Original Article
Homoharringtonine delivered by high proportion PEG of long-circulating liposomes inhibits RPMI8226 multiple myeloma cells in vitro and in vivo

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Abstract: Multiple myeloma (MM) remains an incurable disease in most patients. Homoharringtonine (HHT) is a natural alkaloid produced by various Cephalotaxus species, and is approved by the United States of America Food and Drug Administration to treat patients with acute and chronic myeloid lymphoma. The aim of this study was to develop the high proportion polyethyleneglycol (PEG) of long-circulating HHT liposomes (LCL-HHT-H-PEG) and investigate its therapeutic applicability in vitro and in vivo against RPMI8226 MM. The optimized formulation of LCL-HHT-H-PEG showed a higher association with cytotoxicity against MM RPMI8226 cells than those of low proportion PEG of long-circulating HHT liposomes, liposome-encapsulated-HHT, micelle-HHT, and HHT in vitro. Therapeutic experiments in severe combined immunodeficient mice implanted with MM RPMI8226 cells by the subcutaneous route showed the significant inhibition of tumor growth in LCL-HHT-H-PEG group compared with the HHT group, and other control groups. The analysis of flow cytometry and transmission electron microscopy indicated that LCL-HHT-H-PEG exerted the cytotoxicity against MM by inducing the MM apoptosis in vitro and in vivo. This study suggests that our developed LCL-HHT-H-PEG may be regarded as a promising nano-device to deliver anti-MM drug HHT for treatment of MM patients.

Keywords: Multiple myeloma, homoharringtonine, long-circulating liposomes, polyethyleneglycol, cytotoxicity

Introduction

Multiple myeloma (MM) is a plasma cell neoplasm which constitutes about 10% of all hematologic malignancies, characterized by aberrant expansion of plasma cells within bone marrow and extramedullary sites. The treatment landscape for patients with MM is constantly evolving. Over the last decades, the paradigm of MM therapy has changed dramatically, from the conventional combination of oral melphalan plus prednisone, high-dose chemotherapy with stem cell support to the novel agents such proteasome inhibitors (bortezomib and carfilzomib) and immunomodulatory drugs (thalidomide, lenalidomide, and pomalidomide), which has led to an elongation of patient survival, yet MM runs an aggressive and incurable course in the vast majority of patients [1-3]. Drug resistance remains a major clinical challenge for MM treatment. The main cause of resistance in MM is the minimal residual disease cells that are resistant to the original therapy such as bortezomib and high dose melphalan in stem cell transplant. As such, there is an unmet need for new therapies to increase survival for MM patients [1, 4].

Homoharringtonine (HHT) was originally isolated from the Cephalotaxus evergreen tree and has been widely used in traditional Chinese medicine for treatment of a variety of hematologic malignancies since the 1970s. Also, HHT was approved by the United States of America Food and Drug Administration for treating patients with in acute and chronic myeloid lym-
phoma [5-7]. However, HHT may induce unan-
ticipated side effects in the gastrointestinal
tract, such as diarrhea and nausea/vomiting
as well as cardiac toxicity, which limits its wide
clinical utility, but the mechanisms behind
these severe side effects has not been clarifi-
ced [5].

Accumulating evidence has showed that ther-
apeutic approaches to cancer focus on deve-
loping novel delivery systems to reinforce the
therapeutic efficacy of anticancer agents by
targeted drugs to diseased cells and away from
normal tissues [8, 9]. Liposome encapsulation
of anticancer drugs can alter their pharmacoki-
etics and in vivo distribution, resulting in
reduced toxicity and enhanced efficacy [10-12].
Researchers have developed the long-circulat-
ing dose-independent liposome formulations
containing polyethyleneglycol (PEG) on the
surface of liposomes [9, 13-15], which has
been approved for treatment of varied tumors
[16-19].

In the present study, liposomes with long-circu-
lating properties were used as delivery system
for HHT. We developed conventional and
PEGylated long circulating liposomes contain-
ing HHT to investigate the inhibitory effect of
this novel reagent on MM RPMI8226 cells by in
vitro and in vivo experiments. Here, we showed
that among the diverse formulations, the high
proportion PEG of long-circulating HHT lipo-
somes (LCL-HHT-H-PEG) exhibited encouraging
results of inhibitory MM cells growth in vitro
and in vivo RPMI8226 MM bearing non-obese-
diabetic/severe-combined-immunodeficiency
(NOD/SCID) mice, compared with low propor-
tion PEG of long-circulating HHT liposomes
(LCL-HHT-L-PEG) and other control formula-
tions including general liposome-encapsul-
ated-HHT (L-Enc-HHT), and micelle-HHT (MI-HHT).
The further animal experiments demonstrated
that our developed LCL-HHT-H-PEG appeared
as a new and attractive approach to promote
chemotherapy drug delivery for further clinic tri-
als in treatment of MM patients.

Materials and methods

Cells and mice

Human MM RPMI 8226 cells were ordered
from the Cell Institute in Beijing, People’s
Republic of China. Cells were cultured in com-
plete medium consisting of RPMI 1640, 2 mM
L-glutamine, 100 U/mL penicillin, 100 μg/mL
streptomycin, and 10% fetal bovine serum
at 37°C in a humidified incubator containing
5% CO₂. NOD/SCID mice at 5-6 weeks of age
with 16-18 grams in weight were purchased
from Beijing Weitong Lihua Experimental
Animal Technology Co., Ltd., China. All the mice
were maintained in a pathogen-free facility that
has a 12-hour light/dark cycle and relative
humidity ranged from 40% to 50% at 22°C. All
the animal experiments were performed in
compliance with the Guidelines of the Animal
Research Ethics Board of Southeast University.
Full details of approval of the study can be
found in the approval ID: 20080925.

Main materials

HHT was purchased from Dalian Meilun Bio-
tech Co., Ltd of China. Matrigel was purchased
from BD Biosciences (USA). The annexin
V-PE/7-AAD apoptosis assay kit (KGA1017)
was purchased from Nanjing KeyGen Biotech.
Co., Ltd. 1,2-distearoyl-sn-glycero-3-phospho-
ethanolamine-N- [methoxy (polyethylene gly-
col)- 2000]-biotin (DSPE-PEG 2000-Biotin), and
25-NBD-cholesterol were purchased from
Avanti Polar Lipids, Inc. All other chemicals
were commercially available and of analytical
grade.

Preparation and characterization of diverse
HHT formulations

In preparation of the diverse HHT formulations,
20.42 mg of HHT, 206.24 mg of natural phos-
pholipids, 10.15 mg of cholesterol and 100
mg of DSPE-PEG2000 were synchronously dis-
solved in 1 ml of ethanol with ultrasound. Then
this solution was added in 20 ml pure water
slowly drop by drop with vortex to get the mix-
ture. After removing the organic solvent from
the mixture, liposome suspension was ob-
tained. The suspension was concentrated to
reduce the size of liposomes with probe ultra-
sound. After determination of content, the sus-
pension was freeze dried with 1.2 ml liposome
and 1.2 ml (100 mg/ml) sucrose solution in
each vial. Finally, we got the high pro-
portion PEG of long-circulating HHT liposomes
(1.006 mg/vial), and named it for LCL-HHT-H-PEG.

The LCL-HHT-H-PEG and L-Enc-HHT were pre-
bred by the same method with different pro-

portion of ingredients. The MI-HHT were prepared as follows: 21.01 mg of HHT and 208.07 mg of Solutol®HS15 were dissolved in 2 ml of ethanol with ultrasound. The following steps were performed according to LCL-HHT-H-PEG’s protocol. After determination of content, the suspension was freeze dried with 1.5 ml suspension and 1.5 ml (100 mg/ml) sucrose solution in each vial [20-22].

The morphology of the diverse HHT formulations was observed by using transmission electron microscopy (TEM, JEM-1011, Japan). The mean size, size distribution and zeta potential of HHT were analyzed by using a Malvern mastersizer 2000 (Malvern, UK).

Clonal assay of MM RPMI 8226 cells

For colony formation, the assay protocol followed was previously described in references 23 and 24. Briefly, 2×10^2 MM RPMI 8226 cell suspensions or tumor tissue suspensions were resuspended in 0.8 mL growth media containing 0.3% low melting temperature agarose (Promega, Madison, WI, USA) and a different well (50 ng/ml) at 37°C in 5% CO2 for 72 hours, and cells were stained with FITC-conjugated Annexin V and Propidium Iodide (PI), and resuspended in PBS. The suspended cells at 100 μL were incubated with 5 μL Annexin V-FITC (KeyGen Biotech. Co. Ltd) and 10 μL of 50 μg/mL PI for 15 mins at room temperature in the dark. The rate of cell apoptosis was analyzed within 1 hour by flow cytometry using FACS Caliber (BD, U.S.A) [25, 26].

Apoptosis assay

1×10^6 MM RPMI 8226 cell suspensions or tumor tissue suspensions were seeded into a 6-well plate containing with the different concentration of HHT formulations each well (40 ng/0.8 ml), then were plated in triplicate on 24-well plate over a base layer of 0.8 mL growth media containing 0.6% low melting temperature agarose. The plates were incubated for two weeks until colonies were formed. Colony diameters larger than 75 μm or colony cells more than 50 cells were then counted as 1 positive colony visualized by light a microscope (10 10), according to the previous report [23]. Colony efficiency was calculated as (number of colony/number of cells inoculated) ×100%.

MM xenograft model and therapeutic experiments

In primal animal experiments, 5×10^6 MM RPMI 8226 cells were mixed with matrigel (100 μL in volume), and injected subcutaneously into groin side in NOD/SCID mice. The tumors palpable at the injection sites were examined after around 21 days, and these MM-bearing mice were randomly divided into three groups of equal size (3 mice). Group 1 was respectively treated with 100 ml PBS (left groin side, control) and HHT (right groin side , 150 mg/kg HHT); Group 2 was respectively treated with L-Enc-HHT (left groin side, 150 mg/kg L-Enc-
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HHT) and LCL-HHT-L-PEG (right groin side, 150 mg/kg LCL-HHT-L-PEG); Group 3 was respectively treated with LCL-HHT-H-PEG (left groin side, 150 mg/kg LCL-HHT-H-PEG) and MI-HHT (right groin side, 150 mg/kg MI-HHT). Each mouse tumor was locally treated with the above different reagents (100 μL in volume) 8 days after tumor formation, once three days, in total of 8 times. In further animal experiments, 5×10^6 MM RPMI 8226 cells were injected subcutaneously into dorsal side in NOD/SCID mice, and the tumor treatment was respectively treated with PBS, HHT, LCL-HHT-H-PEG, and LCL-H-PEG. The administration times and dosage were same as the primal animal experiment. The tumour growth in mice was monitored once three days for tumor volume, the general health indicators, such as overall behavior, feeding, body weight and appearance of fur after treatment. The endpoint for this study was the diameter of tumor ≥15 mm, at which point mice were euthanized. Mouse tumour tissues and bones were collected for further analysis. The mouse bone mineral density of the humerus, femurs and vertebra was measured by in vivo Micro-CT imaging with MCT-1108 (China) at a setting of voltage 45 kV and electric current 80 μA [27].

**Observation of ultra-structure by electron microscope**

Tumor tissue suspensions were collected and fixed with 2% paraformaldehyde/2.5% electron microscope grade glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.4) at 37°C. Ultrathin sections were cut and placed on formvar-coated slot copper grids. Sections were then counterstained with uranyl acetate and lead citrate and viewed with TEM. Digital images were acquired with a camera (ORIUS SC200, Gatan, U.S.A) [25].

**Statistical analysis**

Data are expressed in the mean and standard deviations. The Student’s t-test was used to
evaluate the differences between the different experimental groups. Bonferroni correction was used where multiple comparisons were made. \( P \) values < 0.05 were taken as statistically significant.

Results

Characterization of the diverse HHT formulations

The focus of this assay lies on the proportion of DSPE-PEG2000 to liposomes’ characteristic. The diverse HHT formulations, including high and low proportion PEG of long-circulating HHT liposomes, general liposomes and micelles, were shown in Figure 1. It is known that lipids make the most part of carries, and cholesterol enhances the intensity of the carry structure. DSPE-PEG2000 makes carries more hydrophilic to circulate long time in vivo so that HHT in lipids releases slowly with circulation. Micelles were made with surfactant Solutol® HS15 that has hydrophilic-hydrophobic property to make a drug capsule.

As shown in Figure 2, the characterizations of LCL-HHT-H-PEG was performed by using TEM. The morphology of LCL-HHT-H-PEG showed that it was maintained in the spherical shape, and that there were some floccules in the hollow carries. The carries became well-demarcated, and no rupture of the capsule wall was found, strongly suggesting that a typical vesicle structure had been formed. The resulting liposomes exhibited a narrow size distribution, with a diameter of approximate 73.63 nm. The zeta potential of the liposomes was -31 mV that means the suspension of the LCL-HHT-H-PEG has good stability as is shown in Figure 2. Compared to the LCL-HHT-H-PEG, the size of other carries are bigger and the diameter distribution are wider. The size of LCL-HHT-L-PEG are 82.90 nm with the protein dispersibility index (PDI) of 0.179 and the general liposomes are 74.71 nm with PDI of 0.290, and the micelles are 97.34 nm with PDI of 0.199. For zeta potential, other three carries are less than -30 mV that means the carries are not stable sometimes. From the above data, we concluded that the LCL-HHT-H-PEG have the smallest size, most narrow diameter distribution and best stability among all diverse HHT formulations.

Effect of the LCL-HHT-H-PEG on clone formation and apoptosis of MM RPMI 8226 cells in vitro

To test the inhibitory efficacy of the diverse HHT formulations on human MM RPMI 8226

![Figure 3](image-url)
Figure 4. In vitro MM RPMI 8226 cell apoptosis assay. A. Apoptosis ratio of MM RPMI 8226 cells in vitro as described in the section of Materials and methods. B. Quantification of apoptosis ratio. *p < 0.05, **p < 0.01, and ***p < 0.005.
Effect of LCL-HHT-H-PEG on RPMI8226 MM cells, we first observed the ability of cell clone formation when MM RPMI 8226 cells were cultured with the diverse HHT formulations, respectively. Figure 3A, 3B showed that clone formation ratio in LCL-HHT-H-PEG group was a significant lower than that of control group after 14 day culture (2.3±0.81% versus 23±1.92%, \( P < 0.001 \)) or than that of HHT group (2.3±0.81% versus 7.3±1.15%, \( P < 0.05 \)). The clone formation ratio among LCL-HHT-H-PEG, LCL-HHT-L-PEG, L-Enc-HHT, and MI-HHT groups were accounting for 2.3±0.81%, 2.8±0.95%, 3.8±2.29%, and 3.5±2.21% in order, which exhibited a little bit difference, however, there is no significant difference among these groups (\( P < 0.05 \)).

Subsequently, we analyzed these diverse HHT formulations that induced the apoptotic efficacy on MM RPMI 8226 cells. The apoptotic ratio was the highest (60±1.10%) in LCL-HHT-H-PEG group among all experiment groups when cells were incubated with the different reagents for 72 hours. It was found that other apoptotic ratio was around 47±1.36% for the HHT group, 52±1.09% for the L-Enc-HHT group, 56±4.07% for the LCL-HHT-L-PEG group, and 54±1.10% for the MI-HHT group, respectively, which was statistically significant (\( P < 0.01 \)) compared with control group (Figure 4A, 4B). These results suggested that the diverse HHT formulations had obvious inhibitive effect on MM RPMI 8226 cell growth by inhibition of clone formation and induction of cell apoptosis in vitro.

LCL-HHT-H-PEG inhibits the xenograft growth in MM bearing mice

Next, we wanted to know whether our in vitro observations would translate into anti-RPMI 8226 MM efficacy in vivo. To this end, we estab-
Established the murine xenograft model by injection of $5 \times 10^6$ human MM RPMI 8226 cell to the NOD/SCID mice. All the injected mice developed tumors that were felt by touch in around 21 days. We evaluated the anti-tumor efficacy by treatment of MM bearing NOD/SCID mice with the LCL-HHT-H-PEG 8 days after tumor formation. Figure 5A gives a treatment protocol in mice, and Figure 5B shows the images of tumor sizes on day 27 after the mice were initially treated with the diverse HHT formulations in total 8 times, once 3 days. All the treated mice had smaller tumors than that of control group mice, but the smallest tumor sizes was seen in the mice treated with LCL-HHT-H-PEG. The difference was statistically significant between the LCL-HHT-H-PEG and the control groups ($p < 0.01$), between the LCL-HHT-H-PEG and the LCL-HHT-L-PEG groups, and between the LCL-HHT-H-PEG and the HHT groups ($p < 0.03$); however, there was no significant difference in the tumor sizes between the LCL-HHT-H-PEG and the LCL-HHT-L-PEG groups, between the LCL-HHT-H-PEG and the L-Enc-HHT or MI-HHT groups ($p > 0.05$) although a larger decrease of tumor sizes was found in the LCL-HHT-H-PEG group, which were shown in Figure 5B and 5C. From the above results, we concluded that the LCL-HHT-H-PEG have a significant inhibition of tumor growth compared with other HHT formulation’s treatments. However, there were some confounding factors in this model due to two reagents for treatment of tumors of two sides in one mouse concurrently. It is a little of difficult to objectively evaluate the anti-RPMI 8226 MM efficacy in the model. For this reason, we focused the effect of LCL-HHT-H-PEG on the xenograft growth in MM bearing mice in further animal experiments as described in section of Materials and Methods.

Images of Figure 6A, 6B show the tumor bearing mice and the tumor sizes on day 27 after the mice were treated with the LCL-HHT-H-PEG and other control reagents. Compared with

Figure 6. LCL-HHT-H-PEG inhibits RPMI8226 MM in further animal experiments. A. Images of RPMI8226 MM bearing NOD/SCID mice. B. Images of tumor sizes stripped from the mice treated with the LCL-HHT-H-PEG and other control reagents as described in the section of Materials and methods. C. Quantification of tumor sizes. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$. 

Effect of LCL-HHT-H-PEG on RPMI8226 MM
Effect of LCL-HHT-H-PEG on RPMI8226 MM

A

Normal | Control | HHT | LCL-HHT-H-PEG | LCL-H-PEG

2197 | 2727 | 1343 | 1677 | 1489 | 1793 | 1349 | 1972 | 1602 | 1826

1697 | 2136 | 2117 | 1376 | 1445 | 1335 | 1450 | 1424 | 1628 | 1342 | 1501

B

Control | HHT | LCL-HHT-H-PEG | LCL-H-PEG

Humerus

2080 | 2277 | 2393 | 2096

Femur

1419 | 1673 | 1768 | 1502

Vertebra

2094 | 2328 | 2589 | 2042

C

Bone mineral density

Humerus | Femur | Vertebra

D

Bone mineral density

Humerus | Femur | Vertebra

other three control groups, the LCL-HHT-H-PEG formulation has shown to be a significant inhibition of tumor growth in RPMI 8226 MM mouse model. The difference was statistically significant (Figure 6C).

Lytic bone lesions in the MM RPMI 8226 cell-engrafted mice

Having found that the therapeutic efficacy of LCL-HHT-H-PEG formulation was efficient in the RPMI 8226 MM-bearing murine model, we next wanted to determine whether this effect had an influence on the improvement of lytic bone lesions in mice. Figure 7A shows the representative clinical pictures taken on day 27, scanned by in vivo Micro-CT imaging after mice were treated with PBS, HHT, LCL-HHT-H-PEG, and LCL-H-PEG respectively, and the left picture of mouse (Figure 7A) was used in normal control, in where the numbers represent the mouse bone mineral density. The difference was statistically significant ($p < 0.01$) as is shown in Figure 7C. After treatment, mice were sacrificed, and their bones (femurs, humerus and vertebra) were collected, and the bone mineral density was analyzed (Figure 7B). The treatment of LCL-HHT-H-PEG formulation significantly increased of the bone mineral density and ameliorated lytic bone lesions compared with the HHT and other control in the MM RPMI 8226 cell xenograft mice, which was statistically significant ($p < 0.01$) as is shown in Figure 7C. The treatment of LCL-HHT-H-PEG formulation significantly increased of the bone mineral density and ameliorated lytic bone lesions compared with the HHT and other control in the MM RPMI 8226 cell xenograft mice, which was statistically significant shown in Figure 7D. The results showed that the application of LCL-HHT-H-PEG to RPMI 8226 MM bearing NOD-SCID mice increased significantly mouse bone mineral density compared with other control mice.

LCL-HHT-H-PEG reduces the clone formation and increases the apoptosis of MM cells ex vivo

Further, we sought to know the possible mechanisms of this inhibitory efficacy. The tumor cells were isolated from the tumor tissues dissected from the RPMI8226 MM bearing mice, and incubated with the LCL-HHT-H-PEG, HHT and other control formulations, respectively, whose concentration was same as the in vitro assays. The results of the clone formation assay ex vivo showed that the clone formation ratio was dramatically inhibited in LCL-HHT-H-PEG group compared to HHT and control groups (Figure 8A), and refer to the statistically significant differences as indicated in histogram of Figure 8A. Consistently, the quantitative analysis by FCM showed that, upon incubation of MM cell-derived from the RPMI8226 MM bearing mice with LCL-HHT-H-PEG for 72 hours, cells underwent to significant apoptosis ex vivo in contrast to HHT and control groups. The apoptosis ratio was shown in histogram of Figure 8B.

Finally, the ultrastructural morphology of the MM cells was further analyzed by TEM. TEM imaging from the Figure 8C indicates that the ultrastructure of MM cell-derived from the RPMI8226 MM bearing mice treated with PBS, HHT, LCL-HHT-H-PEG, and LCL-H-PEG respectively, in where the LCL-HHT-H-PEG group exhibited the apoptotic morphologic characteristics and showed a chromatin condensation and margination to a crescent shape. These were not found in the PBS control or LCL-H-PEG treated MM cells. However, the HHT treated cells showed some degrees of chromatin condensation too.

Discussion

From the present study, we have shown that the LCL-HHT-H-PEG formulation exhibited more obvious inhibitory effects on clone formation and more apoptosis of MM RPMI8226 cells than any other formulation controls did in vitro. In vivo primal animal experiments, human MM cell (the RPMI8226 cell line) xenografts established in SCID mice showed that treatment with LCL-HHT-H-PEG significantly inhibited the tumor growth compared with that of mice treated with low dose of LCL-HHT-L-PEG, L-Enc-HHT, MI-HHT and free drug, respectively. Although the LCL-HHT-L-PEG also exhibited a good inhibitory effects on RPMI8226 MM cells in vitro and in vivo compared with L-Enc-HHT or MI-HHT, this effect was not so strong as LCL-HHT-H-PEG did. This is because that long-circulating, high dose of PEGylated, liposome-encapsulated HHT may
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Figure 8. Obvious inhibition of clone formation and induction of MM apoptosis. A. Clone formation ratio of MM cells derived from treated MM mice as described in the section of Methods. B. Apoptosis of MM cells derived from treated MM mice was analyzed by FCM. C. Ultrastructural morphology of MM cells derived from treated MM mice was analyzed by TEM. *p < 0.05, **p < 0.01 and ***p < 0.005.
have the efficacy of a steric hindrance and a hydrophilicity of membrane surface, which protects liposome-encapsulated HHT from the degradation by enzyme digestion or other biological activity materials. Therefore, the LCL-HHT-H-PEG formulation generally outperforms the conventional LCL-HHT-L-PEG, suggesting the importance of LCL-HHT-H-PEG caused by enhanced permeability, delivered high amount of HHT, and reserved therapeutic compound in MM, which leads to reinforced antitumor efficacy and decreased side effects compared to LCL-HHT-L-PEG and other control reagents [28]. Furthermore, the high inhibitory efficacy of LCL-HHT-L-PEG on the RPMI8226 MM in vitro was further confirmed by the following animal experiments, especially in the marked induction of MM cell apoptosis that was demonstrated by TEM analysis.

MM is the most common cancer type in haematological system. In most cases, no curative treatment options are available for refractory/relapsed MM as the tumors are highly resistant to chemotherapy. Targeted drug delivery by using liposomal drug delivery system is an attractive approach to enhance the efficacy of anticancer drugs and reduce side effects, thereby potentially promoting the therapeutic index, particularly in the increasing survival of elderly MM patients who are generally more susceptible to adverse events [1-3]. Indeed, reduced adverse effects would be a key attribute for new agents to facilitate their use in a greater proportion of MM patients. In most preclinical models, PEGylated long circulating liposomes containing anticancer drugs were shown to decrease disease burden with reduced drug toxicity in B-cell lymphoma, colon, breast, lung cancers etc [8, 9, 14, 15, 17, 28].

Using conventional HHT or PEGylated long-circulating liposome-engrafted HHT for acute and chronic myeloid leukemia has been reported in the last decades [5-7, 29-33], but has never been studied in RPMI8226 MM. For this reason, we investigated the therapeutic effect of LCL-HHT-H-PEG on RPMI8226 MM. To really evaluate the function of LCL-HHT-H-PEG, we used MI-HHT as control formulation. It is known that lipid micelles serve as versatile and effective drug carriers for cancer therapy and have shown to overcome several limitations of poorly soluble drugs such as non-specific biodistribution and targeting, lack of water solubility and poor oral bioavailability [34, 35]. In the primal study, MI-HHT indeed exhibited stronger therapeutic effect in reduction of clone formation and induction of cell apoptosis in vitro as well as inhibition of tumor growth in vivo than HHT and L-Enc-HHT did, but the LCL-HHT-H-PEG formulation resulted in greater intratumoral drug accumulation over time and reduce tumor growth than that of MI-HHT in the primal study or than that of HHT in the further study. Nevertheless, the different carriers provide specific advantages, which should be considered when formulating optimal combination therapies.

In conclusion, the data presented here indicates the LCL-HHT-H-PEG are effective for treatment of RPMI8226 MM in vitro and in vivo bearing NOD/SCID mice. The findings suggests LCL-HHT-H-PEG may be as a potential anti-MM drug for enhancing MM treatment outcome with decrease of chemotherapeutic side effects.

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Disclosure of conflict of interest

None.

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