

A temperature-sensitive phase-change hydrogel of topotecan achieves a long-term sustained antitumor effect on retinoblastoma cells

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Background: Retinoblastoma (Rb) is one of the most common malignancies among children. Following early diagnosis and prompt treatment, the clinical outcome or prognosis of Rb is promising. However, the prognosis or survival rates of patients with late-stage Rb remain poor. Current therapeutic strategies for advanced Rb mainly involve the use of advanced chemotherapeutic options. However, the efficacy of these strategies is not satisfactory. Therefore, the development of novel strategies to achieve a more effective antitumor effect on late-stage Rb is of crucial importance.

Methods and materials: Topotecan was dissolved in phosphate-buffered saline and prepared into a temperature-sensitive phase-change hydrogel (termed Topo-Gel). Moreover, Topo-Gel was injected into tumor tissues formed by Y79 cells (an Rb cell line) in nude mice to examine the long-term release and long-acting antitumor effect of Topo-Gel on Rb tumors.

Results: Topo-Gel transforms from liquid to a hydrogel at near body temperatures (phase-change temperature $[T_{1/2}]$ was 37.23 ± 0.473 °C), and maintains the slow release of topotecan in Rb tumor tissues. Following the subcutaneous injection of Topo-Gel, the treatment induced long-acting inhibition of tumor growth and relieved the adverse effects associated with topotecan. Topo-Gel, a temperature-sensitive phase-change hydrogel, is a slow-release system that prolongs the presence of topotecan in Rb tissues, and preserves the efficacy of topotecan in the long term.

Conclusion: Preparation of topotecan into a temperature-sensitive phase-change hydrogel achieves a long-term sustained antitumor effect on Rb cells, and may be a useful strategy for the treatment of intraocular Rb.

Keywords: retinoblastoma, temperature-sensitive phase-change hydrogel, slow-release system, long-acting antitumor effect

Introduction

Retinoblastoma (Rb) is one of the most common malignancies among children. Following early diagnosis and prompt treatment, the clinical outcome or prognosis of Rb is promising.¹⁻⁴ However, the prognosis or survival rates of patients with late-stage Rb remains poor.⁵⁻⁷ Current therapeutic strategies for advanced Rb mainly include the use of advanced chemotherapeutic agents, such as melphalan (Alkeran) or topotecan.⁸⁻¹¹ However, the efficacy of these drugs in the treatment of advanced Rb is not satisfactory.^{12,13} Moreover, Rb occurs in children, and these patients have weaker body functions compared with adults.^{14,15} The adverse effects of long-term chemotherapy on the health of children cannot be ignored. Of note, the

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oral or intravenous administration of chemotherapy can lead to the widespread distribution of the drugs to various organs throughout the body. This results in insufficient effective concentrations of the chemotherapeutic drugs in local tumor tissues. Therefore, the preparation of a novel sustained-release formulation of antitumor drugs is of great importance. This approach allows direct intratumoral injection of drugs, which avoids adverse effects or damage to organs of the whole body. In addition, the drugs can exert long-term effects after a single administration, and the approach can improve the compliance of patients receiving antitumor treatment.

Currently, the most commonly used drugs against Rb are carboplatin, etoposide, vincristine, melphalan, or topotecan. Carboplatin, etoposide, and vincristine are widely used in antitumor therapy of Rb, and are administered via intravenous drips.^{16,17} Melphalan or topotecan can be used to treat Rb via intra-arterial chemotherapy (IAC).^{8–10} During intra-arterial chemotherapy, melphalan or topotecan is injected into the tumor tissue in the eye via the ophthalmic artery. Injections of melphalan or topotecan are in the form of solutions that could lead to the complete and rapid clearance of tumor tissues. This results in the short duration of the efficacy of drugs, leading to the requirement for multiple or frequent doses. Therefore, it is valuable to investigate and develop new pharmaceutical formulations that can offer the sustained release of drugs in the tumor tissues, and ultimately provide long-term antitumor efficacy of drugs through a single/one-time administration.

Topotecan is easier to dissolve in water compared with melphalan. The establishment of a topotecan formulation can result in the administration of a larger dose of topotecan in a smaller volume versus that of melphalan. In the present study, a temperature-sensitive phase-change hydrogel of topotecan (Topo-Gel) was prepared. Topo-Gel was directly injected into tumor tissues to examine the duration of its antitumor effect on Rb cells.

Materials and methods

Cell culture and agents

The Rb cell line Y79 was purchased from the National Infrastructure of Cell Line Resource, Chinese Academy of Medical Sciences (Beijing, China), an organization possessing typical biological samples of the Chinese government. Cells were cultured in Dulbecco's modified eagle medium (Thermo Fisher Scientific Corporation, Waltham, MA,

USA), supplemented with 20% fetal bovine serum (Thermo Fisher Scientific Corporation, Waltham, MA, USA) in an incubator at 37 °C and 5% CO₂. Topotecan (Cat. No.: S1231) was purchased from Selleck Corporation, Houston, Texas, USA.

Preparation of topotecan formulations

The formulations of topotecan were prepared as described by Wang YL et al and Tang ZG et al (2018).^{18,19} Briefly, topotecan was fully solubilized in phosphate-buffered saline (PBS) to produce a topotecan solution (termed Topo-Sol). Subsequently, Topo-Sol was repeatedly filtrated using a 0.1-μm micron filter, and termed topotecan solution-1 (Topo-Sol-1). A temperature-sensitive phase-change hydrogel of topotecan (termed Topo-Gel) was generated by mixing Topo-Sol with poloxamer 407 (FREDA Corporation, Jinan City, People's Republic of China). The topotecan in the formulations was examined using liquid chromatograph mass spectrometer/mass spectrometer (LC-MS/MS) methods, as described by Ye et al (2013), Li et al (2010), Holleran et al (2010), and Muenster et al (2008).^{20–23}

For the phase-transition experiments, topotecan was prepared into Topo-Gel and Topo-Gel was incubated at the indicated temperature. To examine the effect of pH on the phase-transition of Topo-Gel, topotecan was diluted using physiological saline at the indicated pH values (6.6, 6.8, 7.0 or 7.2), and fixed with poloxamer 407 to produce the Topo-Gel. Subsequently, Topo-Gel was incubated at the indicated temperature for the phase-transition or in vitro release experiments.

The in vitro release of topotecan from Topo-Gel was examined. Topo-Gel (100 μl) was placed in an EP (eppendorf) tube, incubated at 37 °C for approximately 5 mins. Subsequently, physiological saline (~900 μl) was slowly added, and the solution was incubated at 37 °C. Topo-Gel samples were collected daily for LC-MS/MS, and the in vitro release profile of Topo-Gel was determined based on the sustained presence of topotecan in the hydrogel (topotecan in Topo-Gel can be released into physiological saline).

Release of topotecan from Topo-Gel or clearance of topotecan from tumor tissues injected with Topo-Gel

All animal experiment protocols were approved by the Institutional Animal Care and Use Committee of

National Beijing Center for drug safety assessment, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing, China (Certification ID: IACUC-2019-002). All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act of 1986 and associated guidelines. The preparation of samples was performed as described by Wang et al (2018) and Xie et al (2018).^{18,19} LC-MS/MS experiments were performed, and the curves of sustained topotecan presence in tumor tissues were obtained through calculations as described by Ye et al (2013), Li et al (2010), Holleran et al (2010), and Muenster et al (2008).^{20–23} Briefly, Y79 cells were cultured and injected into the left hind limb sites of nude mice to form subcutaneous tumors. Following the growth of subcutaneous tumors to a volume of approximately 1,500–2,000 mm³, intratumoral administration (intratumoral injection) experiments were performed. Animals (nude mice) were divided into the following three groups: solvent control injection, Topo-Sol injection, and Topo-Gel injection. Agents were directly injected into subcutaneous tumors in nude mice, and each tumor was injected with approximately 50 µl of liquid. Animals were harvested and tumor tissues were collected. The tissues were milled using liquid nitrogen, and the topotecan in the tissue was extracted using acetonitrile. The amount of topotecan was examined using LC-MS/MS.

In vivo antitumor effects of Topo-Gel on Rb tissues formed by Y79 cells

BALB/c nude mice (thymus-deficient) were purchased from SI-Bei-Fu Corporation, Beijing, China. Y79 cells were injected into nude mice to form subcutaneous tumors.^{24–26} When the tumor volumes reached 1,000–1,200 mm³, the mice received oral administration of topotecan or an intratumoral injection with topotecan formulations. For the oral administration of topotecan, the mice received a gastrointestinal administration of the indicated dose of topotecan (ie, 3 mg/kg, 2 mg/kg, 1 mg/kg, or 0.5 mg/kg) over the course of 2 days. For the intratumoral injection of topotecan formulations, the solvent control, Topo-Sol, or Topo-Gel was directly injected into the subcutaneous tumors. After 14–20 days, the mice were sacrificed via cervical dislocation, and tumors were harvested. Tumor weights were measured using precision balances, and tumor volumes were calculated as length × width × width/2.

Western blotting

Subcutaneous tumors received an intratumoral injection of Topo-Sol or Topo-Gel. After 2–3 weeks, subcutaneous tumors were harvested and protein samples were extracted. The protein levels of Pro-PARP, cleaved-PARP, and CHEK1, or the phosphorylation of CHEK1 (S296 and S345 points) were determined through Western blotting experiments as described by Liang et al (2017) and Ji et al (2017).^{27,28}

The adverse effects of topotecan formulations on the body health of mice

Nude mice, which were injected with Y79 cells to form subcutaneous tumors, received an oral administration of topotecan, or an intratumoral injection of Topo-Sol or Topo-Gel. After 2–3 weeks of treatment, the body weight, hematological parameters, and mass of the main organs were examined.

Statistical analysis

A statistical analysis was performed through the Bonferroni correction with a two-way analysis of variance using the SPSS Statistics software (IBM Corporation, Armonk, NY, USA). The $T_{1/2}$ value of the hydrogel was calculated using the Origin software (Version No 6.1; OriginLab Corporation, Northampton, MA, USA). A $P < 0.05$ denoted statistical significance between groups.

Results

Preparation and identification of topotecan solutions

Initially, the characteristics of topotecan formulations were examined using LC-MS/MS. As shown in [Figure 1](#), topotecan can be easily dissolved in PBS to produce a topotecan solution (termed Topo-Sol). The concentration of topotecan in Topo-Sol was >30 mg/ml (30.05 ± 1.25 mg/ml). Subsequently, the uniformity of Topo-Sol and the dissolution of topotecan in Topo-Sol were examined. Topo-Sol was filtered using a 0.1-µm pore size filter to extract potential undissolved topotecan microparticles. As shown in [Figure 1A–D](#), there was no significant difference between the concentrations of topotecan in Topo-Sol and Topo-Sol-1 (Topo-Sol after multiple filtrations using a 0.1-µm aperture). The concentration of topotecan in Topo-Sol-1 was 30.14 ± 1.33 mg/ml ([Figure 1D](#)). The results are shown as

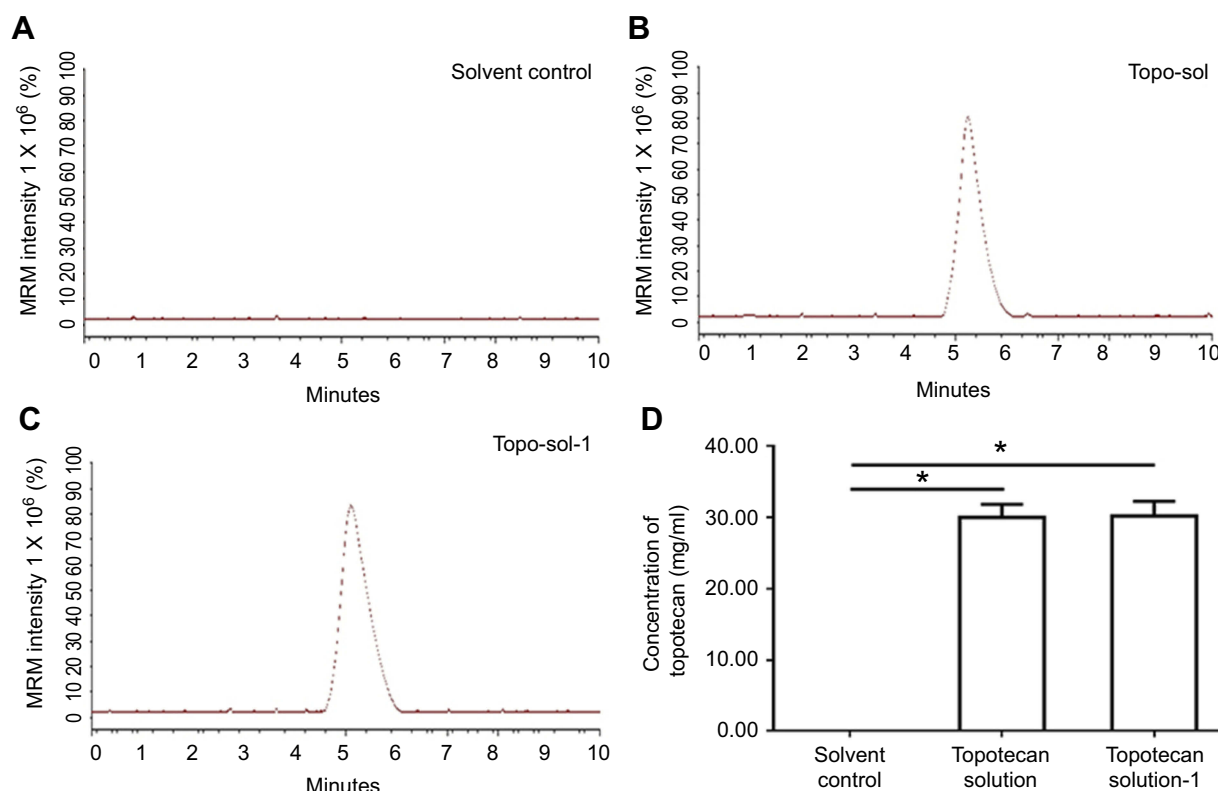


Figure 1 Concentration of topotecan in the solution. Topotecan was dissolved in PBS to form a solution (termed Topo-Sol). Topo-Sol was filtered using a 0.1- μ m pore diameter filter membrane, and the resultant solution was termed Topo-Sol-I. Topotecan was extracted from the solutions using acetonitrile for investigation through LC-MS/MS. The results are shown as images of LC-MS/MS for the solvent control (A), Topo-Sol (B), Topo-Sol-I (C), and concentration of topotecan in the solutions (D). * $P < 0.05$.

images from the LC-MS/MS or quantitative analysis. Therefore, it was concluded that the topotecan solution was successfully prepared, and topotecan is fully soluble or highly soluble in PBS.

Preparation of topotecan hydrogel and the in vitro release of topotecan from the topotecan hydrogel

Subsequently, the solution of poloxamer 407 dissolved in PBS was prepared at 10 °C. Topo-Sol was added and the poloxamer 407 solution was stirred at 10 °C. The apparent viscosity (η [Pa/s]) of Topo-Gel containing different doses of poloxamer 407 as a function of temperature (°C) was examined to reveal whether the topotecan hydrogel (termed Topo-Gel) could transform from the liquid form to a hydrogel form. In addition, the phase-transition temperature ($T_{1/2}$) of Topo-Gel was calculated based on the apparent viscosity-temperature curves. As shown in Figure 2 and Table 1, Topo-Gel can transform from a liquid to a hydrogel, and the $T_{1/2}$

value of Topo-Gel containing 10.0% poloxamer 407 was similar to body temperature. Therefore, a 12.5% concentration of poloxamer 407 was used for the subsequent steps of the experiment. Moreover, there was no significant difference in the $T_{1/2}$ value of Topo-Gel with a 10.0% concentration of poloxamer 407 at the indicated pH (Figure 3 and Table 2). Topotecan did not alter the phase-transition temperatures of topotecan hydrogel with added poloxamer 407 (Table S1).

Subsequently, the in vitro release of topotecan from Topo-Gel was determined to examine whether topotecan prepared as Topo-Gel resulted in the slow release of topotecan. As shown in Figure 4, Topo-Gel achieved the long-term sustainability of topotecan. The presence of topotecan was sustained in Topo-Gel for >400 h. The half-life time ($t_{1/2}$ value) of topotecan release from Topo-Gel was 151.94 \pm 19.32 h. Moreover, there was no significant difference in the $t_{1/2}$ value of topotecan release from Topo-Gel at different pH values (Table 3). Therefore, the preparation of the Topo-Gel formulation achieved the long-term sustainability of topotecan in Topo-Gel.

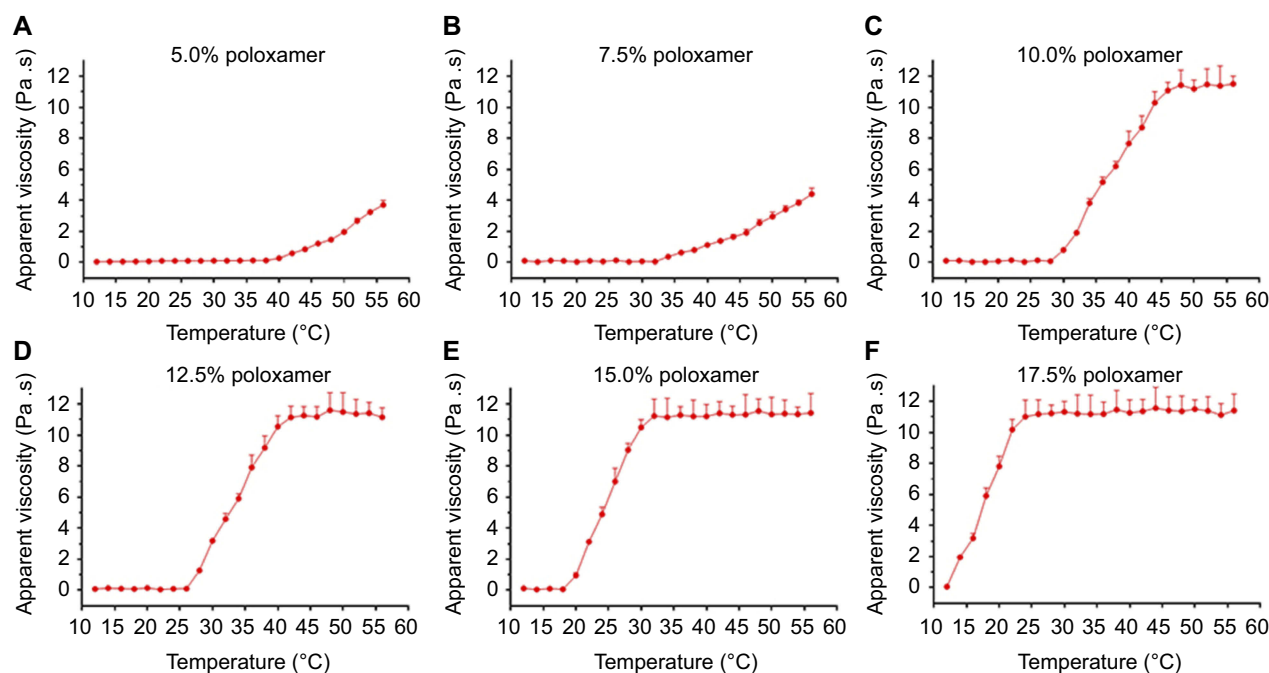


Figure 2 Phase-transition of the topotecan hydrogel with indicated concentration of poloxamer 407. The apparent viscosity (η [Pa/s]) of Topo-Gel containing different doses of poloxamer 407 as a function of temperature ($^{\circ}\text{C}$) was examined. The apparent viscosity-temperature curves of the topotecan hydrogel with 5% (A), 7.5% (B), 10% (C), 12.5% (D), 15% (E), or 17.5% (F) poloxamer 407 are shown.

Table 1 Phase-transition temperatures of Topotecan hydrogel with indicated Poloxamer 407

Poloxamer 407 (%)	Phase-transition temperature ($T_{1/2}$, $^{\circ}\text{C}$)
5.0	-
7.5	-
10.0	37.23 ± 0.473
12.5	32.97 ± 0.22
15.0	25.02 ± 0.65
17.5	22.33 ± 0.26

In vivo release of topotecan from tumor tissues injected with topotecan formulations

Subcutaneous tumors were formed by injecting Y79 cells into nude mice to further assess the slow-release of Topo-Gel. Subsequently, topotecan formulations were directly injected into the subcutaneous tumor tissues, and tumors were harvested for LC-MS/MS to examine the sustainability of topotecan. As shown in Figure 5, topotecan was almost completely removed from subcutaneous tumors within 48 h after injection of Topo-Sol. The $t_{1/2}$ value of topotecan in tumor tissues after injection of Topo-Sol was 10.07 ± 1.10 h. An injection of Topo-Gel resulted in long sustainability or slow release of topotecan in subcutaneous tumor tissues ($t_{1/2}$ value:

94.07 ± 11.58 h). Following the injection of Topo-Gel, topotecan was detected in tumor tissues, even after 408 h.

The concentration of topotecan in the blood of nude mice after intratumoral injection of the topotecan formulations was also examined. As shown in Figure 6, after injection of Topo-Sol, topotecan was rapidly cleared from the tumor tissue, and its blood concentration peaked at the 12-h time point (Figure 6). After injection of Topo-Gel, the clearance of topotecan from the tumor tissues was extremely slow, and its concentration in the blood was constantly low (Figure 6). Notably, topotecan was detected in the blood >400 h after injection (Figure 6). The peak concentration of topotecan after an intratumoral injection of Topo-Sol was almost 72.85-fold higher than the peak concentration of topotecan after an intratumoral injection of Topo-Gel. Therefore, the preparation of topotecan into Topo-Gel achieved the long sustainability of topotecan in tumor tissues.

Injection of Topo-Gel induced the long-acting antitumor effect of topotecan

The aforementioned results showed that an injection of Topo-Gel could induce the long sustainability or slow release of topotecan. Topotecan formulations were injected into

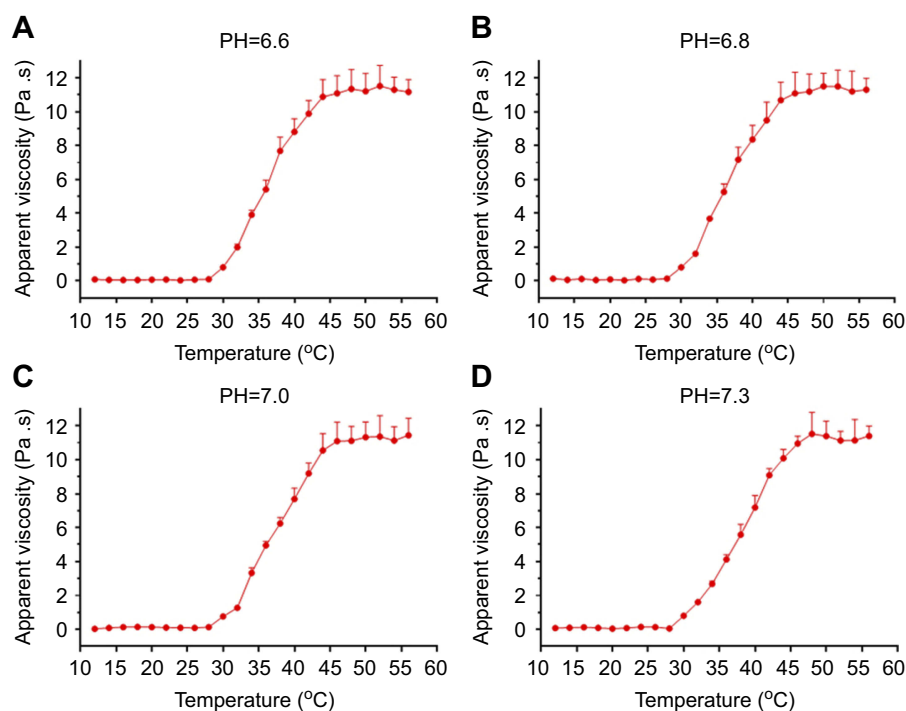


Figure 3 Phase-transition of the topotecan hydrogel with 10% poloxamer 407 at indicated pH values. The apparent viscosity (η [Pa/s]) of Topo-Gel containing 10% poloxamer 407 dose as a function of temperature ($^{\circ}\text{C}$) was examined. The apparent viscosity-temperature curves of the topotecan hydrogel with 10% poloxamer 407 at pH=6.6 (A), pH=6.8 (B), pH=7.0 (C), or pH=7.2 (D) are shown.

Table 2 Phase-transition temperatures of Topo-Gel with 10% Poloxamer 407 at indicated PH-value

PH values	Phase-transition temperature ($T_{1/2}$, $^{\circ}\text{C}$)
6.6	36.37694 \pm 0.11262
6.8	36.79925 \pm 0.12918
7.0	37.28394 \pm 0.14601
7.2	37.93283 \pm 0.17973

subcutaneous tumors to further examine whether an injection of Topo-Gel could achieve the long-acting feature of topotecan. As shown in Figure 7, a single injection of Topo-Sol did not inhibit the subcutaneous growth of Y79 cells in nude mice. In contrast, a single injection of Topo-Gel significantly inhibited the subcutaneous growth of Y79 cells in nude mice. Moreover, the results of the Western blotting analysis

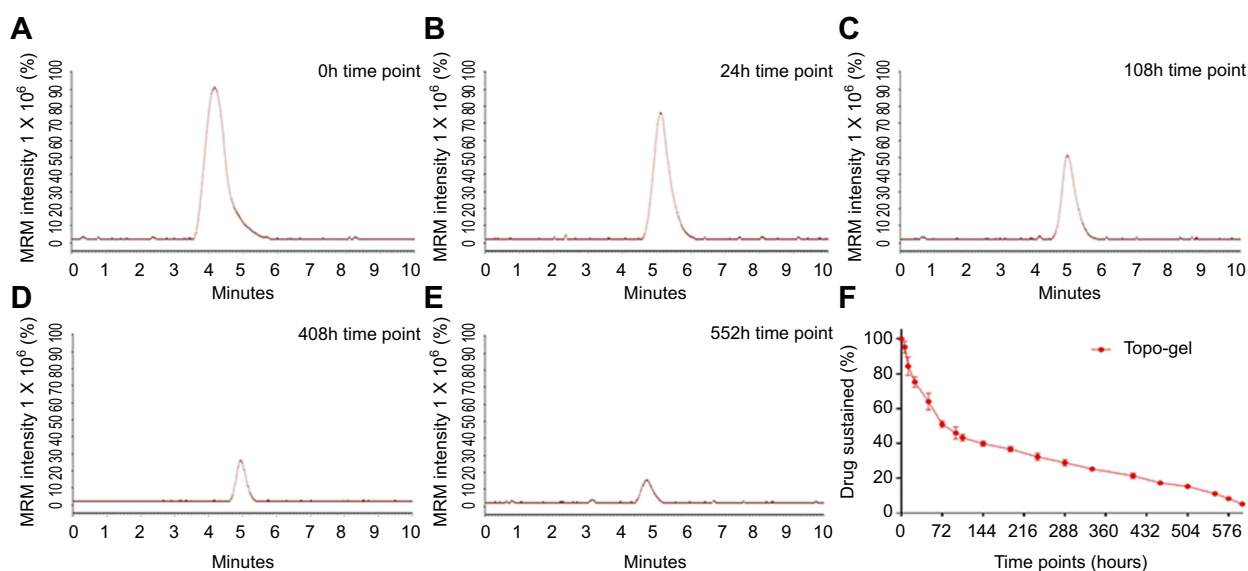


Figure 4 In vitro release of topotecan from the hydrogel (Topo-Gel). The in vitro release of topotecan from the hydrogel (Topo-Gel) was examined at indicated time points through LC-MS/MS. The results are shown as images of the LC-MS/MS results (A–E) and the drug-sustaining curve of topotecan in Topo-Gel (F).

Table 3 Half-life time of Topotecan releasing from Topo-Gel at indicate PH-values

PH values	Half-life time ($t_{1/2}$, Hours)
6.6	148.45±40.42
6.8	154.94±36.61
7.0	151.87±24.64
7.2	156.47±18.87
7.4	158.45±22.53

showed that, unlike Topo-Sol, a single injection of Topo-Gel enhanced the cleavage of PARP. Unlike Topo-Sol, an injection of Topo-Gel induced the phosphorylation of CHEK1 at the S296 or S345 residue (Figure 8). Therefore, the direct injection of Topo-Gel into tumor tissues achieved the long-acting antitumor effect of topotecan.

Topo-Gel reduces the adverse effects of topotecan on the health of mice

An injection of Topo-Gel can induce a high concentration of topotecan in tumor tissues and a low concentration of topotecan in the organs of nude mice. Therefore, the body weight,

hematological parameters, and mass of the main organs were examined to reveal the adverse effects of topotecan formulations. As shown in Figure 9, an oral administration of 3 mg/kg, 2 mg/kg, or 1 mg/kg of topotecan significantly inhibited the subcutaneous growth of Y79 cells in nude mice. The antitumor effect observed after oral administration of a 3 mg/kg topotecan is similar to that reported after a single intratumoral injection of Topo-Gel (Figure 9). Meanwhile, the antitumor effect observed after the oral administration of 2 mg/kg or 1 mg/kg topotecan was weaker than that reported after a single intratumoral injection of Topo-Gel (Figure 9). The oral administration of 0.5 mg/kg topotecan could not inhibit the subcutaneous growth of Y79 cells in nude mice (Figure 9). Moreover, the oral administration of topotecan can cause serious damage to the bodily health of nude mice, leading to a decrease in hematological parameters, weight loss, and weight loss in major organs (Tables 4 and 5, and Figure 10). It is worth noting that the oral administration of topotecan caused these effects in a dose (0.5 mg/kg) without antitumor activity. The injection of Topo-Sol did not exert a significant antitumor effect, and did not affect the hematological parameters, body

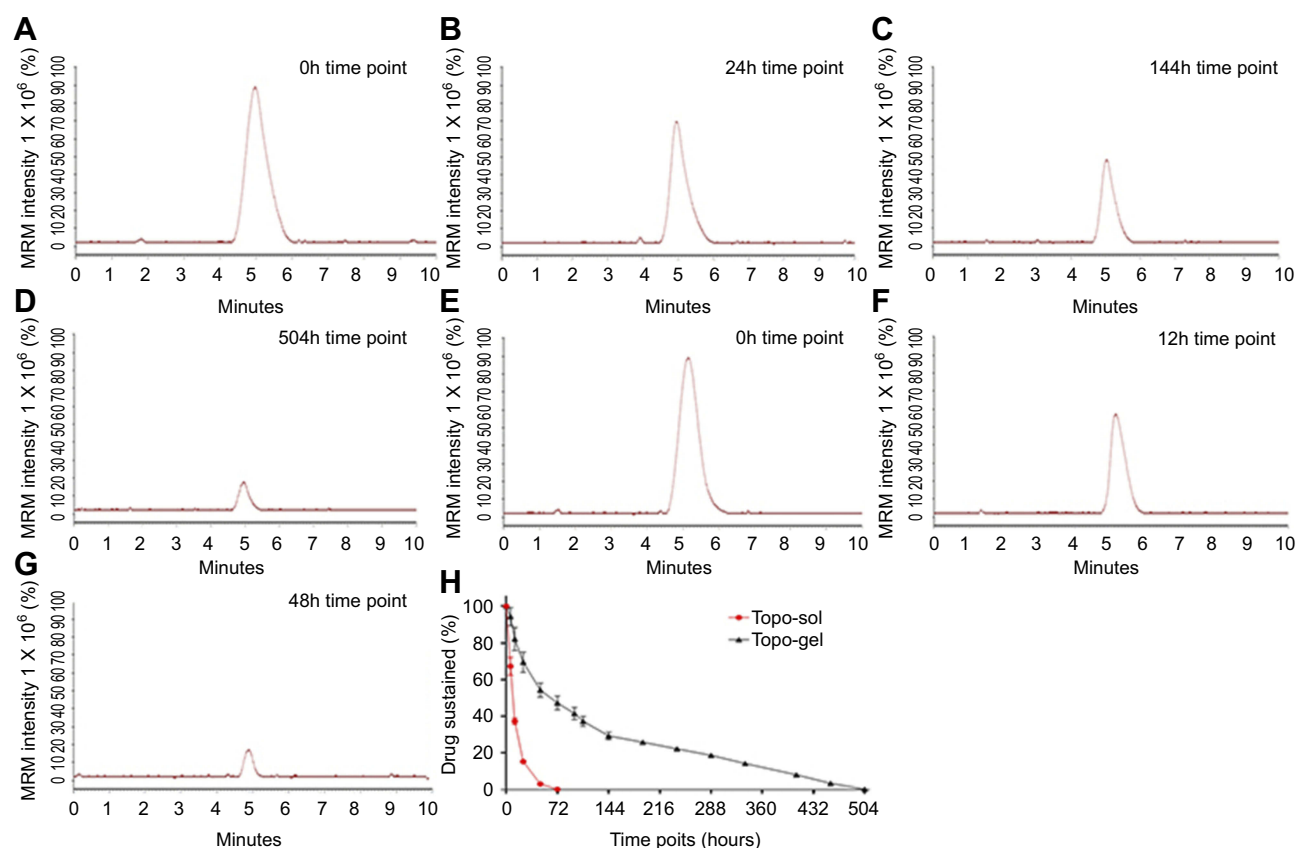


Figure 5 In vivo release of topotecan from tumor tissues injected with topotecan formulations. Y79 cells were injected into nude mice to form subcutaneous tumors. Topotecan formulations (Topo-Gel, Topo-Sol) were injected into tumor tissues. At each time point, subcutaneous tumors were harvested for investigation through LC-MS/MS. The results are shown as images of the LC-MS/MS results (A–G) from the Topo-Gel injection group (A–D) or Topo-Sol group (E–G), and the drug-sustaining curve of topotecan in tumor tissues (H).

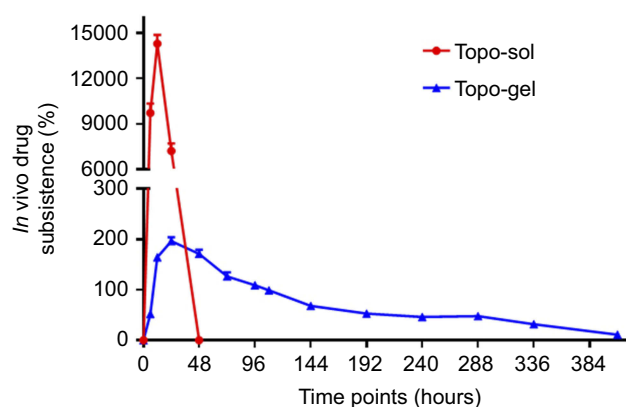


Figure 6 In vivo release of topotecan from tumor tissues injected with topotecan formulations – measurement of the concentration of topotecan in the blood. Y79 cells were injected into nude mice to form subcutaneous tumors. Topotecan formulations (Topo-Gel, Topo-Sol) were injected into tumor tissues. At each time point, blood was harvested for investigation through LC-MS/MS. The results are shown as the concentration curve of topotecan in the blood of nude mice. $n=10$ for each group at each time point.

weight, and weights of major organs (Tables 4 and 5, and Figure 10). A single intratumoral injection of Topo-Gel exerted a similar antitumor effect to that observed following the oral administration of 3 mg/kg topotecan. However, the injury induced in mice was significantly less than that noted after the oral administration of each dose of topotecan (Tables 4 and 5, and Figure 10). The results are displayed as a data Table (Tables 4 and 5) or a heat map drawn from the data (Figure 10). Therefore, it was concluded that Topo-Gel reduces the adverse effects of topotecan on the health of mice.

Discussion

Chemotherapy continues to be the mainstay for the treatment of Rb.^{29,30} However, the current strategies for the administration of these drugs are not satisfactory. In this study, topotecan was prepared as a hydrogel that can be transformed

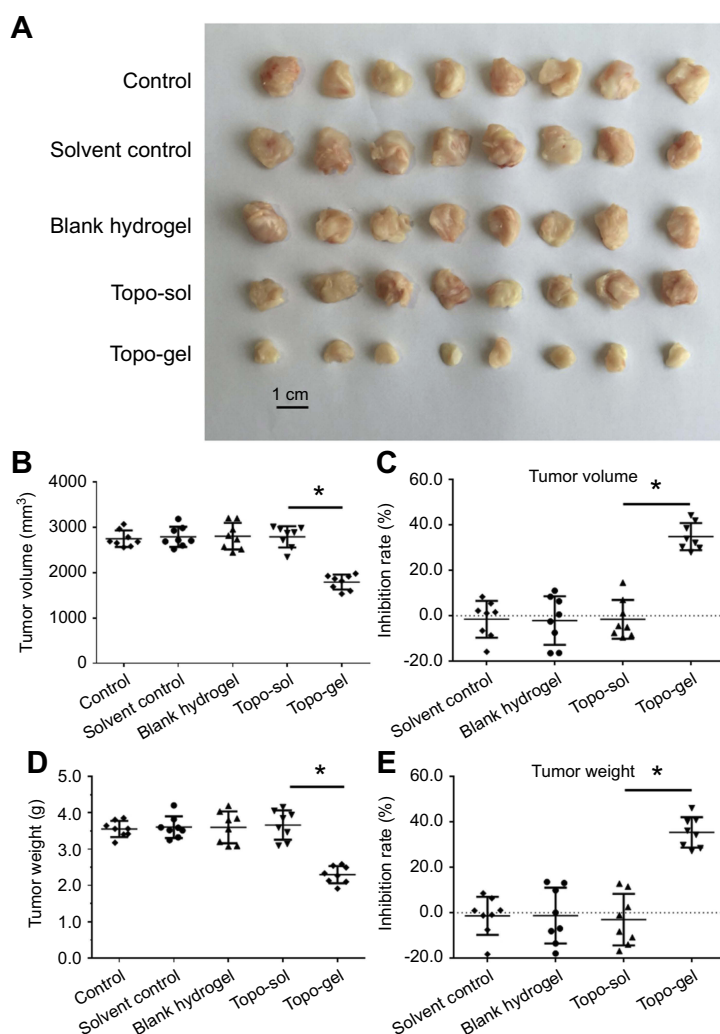


Figure 7 Investigation of the long-acting antitumor effects of the topotecan formulations in the Rb subcutaneous tumor model. Y79 cells were injected into nude mice to form subcutaneous tumors. A single injection of solvent control (PBS), blank hydrogel (PBS with 10% poloxamer 407), or topotecan formulations (Topo-Gel, Topo-Sol) was performed into tumor tissues. After 3–4 weeks of growth, tumors were harvested, and tumor volumes and weights were calculated. The results are shown as (A) tumor images, (B) tumor volumes, (C) inhibition rates from tumor volumes, (D) tumor weights, and (E) inhibition rates from tumor weights. $*P<0.05$.

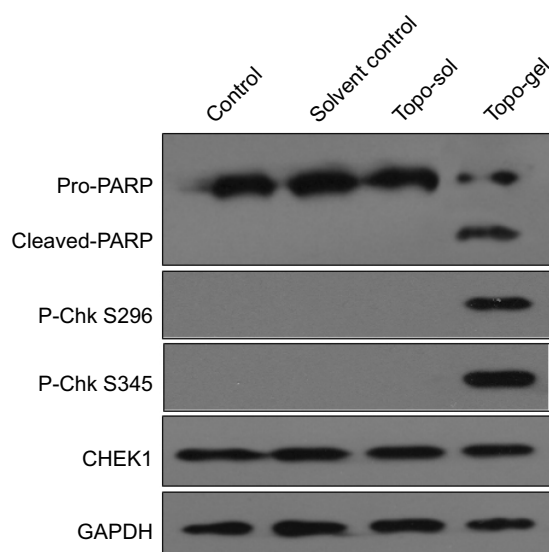


Figure 8 Investigation of the long-acting antitumor effect of topotecan formulations in the Rb subcutaneous tumor model through Western blotting. Y79 cells were injected into nude mice to form subcutaneous tumors. A single injection of solvent control (PBS), blank hydrogel (PBS with 10% poloxamer 407), or topotecan formulations (Topo-Gel, Topo-Sol) was performed into tumor tissues. After 3–4 weeks of growth, tumors were harvested, and the levels of PARP cleavage and expression/ phosphorylation of CHEK1 were examined through Western blotting.

from a liquid into a hydrogel at body temperature. Topotecan exhibits better solubility compared with melphalan, allowing topotecan formulations to deliver a larger amount of the drug in a smaller volume. In the present study, topotecan formulations (Topo-Sol or Topo-Gel) yielded a high dose of topotecan (almost 30 mg/ml). Topo-Gel, which is in liquid form at room temperature, can be directly injected into the tumor tissue to ensure precise drug administration. Moreover, at the body temperature of an animal, Topo-Gel can be sustained in the tumor tissues for a long time in the form of a hydrogel. Topotecan can be slowly released from Topo-Gel into tumor tissues via dissolution of the hydrogel by the cells, and exerts its long-term antitumor effect via a single administration.

Topotecan, which functions as a topoisomerase I inhibitor, is often used as a DNA damage-inducing treatment.^{31,32} In cancer cells, topotecan can also be used as a therapeutic strategy for recurrent cisplatin-resistant tumors (eg, ovarian cancer).^{33,34} In the present study, topotecan was rapidly cleared from tumor tissues after a single injection of Topo-Sol, whereas topotecan was detected in tumor tissues for a

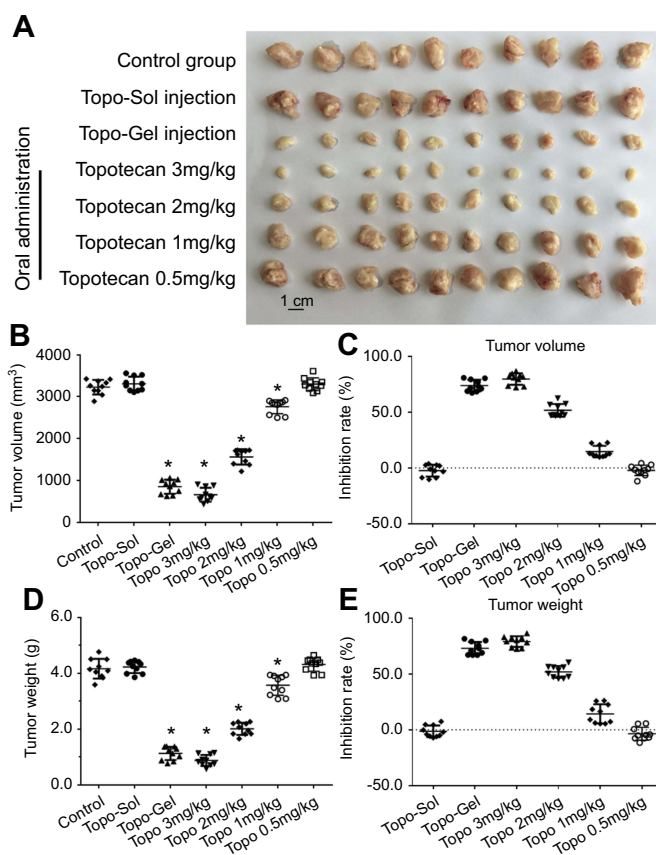


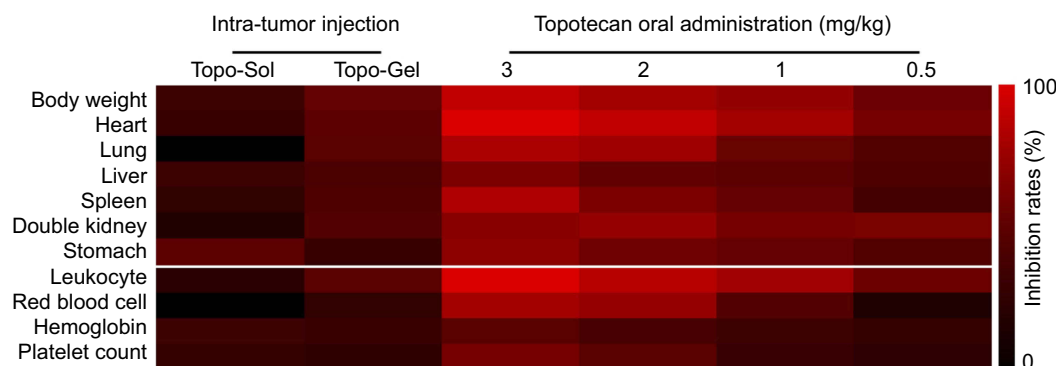
Figure 9 Investigation of the long-acting antitumor effect of topotecan formulations in the Rb subcutaneous tumor model. Y79 cells were injected into nude mice to form subcutaneous tumors. A single injection of topotecan formulations (Topo-Gel, Topo-Sol) was injected into tumor tissues. Alternatively, nude mice received oral administration of topotecan (3 mg/kg, 2 mg/kg, 1 mg/kg, or 0.5 mg/kg) once per 2 days. After 3–4 weeks of growth, tumors were harvested, and tumor volumes or tumor weights were calculated. The results are shown as (A) tumor images, (B) tumor volumes, (C) inhibition rates according to tumor volumes, (D) tumor weights, and (E) inhibition rates according to tumor weights. * $P < 0.05$ Topo-Gel versus solvent control; * $P < 0.05$ oral administration of topotecan versus solvent control.

Table 4 The effect of Topotecan on nude mice's hematological parameters

Hematological parameters	Control group	Intra-tumor injection		Topotecan oral administration (mg/kg)			
		Topo-Sol	Topo-Gel	3.0	2.0	1.0	0.5
Leukocyte ($10^9/L$)	3.33±0.98	3.26±1.22	2.75±1.31	1.45±0.80	1.80±0.71	2.03±0.97	2.59±1.23
Red blood cell ($10^{12}/L$)	9.35±0.77	10.26±0.61	8.97±0.63	5.63±0.21	6.03±0.61	7.91±0.69	9.51±0.70
Hemoglobin (g/L)	155.48±11.25	142.61±15.26	144.77±35.80	129.08±13.01	137.95±7.55	141.76±8.34	147.13±14.58
Platelet count ($10^9/L$)	615.33±101.95	581.43±93.60	592.30±76.92	453.93±45.71	510.25±102.56	576.12±73.20	594±60.65

Table 5 The effect of Topotecan on nude mice's body weight and main organs mass

Main organs mass	Control group	Intratumor injection		Topotecan oral administration (mg/kg)			
		Topo-Sol	Topo-Gel	3.0	2.0	1.0	0.5
Body weight (g)	20.34±1.88	19.71±1.82	17.61±2.93	12.87±3.45	14.43±2.85	15.16±3.72	17.18±2.86
Heart (mg)	108.91±8.91	107.42±10.69	96.52±25.73	61.98±8.34	69.18±12.86	77.21±16.37	89.29±9.76
Lung (mg)	141.78±21.43	158.09±31.52	126.77±19.29	97.13±14.50	101.40±35.08	120.78±36.25	129.07±17.54
Liver (mg)	658.41±18.42	638.59±16.49	610.52±31.57	530.02±33.91	574.55±28.53	582.63±15.78	605.27±48.53
Spleen (mg)	16.73±6.32	16.78±7.99	15.43±8.59	11.34±5.02	13.50±8.77	14.35±7.71	15.87±3.450
Double kidney (mg)	237.62±56.67	247.14±35.08	215.73±20.99	183.74±26.90	176.52±46.78	195.20±36.25	192.21±46.19
Stomach (mg)	128.47±30.05	113.74±38.85	126.31±22.57	98.09±19.41	106.77±19.29	110.75±29.23	117.14±35.08

**Figure 10** The effect of topotecan on the hematological parameters, body weight, and mass of the main organs of nude mice (Tables 4 and 5). The inhibition rates in each group were calculated and shown as a heat map.

long time after a single injection of Topo-Gel. Unlike Topo-Sol, an injection of Topo-Gel induced the activation of apoptotic or DNA damage-related pathways, including cleavage of PARP or phosphorylation of CHEK1.³⁵ Moreover, the adverse effects of topotecan (ie, low blood counts, nausea, vomiting, diarrhea, or fatigue) limit the use of topotecan. The present results showed that, when an equal antitumor effect was achieved via the oral administration of topotecan or injection of Topo-Gel, the observed toxicity in nude mice of the former group was much more serious. Therefore, the development of the Topo-Gel induced the slow release or long-acting effects of

topotecan in tumor tissues, and improved the adverse effects caused by topotecan. Moreover, it is difficult to establish immunodeficient animals and corresponding tumor models in large animals (eg, beagle dogs). Immunodeficient mice are a common model for oncological research. However, their size is insufficient to establish tumor tissue in the mouse eye. Thus, a subcutaneous tumor model in nude mice is established, and drugs are injected into solid tumors. In vivo imaging of small animals can reflect the growth of tumor tissue in vivo.^{36–40} In order to inject drug preparations into solid tumor tissues, we established a subcutaneous tumor model. The results fully

demonstrated the antitumor effect of the drug. Subcutaneous tumors are easy to observe and can reflect the antitumor effect of drugs by measuring tumor volume and weight.

Currently, various image-guided interventional strategies enable precise drug delivery in tumor tissues.^{41–44} For tumor tissues with large vascular invasion, transcatheter arterial chemoembolization can be used to mix lipiodol with anti-tumor drugs for injection into the tumor tissue through the large blood vessels. This approach results in tumor vascular occlusion and antitumor effects.^{45–47} For tumor tissues without large vascular invasion, the drug can be injected into the tumor tissue through a puncture under the guidance of computed tomography.^{48,49} The Topo-Gel prepared in this study can be used for the long-acting and sustained release of drugs, as well as the embolization of tumor blood vessels. Poloxamer, a polymer material used in the preparation of Topo-Gel, is a pharmaceutical excipient approved by the Chinese Pharmacopoeia (the fourth part of Chinese Pharmacopoeia [2015 Version], page 531–532.). It can be used in injections, emulsifiers, etc., and it has a good safety profile. In addition, poloxamer can be biodegraded without posing a potential risk to the health of the patient (eg, polymer residue in the organs of the patient). Interestingly, Taich et al (2016) prepared a sustained-release hydrogel of topotecan using another material, namely poly(ϵ -caprolactone)-poly(ethyleneglycol)-poly(ϵ -caprolactone).⁵⁰ Majumder et al (2018) designed a multicompartiment hydrogel that can facilitate the time-resolved delivery feature of combination therapy on glioblastoma. Furthermore, Li et al produced a systematic summary and introduction for the design of hydrogels targeting controlled drug delivery.^{51,52} Meng et al prepared a temperature-sensitive phase-change hydrogel of tamoxifen achieving long sustainability of aggr in tumor tissues and a long-acting antitumor effect.^{48,53} Studies are warranted to examine the differences between hydrogels prepared using different materials.

Conclusion

Topo-Gel, a temperature-sensitive phase-change hydrogel, is a slow-release system allowing the long-term sustainability of topotecan in Rb tissues and preserving the efficacy of topotecan in the long term.

Abbreviations

Rb, Retinoblastoma; PARP, poly ADP-ribose polymerase; CHEK1, checkpoint kinase 1; TACE, transcatheter arterial chemoembolization; CT, Computed Tomography.

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Author contributions

All authors made substantial contributions to the design and conception, acquisition, analysis or interpretation of data. All authors took part in either drafting or revising the manuscript. At the same time, authors gave final approval of the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Topotecan did not alter the phase-transition temperatures of Topotecan hydrogel with indicated Poloxamer 407

Poloxamer 407 (%)	Phase-transition temperature ($T_{1/2}$, °C)	
	Without topotecan	With topotecan
5	-	-
7.5	-	-
10	37.23±0.47	37.30±0.55
12.5	32.97±0.22	32.41±0.30
15	25.02±0.65	24.88±0.38
17.5	22.33±0.26	22.45±0.11

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