 0.2 as shown in Fig. 1a. Further, the size and shape were confirmed by TEM image, which showed a perfect, well-maintained spherical shape with a clear boundary between each particle (Fig. 1b). The TEM results were consistent with the average particle size observed in DLS. The formed micelles were physically and chemically stable for more than six months at room temperature (data not shown). The gelation temperature was 33.0 ± 0.4°C as shown in Fig. 1c. The viscosity of the liquid suppository gradually increased as the temperature rose from 10°C to 40°C and was around 196 ± 2 mPa·s at 25°C with a gel strength of 9,250 ± 50 mPa·s and gelation time of 7.5 min. When placed at physiological temperature, 36.5°C, the nanomicelles formed a gel phase instantly due to a relatively fast gelation time and aqueous gel strength (Fig. 1c). The bioadhesive force of the optimized formulation was 2,100 ± 140 dyn/cm^2.

Pharmacokinetic Parameters

In order to compare the pharmacokinetic behavior of DCT-loaded nanomicelles-based liquid suppository to that of oral and IV docetaxel solution, we carried out pharmacokinetic studies in rats. In this study, the DCT solution for oral and IV administration was prepared by diluting the commercial injectable product (Taxotere®) with 13% ethanol in water for injection. The IV DCT solution is used as a control in order to determine the absolute bioavailability of this liquid suppository via rectal route and compare to present commercial product. Furthermore, the oral DCT solution is used as the other control for checking the effect of liquid suppository on the multidrug efflux mediated by P-glycoprotein via rectal route. Docetaxel was entirely soluble in the oral DCT solution, leading to solving the poor water-solubility of DCT in the oral preparation. However, this DCT solution was predicted to be poorly absorbed in GI tract due to multidrug efflux mediated by P-glycoprotein. In addition, the rectal DCT solution was not considered as another control, because it is impossible to administer the clear DCT solution rectally due to a very low viscosity and lack of adequate visco-elastic properties to withstand in the upper part of rectum.

Figure 2 depicts the plasma concentration-time curves of DCT after oral and intravenous administration of DCT solution, and rectal administration of DCT-loaded liquid suppository to rats. The oral dose of DCT was fixed at 30 mg/kg compared to 5 mg/kg for the intravenous and rectal routes, because DCT at lower dose was undetectable in the plasma due to its low absorption rate after oral administration. IV dose was kept low in order to avoid the

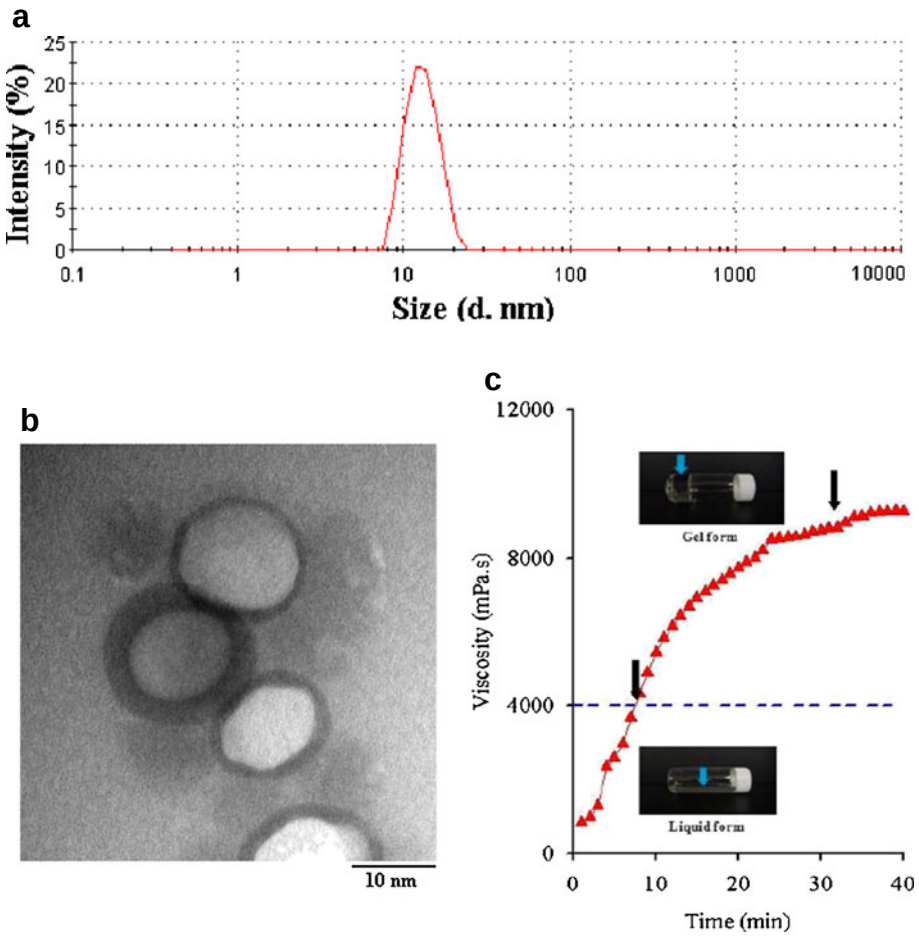

Fig. 1 (**a**) Particle size distribution and (**b**) TEM images (20,000×) of docetaxel-loaded nanomicelles illustrating monodispersed spherical particles. (**c**) Rheological behavior of docetaxel-loaded nanomicelles-based liquid suppository at 36.5°C.

unnecessary drug-related systemic side effects. The plasma concentration of DCT rapidly slumped to <10 ng/mL at 4 h after intravenous administration of DCT solution, while it was approximately 100 ng/mL at 1 h after the oral administration and gradually decreased until 8 h. However, DCT attained a maximum plasma level of about 140 ng/mL at 1 h after rectal administration of the liquid suppository and maintained a high level of 50–80 ng/mL until 8 h. The plasma levels of DCT from rectal route were significantly higher than those from the oral or intravenous route until 12 h ($P<0.05$).

The corresponding pharmacokinetic parameters are listed in Table I. As shown in Table I, DCT from rectal route has relatively high $t_{1/2}$ and low elimination rate (K_{el}) compared to that from the other two routes and formulations. To be specific, even though the dose of the liquid suppository was six-fold lower than that of the oral solution, the liquid suppository showed a 1.25-fold higher AUC than the oral solution. In addition, the liquid suppository exhibited a 10-fold higher absolute bioavailability (BA) (29.0%) than the oral solution. Such remarkable success complies with our hypothesis that liquid suppository administered via the rectal route will increase the bioavailability of DCT.

In Vivo Anti-Tumor Efficacy

The anti-tumor efficacy of rectally administered DCT-loaded liquid suppository was compared with that of DCT solutions

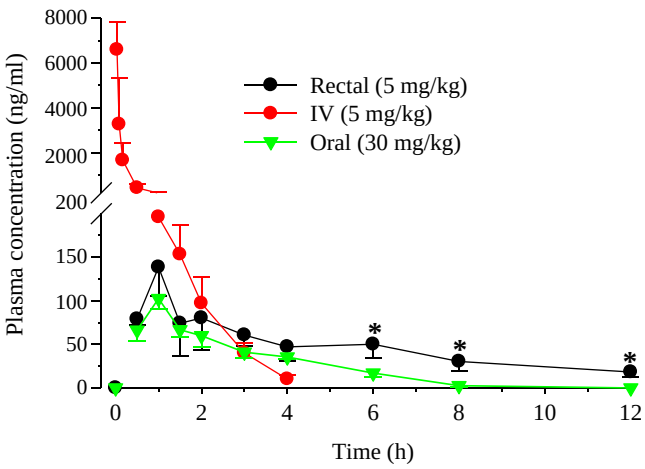

Fig. 2 Plasma concentration-time profiles of rectal docetaxel-loaded liquid suppository, oral docetaxel solution, and intravenous docetaxel solution in rats. The docetaxel-loaded liquid suppository was composed of docetaxel/P407/P188/Tween 80/0.25/11/15/10%. Each value represents the mean ± S.D ($n=6$). * Significantly smaller than docetaxel solution group ($p<0.05$).

Table I Pharmacokinetic Parameters of Rectally Administered Docetaxel Liquid Suppository, Oral Docetaxel Solution and Intravenous Docetaxel Solution

Parameters	Rectal (5 mg/kg)	Oral (30 mg/kg)	IV (5 mg/kg)
AUC (h·ng/ml)	455.6 ± 103.1	364.5 ± 40.8	1573.0 ± 481.6
T_{max} (h)	0.98 ± 0.10	0.58 ± 0.24	–
C_{max} (ng/ml)	138.6 ± 32.7	104.9 ± 11.5	11162.9 ± 4118.1
$t_{1/2}$ (h)	4.0 ± 0.6	2.3 ± 0.8	0.6 ± 0.1
K_{el} (h^{-1})	0.17 ± 0.03	0.30 ± 0.09	0.63 ± 0.11
Absolute bioavailability (%)	29.0	2.8	–

Absolute bioavailability(%) = $(AUC_{oral}/Dose_{oral})/(AUV_{iv}/Dose_{iv}) \times 100$
Each value represents the mean ± S.D. ($n = 6$).

administered orally or IV in tumor-bearing nude mice (Fig. 3). Phosphate-buffered saline (PBS) and the respective bases were used as controls. As seen in Fig. 3a–c, DCT induced a significant reduction in tumor volume compared to PBS and their respective bases. Neither PBS nor the respective bases had any effect on tumor growth, and the maximum tumor volume was attained with each. The tumor size was similar for all groups until day 7 when they received the first injection. On the other hand, mice given with the liquid suppository had significant smaller tumor volumes than did controls after 20 days (Fig. 3a; $P<0.01$ at 20 days, $P<0.05$ at 23–30 days). In Fig. 3b, mice given the IV solution showed smaller tumor volumes compared to mice treated with phosphate buffered saline and the solution base. Moreover, there were statistically significant differences in tumor volume between the IV solution and controls after 17 days (Fig. 3b; $P<0.01$ at 17 days, $P<0.05$ at 20–30 days). Our results suggest that the liquid suppository and the IV solution had very effective antitumor efficacy, even if the former is less effective than the latter. In contrast, the tumor volume in orally administered group increased constantly and showed no difference from the tumor volume of the plain solution base. Furthermore, the liquid suppository and IV DCT solution induced a significant 4-fold reduction in tumor size compared to 1.5-fold by the oral DCT solutions (Fig. 3d). This observation further reinforces the fact that a liquid suppository can remarkably increase the bioavailability and the anti-tumor effect of DCT in tumor-induced mice.

Qualitative *In Vivo* Identification

The localization of liquid suppository in rectum was observed after mixing 0.01% of blue lake dye to liquid suppository. The blue color of the suppository was clearly seen in the rectum after 5 min of administration (Fig. 4a). However, at 12 h, the blue color of suppository faded due to dilution in the body fluid (Fig. 4b). It is worth noting that although the blue color faded with time, the position of the gelled liquid suppository in the rectum did not change significantly.

Safety

The change in body weight was considered an indicator of the toxicity profile of drug treatment. The same groups of mice that were used for anti-tumor studies were monitored

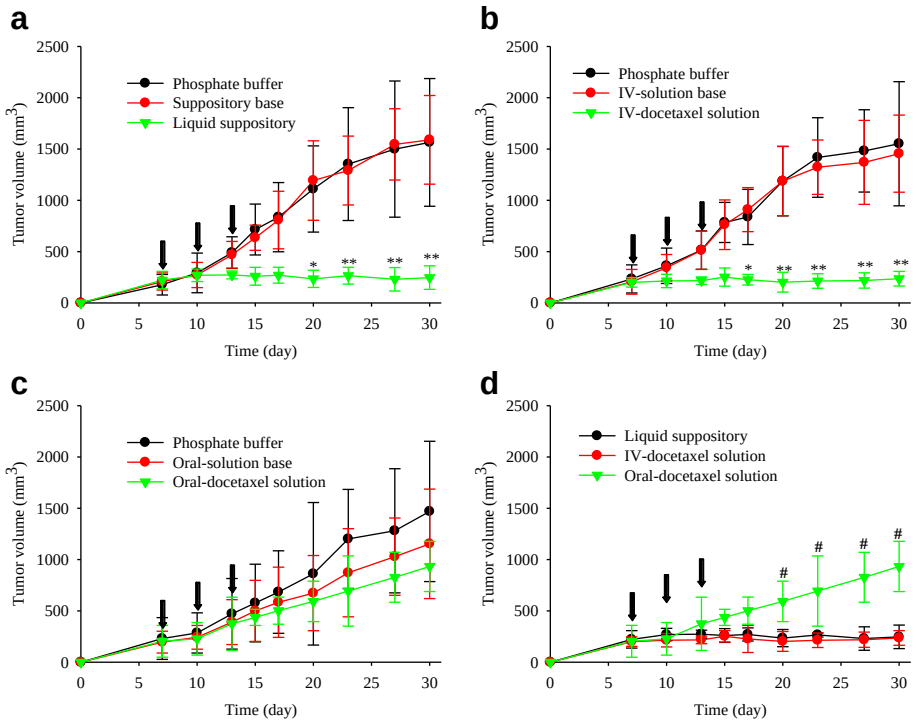

Fig. 3 Antitumor efficacy of (**a**) rectally administered docetaxel-loaded liquid suppository, (**b**) intravenously administered docetaxel solution and (**c**) orally administered docetaxel solution to tumor-bearing nude mice on days 7, 10 and 13. The last graph (**d**) represents the comparative antitumor effects of all three formulations. *Significantly smaller than PBS and suppository base ($p < 0.05$). **Significantly smaller than PBS and suppository base ($p < 0.01$). #Significantly larger than docetaxel-loaded liquid suppository and intravenous docetaxel solution ($p < 0.05$). Each values represents the mean ± S.D. ($n = 5$).

for body weight change. As shown in Fig. 5, throughout all groups, PBS and the respective formulation bases had no effect on the body weight changes. The mice treated with DCT-loaded suppository and IV DCT solution did lose some weight on days 10 and 13 following the 2nd and 3rd drug treatments. However, the liquid suppository led to body weight loss compared to phosphate buffered saline and the solution base, but there were no significant differences except at 13–15 days (Fig. 5a; $P<0.01$ at 13 days, $P<0.05$ at 15 days). Our results indicate that the liquid suppository did not lead to body weight loss until day 10, followed by body weight loss at 13–15 days. Then, from 17 days, body weight gradually recovered, since there were no significant differences in body weight loss between mice given the liquid suppository and controls. Similarly, the intravenously administered DCT solution led to body weight loss compared to phosphate buffered saline and the solution base. It led to significant body weight loss at 13–20 days (Fig. 5b; $P<0.01$ at 13–17 days, $P<0.05$ at 20 days). Therefore, it caused no body weight loss until day 10, followed by body weight loss from 13 to 20 days, and body weight recovery from day 23. These results suggest that this formulation resulted in body weight loss for a longer period of time compared to the liquid suppository. Thus, the liquid suppository reduced the side effects compared to the IV solution. The oral administration of DCT solution did not induce any body weight loss; indeed, these mice had gained 20% of body weight at the end of the study (Fig. 5c), and the orally administered DCT solution had no antitumor efficacy due to poor absorption of DCT. Regardless of the fact that DCT leads to loss of body weight, DCT-loaded liquid suppository was quite safe even after repeated administration.

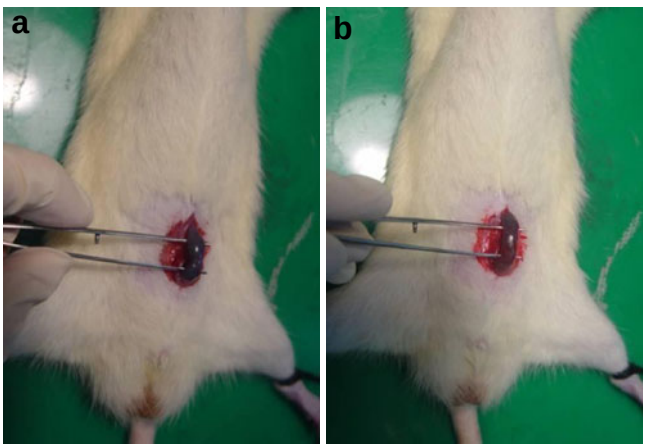

Fig. 4 *In vivo* localization of the docetaxel loaded liquid suppository in the rectum at (**a**) 5 min and (**b**) 12 h after rectal administration. The docetaxel-loaded liquid suppository composed of docetaxel/P 407/P 188/Tween 80 (0.25/11/15/10,%) with 0.01% blue lake was administered into the rectum of a rat. The localization of the liquid suppository in the rectum was identified by a *blue color*.

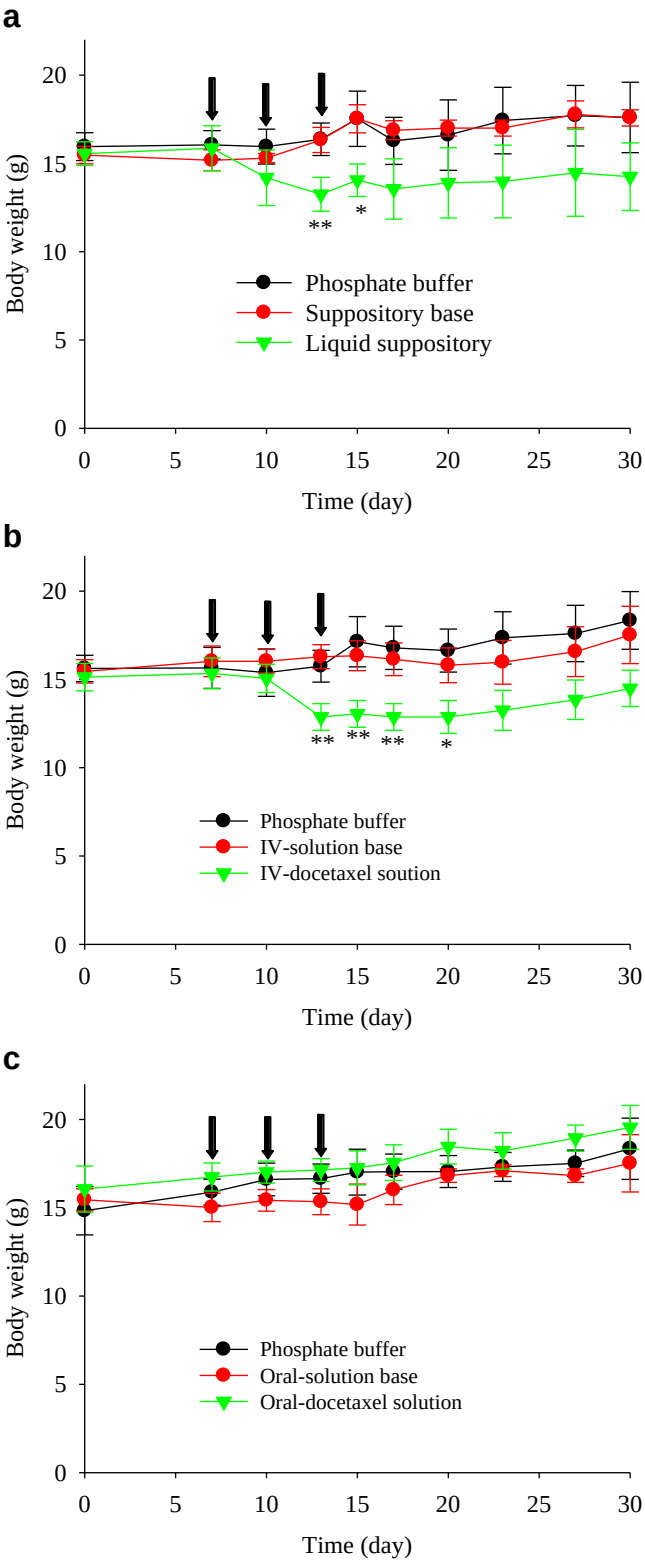

Fig. 5 Body weight changes of tumor-bearing nude mice after intravenous administration of (**a**) rectal administration of docetaxel-loaded liquid suppository, (**b**) intravenously administered docetaxel solution and (**c**) oral administration of docetaxel solution according to a dose schedule regimen of three administrations on days 7, 10 and 13. *Significantly smaller than PBS and suppository base ($p<0.05$). **Significantly smaller than PBS and suppository base ($p<0.01$). Each value represents the mean ± S.D. ($n=5$).

The irritation index of rectal tissue in response to DCT-loaded liquid suppository was investigated at 1, 3 and 5 days after the repeated administration of the suppository base (Fig. 6d, f and h) or liquid suppository (Fig. 6c, e and g) on days 0, 2, and 4, respectively (Fig. 6). No remarkable differences between control (Fig. 6a and b) and suppository-treated group were observed upon the repeated administration of DCT-loaded liquid suppository at several time points. No signs of severe irritation such as hemorrage or epithelial necrosis were observed. In addition, the morphology and structure of rectal mucosa or colonic gland layer remained similar throughout the treatment period. Thus, the morphology of the rectal tissues suggested that the repeated rectal administration of this liquid suppository did not cause irritation or damage (25).

DISCUSSION

Docetaxel is a widely used potent anti-neoplastic agent effective against a broad spectrum of cancers. DCT binds to β-tubulin and thereby induces cell cycle arrest, which leads to cell apoptosis (27). However, its low bioavailability and limited solubility in water make it a poor choice of drug for oral administration (2). In addition, high hepatic first-pass metabolism and P-gp mediated multidrug efflux contribute to its inefficiency via the oral route. Further, DCT in injectable formulations such as 'Taxotere®' is known to cause pronounced side effects such as fluid retention, neutropenia, and neurotoxicity due to the solvent system and the drug itself (4).

In this regard, rectal delivery is preferred over the oral route as the drug is immediately delivered to systemic circulation, first-pass metabolism is avoided and the rectum has very few P-glycoprotein membrane transporters (10,28). A conventional solid suppository would be highly uncomfortable to patients as it will melt or soften over a period of time, which may lead to patient refusal. In a preliminary study, DCT was not absorbed by the rectum of rats from a solid suppository (comprised of 0.25% DCT in 99.75% PEG 6000) because the drug failed to dissolve in it; moreover, the solid suppository was non-bioadhesive to the rectal mucosa (data not shown). Thermo-reversible liquid suppository, on the other hand, would gel instantly at physiological body temperature and would also be adhesive to the rectal mucosa (29). Earlier, our research group compared the potential of acetaminophen-loaded conventional solid suppository and liquid suppository in human subjects. Although plasma profile of solid suppository was equally effective to that of liquid suppository, liquid suppository gave significantly faster time to reach the maximum plasma concentration (T_{max}) due to its fluidity and bio-adhesive force. Thus, liquid suppository would allow faster absorption of drug and eventually the gel will be cleared from the body with time without causing any discomfort to the patients. Taking all this into consideration, we attempted to develop a novel thermosensitive and bioadhesive DCT-loaded nanomicelles based liquid suppository to overcome the aforementioned limitations.

One of the prerequisites of a liquid suppository is that the gelation temperature should range from 30–36°C (21). Anything less than this will lead to gelation at room

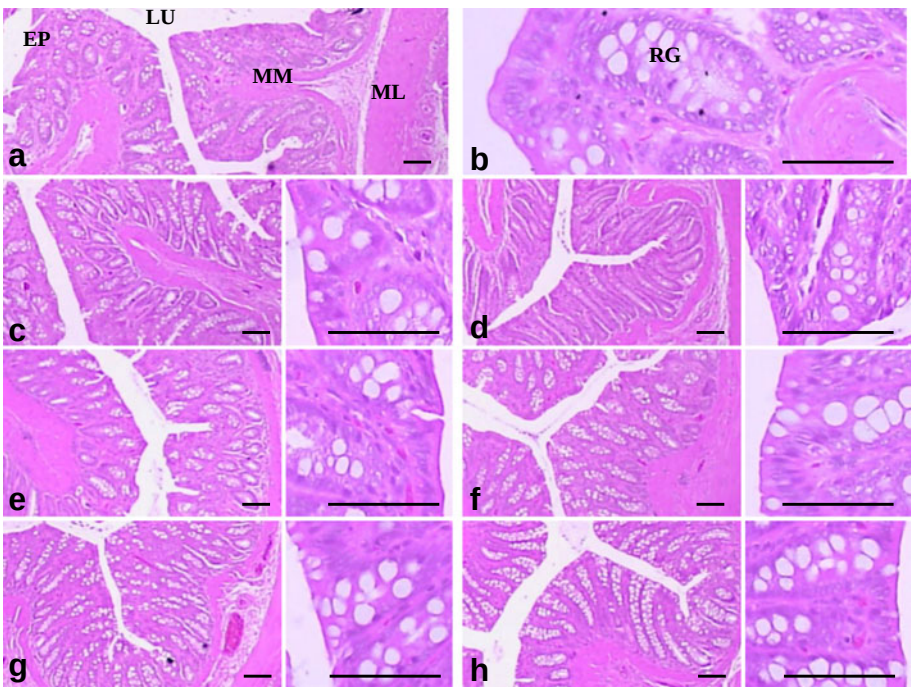

Fig. 6 Morphology of the rectal mucosa of rats after consecutive rectal administration of the DCT-loaded liquid suppository and suppository base: (**a**,**b**) untreated rats before administration (control); (**c**,**d**) DCT-loaded liquid suppository and suppository base on day 1; (**e**,**f**) DCT-loaded liquid suppository and suppository base on day 3; (**g**,**h**) DCT-loaded liquid suppository and suppository base on day 5. The liquid suppository was repeatedly administered at a dose of 1.5 g/kg into the rectum of rats at 4 cm above the anus using a stomach sonde needle on three consecutive days (days 0, 2 and 4). Each rectum was isolated on days 1, 3 and 5, respectively. *EP* Epithelium; *LU* lumen; *RG* rectal gland; *MM* muscularis mucosa; *ML* muscle layer. All H&E stain; scale bars = 160 μm.

temperature, making it difficult to handle and manufacture, while a gelation temperature higher than 36°C will cause a leakage problem so that the drug is not released inside the body (30). Therefore, in this study, poloxamer mixtures of P 407 and P 188 were selected as liquid suppository bases in order to form thermosensitive nanomicelles that can gel (33°C) below the physiological temperature of the body (31). In addition, P 407 and P 188 have favorable characteristics for drug delivery such as cytocompatibility, low toxicity profiles, low levels of skin irritation, and high solubilizing capacity. Tween 80 (10%) was selected due to its ability to form a eutectic mixture with DCT (32). The eutectic mixture was reported to be more soluble in water than the drug itself (21). In addition, it acts as an effective absorption enhancer in the rectum for poorly water soluble drugs.

Syringe-ability is an important factor for successful injection of a liquid suppository in the body. The viscosity of the liquid suppository was 300 mPa.s at 25°C, allowing easy administration through a syringe. Generally, there is an optimum threshold level for syringe ability, below which it is easy to administer without breaking sonde and syringe, but above which it becomes difficult to administer and both parts of the syringe separate.

Faster gelation time and higher gel strength are critical for the liquid suppository to stay in the rectum without leaking from the anus. The gelation threshold for the poloxamer-based liquid suppository is 4000 mPa·s at 36.5°C (22). Any suppository whose gel strength is below this viscosity level will eventually flow like a liquid and cannot form a thermosensitive gel; however, above this, it will form a stronger gel with good intrinsic gel strength. The time required to change from the liquid state to the gel state is referred to as gelation time. In other words, it is time taken by the liquid suppository to reach a minimum viscosity level of 4000 mPa·s at 36.5°C. In this study, we observed a gel strength of 9250 mPa·s with a gelation time of 7.5 min, satisfying the criteria for an effective thermo-reversible gel formation. Such high gel strength indicates the presence of optimized levels of individual constituents in this formulation.

Similar to the above mentioned physical parameters, the bioadhesive force is one of the important design criteria that determine the success of any liquid suppository. Generally, a strong bioadhesive force can prevent the gel from reaching the anus (22). Furthermore, a longer time of contact of gelled suppository with rectal mucosa will increase the drug absorption, which is very important in the case of cancer chemotherapy. The poloxamer-based nanomicelles exhibited sufficient bioadhesive force (2100 dyn/cm^2) to be retained in the upper part of rectum. The poloxamers (P 407 & P 188) exhibited bioadhesive properties because their oxyethylene and oxypropylene functional groups form hydrogen bonds with the sialic acid of oligosaccharide chains on the rectal mucous membranes (20). As the poloxamer nanomicelles in the gelled suppository provided an excellent bio-adhesive force, they did not reach the end of the colon and thus avoided the P-glycoprotein pump pathway and hepatic first-pass metabolism.

The DCT-loaded liquid suppository [DCT/P 407/P 188/Tween 80 (0.25/11/15/10%)] gave poloxamer nanomicelles with a size of about 10 nm, and the following gel properties: gelation temperature, 33.0°C; viscosity at 25°C, 196 mPa·s; gel strength, 9,250 mPa·s; gelation time, 7.5 min; bioadhesive force, 2,110 dyn/cm^2. Thus, this liquid suppository with thermosensitive and bioadhesive nanomicelles was easy to administer rectally, which should alleviate any discomfort and refusal during application. It gelled rapidly in the body, and did not leak out from the anus. Moreover, it attached to the rectal membranes as bioadhesive nanomicelles until drug absorption was complete, without reaching the end of the colon, since the bioadhesive force of the poloxamer nanomicelles was strong enough to hold the gelled suppository in the rectum for a long time.

The pharmacokinetics of DCT-loaded liquid suppository was compared with those of DCT solutions via oral and IV routes (Fig. 2). The plasma concentration-time profiles clearly demonstrated that the concentration of DCT from the liquid suppository was significantly higher than that from DCT solution. Further, a high plasma concentration was maintained for 12 h as the prolonged duration of contact between the suppository and mucosa ensured that the DCT was absorbed slowly but consistently. These results revealed that the liquid suppository attained 1.25-fold higher AUC than the oral solution, even though the dose of the former (5 mg/kg) was six-fold lower than that of latter (30 mg/kg). Similarly, the liquid suppository exhibited a remarkably higher bioavailability (BA) than oral DCT solution. The elevated BA was attributed to combination of various factors such as excellent bio-adhesive force, high gel strength, and nanomicellar size of the particles which may contribute to its increased uptake in rectal mucosa (19). In addition, a layer of Tween 80 around the nanomicelles protected the DCT from being recognized by multidrug efflux transporters and increased its solubility in the rectal fluid (33,34).

One of the main aims of this study was to increase the tumor killing potential of DCT. The dose of DCT administered via oral and rectal route was 30 mg/kg and 5 mg/kg which correspond to 90 mg/m^2 and 15 mg/m^2 in a human weighing 65 kg (27). The DCT from the liquid suppository exhibited a significantly higher anti-tumor efficacy than the oral solution as it avoided the hepatic first-pass metabolism and P-gp mediated efflux transporter, which is much higher in the GI tract. The tumor volume was around 200 mm^3 in mice treated by liquid suppository and IV injection, which was significantly ($P<0.001$) smaller than the tumor volume of 950 mm^3 in mice treated with oral DCT solution; those tumors grew rapidly and were severely ulcerated before the

end of the study (Fig. 3). No anti-tumor effect was observed for PBS and suppository bases. Although the mechanism of action of IV Taxotere® is not clear yet a combined effect of passive targeting and enhanced sensitization of cancer cells to high DCT could be the reason behind the significant suppression of tumor growth. Tumor volume reduction in case of Taxotere® may also attribute to high toxicity of DCT where the overall mice are weak and tumor could not grow. In case of DCT-loaded nanomicelles, suitable size, core shell architecture, micellar system could confer the enhanced uptake of DCT in tumor cells and so is the tumor suppression. The high anti-tumor effect of DCT-loaded liquid suppository was also attributed to its ability to prolong the half-life of the drug and to reduce the elimination rate constant, which could increase drug accumulation at cancer sites. The liquid suppository showed a steep-dose response curve and maintained the high dose intensity required to ensure its therapeutic success (35). Furthermore, enhanced permeability and retention (EPR) also contributed to its higher drug level inside the tumor cells. However, it is worth noting that, we got an astonishing anti-tumor efficacy result without any specific targeting agent; a mechanistic study is the subject of our ongoing investigation (36).

The very high tumor volume and no weight reduction in animals treated with oral DCT were a proof that a negligible amount of drug was absorbed through the GI tract (37). With the other two formulations, the mice experienced a slight weight loss (10%) following drug administration on the 13^{th} day, which was significant compared to the control group (Fig. 5). However, in the case of the suppository, the weight was gradually recovered before the end of study, strongly suggesting that the suppository was safer. Thus, IV injection (Taxotere®) and liquid suppository induced the same level of tumor regression and a low toxicity profile at a dose of 5 mg/kg, which is presumed to be the optimal dose for epithelial cell carcinoma. It should be noted that Taxotere® exhibited moderate to severe toxicity in other studies (36), but it was relatively safe in our study. This could be due to the difference in dose (5–30 mg/kg) in different studies and its dose-dependent effects.

On repeated administration, the liquid suppository did not irritate or damage the rectal tissues of rats (Fig. 6). Previously, the non-ionic surfactants poloxamers and Tween 80 were reported to be inert, resulting in no damage to mucous membranes (25). However, DCT might irritate mucous membranes due to its cytotoxicity (7,15,24). The lack of irritation associated with repeated administration of liquid suppoistory containing DCT might be explained by the low DCT content (0.25%), which is lower than the tissue-damaging threshold (22). Our results indicated that DCT gave similar antitumor efficacy and reduced the toxicity induced by the drug after anministration to the rectum compared to the commercial DCT-loaded injectable product. Furthermore, in rectal administration, Tween 80 shows no side effects such as hypersensitivity and fluid retention induced by injectable administration (38). P 407 and P 188 are ingredients frequently used in rectal product membranes (20–22). Thus, unlike the commercial DCT-loaded injectable product, this DCT-loaded liquid suppository contained no ingredients that might produce severe side effects in patients due to rectal administration. Moreover, we did not observe any faecal related side effects in liquid suppository-treated mice groups and were healthy throughout the study period. Like general suppository dosage form, this suppository could stay in the rectum for at least 12 h (Fig. 4), followed by evacuation with faces out of the anus within 12 h (20,22). For its practical development, a bowel preparation for inserting in the anus can be designed as a syringe type with long inserting part. Moreover, it should be rectally administered before bedtime as its rectal administration is of less patient compliance and it requires fasting for a long time. Overall, our results indicate that the DCT-loaded liquid suppository shows superior anti-tumor efficacy and is well-tolerated with no obvious side effects, which is very important for its future clinical application.

CONCLUSION

The novel DCT-loaded liquid suppository [DCT/P407/P188/Tween 80 (0.25/11/15/10%)] with thermosensitive and bioadhesive nanomicelles was successfully prepared to improve the bioavailability and anti-tumor potential of DCT. The present study clearly showed that the DCT-loaded nanomicelles-based liquid suppository possessed sufficient bioadhesive force and gel strength to stay in contact with the rectal mucosa for prolonged time. This phenomenon substantially increased the therapeutic concentration of drug in the plasma and its pharmacological action. The anti-tumor study revealed that the liquid suppository can markedly decrease the tumor volume in tumor bearing mice and showed no signs of toxicity. Therefore, these findings suggest that rectal delivery of the present system offers an exciting mode of DCT delivery to improve its chemotherapeutic action.

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