Effect of transferrin-modified liposomal curcumin on proliferation inhibition of oral squamous cell carcinoma

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Research Article

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Abstract

Curcumin can inhibit the proliferation and metastasis of a variety of tumor cells, including oral tumor, but its use is severely limited by its low bioavailability and stability. In this study, transferrin-modified liposomal curcumin was constructed to detect its inhibition on oral squamous cell carcinoma cell line HN4 and compared with liposomal curcumin and curcumin solution (curcumin dissolved in PBS (pH7.4, 0.01Mol/L)). Firstly, curcumin solution (Cur), liposomal curcumin (Cur-Lips) and transferrin-modified liposomal curcumin (Tf-Cur-Lips) were prepared. The regulatory effects of Cur, Cur-Lips and Tf-Cur-Lips on the internal and external disposal of curcumin were investigated by curcumin release in vitro and pharmacokinetics in vivo in rats. Then, HN4 cells were treated with Cur, Cur-Lips and Tf-Cur-Lips at different concentrations, respectively, and cck-8 was used to detect the effect of different experimental groups on HN4 cells proliferation. Finally, to investigate the molecular mechanism of curcumin inhibiting the proliferation and apoptosis of HN4 cells, the expression levels of apoptosis-related genes P53 and Fas was detected and compared by real-time fluorescence quantitative PCR in Cur, Cur-Lips and Tf-Cur-Lips groups. We found that compared with Cur, Cur-Lips metabolic time was significantly prolonged, and transferrin modification could further improve the stability of liposomal curcumin and prolongate liposomal curcumin metabolic time. Compared with Cur and Cur-Lips, Tf-Cur-Lips can significantly enhance the proliferation inhibition of HN4 cells, induce their apoptosis, and up-regulate the expression of apoptosis-related genes P53 and Fas. In conclusion, compared with Cur and Cur-Lips, Tf-Cur-Lips have stronger inhibitory effect on oral squamous cell carcinoma cell line HN4. This study will provide a new option for construction a long-term targeted curcumin delivery system against oral squamous cell carcinoma.

Background

Oral cancer is the most common malignant tumor of head and neck, among which oral squamous cell carcinoma is accounting for about 90% of oral malignant tumors [1, 2]. In the treatment of oral cancer, surgery combined with radiotherapy and chemotherapy is the most effective treatment method at present, but after treatment, patients often lose many important functions, such as facial changes, speech problems, taste disorders and dysphagia, greatly endangering the quality of life of patients [3–5]. Therefore, the prevention and treatment of oral cancer has become a research focus.

Curcumin is a natural plant polyphenolic compound extracted from the rhizome of curcuma. Studies have shown that curcumin can inhibit the growth and metastasis of a variety of tumor cells, including oral cancer [6–9]. At present, the anti-tumor mechanism of curcumin is mainly the regulation of oncogenes and tumor suppressor genes. The activity of cyclooxygenase 2 (COX-2) and induced nitric oxide synthase (iNOS) was inhibited. Down-regulated activity of nuclear transcription factor-κB (NF-κB); Affect protein kinase C signaling pathway; Induce cell apoptosis or cell cycle stagnation; Inhibition of urokinase activity and matrix metalloproteinase 9 (MMP − 9) secretion; Inhibit angiogenesis, inhibit the invasion and metastasis of tumor cells. [10–12]. Curcumin's good anti-tumor properties and small toxic side effects make it a promising anti-tumor drug with strong development and application prospects [13].

But curcumin is hard to dissolve in water, and bioavailability is extremely low, which severely limits its clinical use. In addition, curcumin was stabilized in a solution with a pH < 6.5, and the degradation rate was significantly accelerated as the pH value of the solution increased. Curcumin was incubated in PBS (pH7.2) at 37°C 0.1Mol/L, and 90% of it will be degraded [14–16]. Liposomes are lipid bilayer structures composed of phospholipids and cholesterol, which are often used as drug carriers. Liposomes have the advantages of good biocompatibility, avoiding immune response and reducing toxicity [17, 18]. As a hydrophobic drug, curcumin has high liposomes encapsulation rate, so it has obvious advantages to use the liposomes as the carrier of curcumin. After encapsulating curcumin into liposomes, the water solubility and stability of curcumin were significantly improved [19, 20]. Liposomes, as drug carriers, have a certain targeting property, but in general, the targeting property of liposomes mainly focuses on organs rich in reticuloendothelial cells, such as liver, spleen and kidney, and is passive targeting. If treat other tissues and organs, its active targeting property is not obvious [21, 22]. Although curcumin, as a natural product, rarely causes toxic side effects, it is still a multi-target drug and can cause some side effects, including abdominal pain, nausea, and even severe vomiting or diarrhea [23]. It is also important to develop the active targeting drug delivery system of liposomal curcumin to improve the targeting efficiency of curcumin, enhance the specific lethality of curcumin to tumor cells, and reduce the damage to normal cells [24–26].

Studies have shown that transferrin receptor is a receptor commonly expressed on the surface of tumor cells [27, 28]. The expression level on the surface of tumor cell membrane is usually 2–7 times that of normal cells, and the affinity between transferrin receptor and its ligand transferrin on the surface of tumor cells is 10–100 times that of normal cells. Transferrin is a small molecule glycoprotein. After modifying the drug carrier with transferrin, transferrin can bind to transferrin receptors on the surface of tumor cells and be actively absorbed into cells under the mediation of transferrin receptors, thus achieving the specific transport of anti-tumor drugs to tumor cells [29–33].

Therefore, in this study, transferrin was used to carry out targeted modification of liposomal curcumin to study its metabolism in vivo and in vitro and its inhibitory effect on the proliferation of oral squamous cell carcinoma cell line HN4, so as to establish a long-term drug system for the targeted treatment of oral squamous cell carcinoma and provide new ideas for the targeted treatment and drug development of oral squamous cell carcinoma.

Methods

Materials

Phospholipids (soybean lecithin for injection use, with phosphatidylcholine content > 70%) were purchased from Shanghai Tai-Wei Pharmaceutical Co., Ltd. (Shanghai, China). Cholesterol was obtained from Amresco (Solon, OH, USA). Curcumin was obtained from Sigma (St. Louis, MO, USA). Transferrin was obtained from Sigma (St. Louis, MO, USA). Bovine serum albumin-V was obtained from Amresco (Solon, OH, USA). Improved PRMI-1640 medium, fetal bovine serum and pancreatic enzyme were

purchased from HyClone (Logan, UT, USA). Poloxamer 188 (F68) was obtained from BASF (China) Co., Ltd. (Shanghai, China). All other chemical reagents used in this study were of analytical grade or better. Oral squamous cell carcinoma cell line HN4 was provided by the state key laboratory of west China stomatology, Sichuan university.

Preparation of liposomal curcumin (Cur-Lips)

In this experiment, the modified ethanol injection method according to our previous works was used to prepare Cur-Lips [34]. The specific methods are as follows: Combining curcumin, phospholipids, and cholesterol in ethanol was as an organic phase. The phosphate buffer solution (PBS, 0.01Mol/L, pH 7.4) containing F68 was used as the aqueous phase. F68 was served as a surfactant to narrow the size distributions. Then the organic phase was injected into the aqueous phase under magnetic agitation slowly, and the organic solvent was removed by roto evaporator in a water bath at 37°C for 30 minutes without light. The resulting suspension was first centrifuged at a low speed (3 000r·min^{-1}, 5min) to remove the free curcumin, then centrifuged at a high speed (16 000r·min^{-1}, 10min) to discard the supernatant. Finally, the precipitate was suspended in PBS (pH 7.4) to obtain Cur-Lips. For Cur-Lips, the mass ratio of phospholipid to cholesterol was 5:1, the mass fraction of F68 was 1%, the ratio of organic phase to aqueous phase was 10:1, the final concentration of phospholipid was 10mg/mL, and the final concentration of curcumin was 0.4mg/mL.

Preparation of transferrin-modified liposomal curcumin (Tf-Cur-Lips)

Tf-Cur-Lips were prepared by post-insertion method. Specific methods: Transferrin micelles were obtained by mercaptoylation of transferrin in a water bath at 37°C for 2h. Then the transferrin micelles were incubated with the prepared liposomal curcumin in a water bath at 37°C for 4h and was added to the dialysis bag (with an immobilized molecular weight of 10KDa) for dialysis for 12h [35]. Transferrin was modified to liposomal curcumin by covalent binding of the amine group of transferrin to the carboxyl group of the liposomes. Free curcumin and transferrin were removed to obtain Tf-Cur-Lips.

Characterization of Cur-Lips and Tf-Cur-Lips

The particle size, size distribution and ζ-potential of Cur-Lips and Tf-Cur-Lips were examined by dynamic light scattering (DLS) and electrophoretic light scattering (ELS) technologies, respectively, using the instrument of Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). The morphology of Cur-Lips and Tf-Cur-Lips was observed by scanning electron microscopy (SEM, INSPECT F, FEI, Eindhoven, The Netherlands). Before SEM, one drop of the properly diluted Cur-Lips or Tf-Cur-Lips suspension was placed on a clean glass sheet, followed drying in the air. The samples were coated with gold and observed under the scanning electron microscopy.

The entrapment efficiency of curcumin in Cur-Lips and Tf-Cur-Lips was determined by low temperature and high-speed centrifugation method. An appropriate amount of Cur-Lips or Tf-Cur-Lips was taken in a precise amount and centrifuged at 16,000 r·min^{-1} for 5min. After centrifugation, the supernatant (1mL to

10mL) was diluted to the scale with anhydrous ethanol and filtered through 0.22μm organic filter membrane. The content of free curcumin was calculated by fluorescence spectrophotometry (Ex:458nm, Em:548nm) in the supernatant and presented as W_1, i.e. Then, 1mL Cur-Lips or Tf-Cur-Lips was added to 10mL measuring bottle, anhydrous ethanol was added to the scale, and then filtered through 0.22μm organic filter membrane. The content of free curcumin was determined by fluorescence spectrophotometry in the filtrate and presented as W, i.e. The encapsulation efficiency (EE) of the curcumin was calculated using the following equation: $EE\% = (W - W_1) / W \times 100\%$.

In vitro release study of Cur-Lips and Tf-Cur-Lips

The *in vitro* release property of Cur-Lips and Tf-Cur-Lips was observed using the dynamic dialysis method [36]. As a control, we measured the release rate of curcumin dissolved in PBS (pH7.4, 0.01Mol/L). 1mL curcumin solution, Cur-Lips or Tf-Cur-Lips (the final concentration of curcumin was 2mg/mL in these three groups) were added to dialysis bags with a cutoff molecular weight of 10KDa (only curcumin was allowed to pass, but liposomes were not). After tying the two ends of the dialysis bag tightly, they were immersed in 10mL of the release medium (PBS containing 0.5% Tween® 80, pH 7.4) and placed in a horizontal shaker (37°C, 100 $r \cdot min^{-1}$). All the fluid outside the dialysis bag was removed at a fixed time point (0.5,1, 2, 4, 6, 8, 24, 48, 72,96,168h) and 10mL of fresh release medium was added to the release system. The collected samples were stored in the refrigerator at -80°C. After all samples were collected, the samples were 16000 $r \cdot min^{-1}$ and centrifugated for 5min. A supernatant of 200 μL was taken to analyze the curcumin content (Em:458nm; Ex: 548nm).

Pharmacokinetics of Cur-Lips and Tf-Cur-Lips in rats

Eighteen healthy adult SD male rats of 200-250g were divided into three groups of six. Curcumin solution (dissolved in pH7.4, 0.01Mol/L PBS as a control group), Cur-Lips or Tf-Cur-Lips were intravenously injected into male SD rats with a final curcumin concentration of 200μg/kg (n = 6). At a predetermined time-point (15min, 30min, 1h, 2h, 4h, 6h, 8h, 24h, 48h, 72h), venous blood was taken 200μL and added to the heparin-treated EP tube. An aliquot of 50μL plasma was added into the tubes containing 10ng of emodin which was used as the inner standard. 100μL methanol and 100μL chloroform were added to extracts curcumin and emodin by vortex and sonication for 5 min in sequence. Plasma was separated by 16000 $r \cdot min^{-1}$ centrifugation twice for 3min and 200μL fresh methanol was added to the residue followed by vortex and centrifugation again. The liquid was combined and dried by air flow. The residue was dissolved in 200μL of methanol by vortex for 3 min. The contents of curcumin in plasma at each time point were determined by HPLC internal standard method using a fluorescent detector (Em:458nm; Ex: 548nm), and the plasma drug concentration-time curve of curcumin was drawn to compare the differences among the groups.

Inhibition of Cur-Lips and Tf-Cur-Lips on HN4 cells proliferation

HN4 cells were cultured in vitro, and the logarithmic growth phase cells were taken. After digestion with trypsin, about 5000 cells per well were inoculated into 96-well plates. After the cells were further cultured in an incubator for 24h, curcumin solution (dissolved in pH7.4, 0.01Mol/L PBS as a control group), Cur-Lips or Tf-Cur-Lips were added, respectively. The final concentration of curcumin in each group was diluted to 0,10,20,40,80μmol/L with a modified serum free PRMI-1640 medium, and 5 secondary pores were set in each group. After treatment for 1d and 3d, the culture medium was discarded for each group, and the modified PRMI-1640 medium containing 10% cck-8 was added. After incubation at 37°C for 30min, the absorbance value at 450nm was detected, and the proliferation inhibition rate of HN4 cells was calculated.

Extraction of total RNA, RT-PCR and real-time PCR

HN4 cells were cultured in vitro and logarithmic growth phase cells were added with curcumin concentration of 40μmol/L curcumin solution (dissolved in pH7.4, 0.01Mol/L PBS as a control group), Cur-Lips or Tf-Cur-Lips for 24h, and serum-free modified PRMI-1640 medium was added as the control group. Total RNA, RT-PCR and real-time PCR were extracted to evaluate the expression of apoptosis-related genes P53 and Fas at transcriptional levels in HN4 cells. The total RNA of each group was extracted using the Simply. P total RNA extraction kit (BioFlux, hangzhou, China), and then the PrimeScript RT reagent kit with cDNA Eraser (Takara Bio, Tokyo, Japan) was used to transect 1μg of total RNA into cDNA. Method outlined in 'Molecular Cloning: A Laboratory Manual' (2001, 3rd edn). Total RNA and cDNA were detected using agar-gel electrophoresis. $_{c}$DNA samples were amplified with RT-PCR kit (Tian-gen, Beijing, China) to establish the standard curve of specific genes. The primers of each gene used in this experiment are shown in Table 1. These primers were all from the established GenBank sequence. For real-time PCR, expression of target genes in HN4 cells were quantified using SYBR Premix Ex TaqTM (Perfect Real Time) kit (Takara) carried out on an ABI 7300 system (ABI, Foster City, CA, USA). For each reaction, a melting curve was generated to test primer dimer formation. The relative quantification of mRNA level was carried out by the double-standard curve method. To evaluate the PCR efficiency, the amplification of GAPDH (D-glyceraldehyde-3-phosphate dehydrogenase) was used as the control.

Table 1
Primer sequences of target genes and GAPDH for real-time PCR assay

Gene	Primer sequence (5'–3')
GAPDH	F: GACGGCCGCATCTTCTTGTGC
	R: TGCAAATGGCAGCCCTGGTGA
P53	F: CTTTGAGGTGCGTGTTT
	R: CAGTGCTCGCTTAGTGC
Fas	F: GTGATGAAGGGCATGGTTTAG
	R: GCATTTGGTGTTGCTGGTT

Statistical Analysis

All assays in this study were repeated at least thrice. The t test was used for comparison between the two groups and a one-way ANOVA was used to analyze differences among groups. In all tables and figures, representative data were presented as the mean ± standard deviation. *P* values < 0.05 were considered statistically significant.

Results And Discussion

Characterization of Cur-Lips and Tf-Cur-Lips

The results of particle size, polydispersity index (PDI), and ζ-potential of Cur-Lips and Tf-Cur-Lips measured in this experiment are shown in Table 2, and the typical particle size and ζ-potential diagram are shown in Fig. 1. The results showed that the mean particle size of Tf-Cur-Lips was 194nm, slightly larger than the mean particle size of Cur-Lips at 183nm, with a statistically significant difference. Generally, the smaller the PDI, the more uniform the particle size dispersion is. In this experiment, the PDI of Cur-Lips and Tf-Cur-Lips prepared is less than 0.25, and the difference is not statistically significant, indicating that the particle sizes of Cur-Lips and Tf-Cur-Lips prepared by us are relatively uniform. The mean potential of Cur-Lips was − 28.8mv and that of Tf-Cur-Lips was − 29.3mv, showing no statistically significant difference. The morphology of Cur-Lips and Tf-Cur-Lips was observed by SEM. As shown in Fig. 2, it can be seen that both Cur-Lips and Tf-Cur-Lips are spherical with uniform particle distribution. The particle size of Tf-Cur-Lips is slightly larger than that of Cur-Lips. The results of SEM were consistent with those of particle size derived from DLS measurement.

After calculation, the entrapment efficiency of Cur-Lips was (86.5 ± 1.1) %, while that of Tf-Cur-Lips was (85.9 ± 1.5) %, and the difference was not statistically significant. This indicates that both Cur-Lips and Tf-Cur-Lips prepared in this study have good capacity to carry curcumin, and the presence of transferrin does not affect the encapsulation rate of Cur-Lips.

Table 2
Particle size and surface potential of Cur-Lips and Tf-Cur-Lips

Samples	Size(nm)	polydispersity index	ζ-potential(mv)
Cur-Lips	183 ± 2.8	0.19 ± 0.03	-28.8 ± 0.3
Tf-Cur-Lips	194 ± 2.1*	0.20 ± 0.05	-29.3 ± 0.5
Note: Compared with Cur-Lips, *P< 0.05, Data are presented as mean ± s.d. (n = 3)			

In vitro release study of Cur-Lips and Tf-Cur-Lips

Release of drug in loaded by liposome is an important evaluation index for liposome delivery system. In this experiment, we measured curcumin release rates of Cur-Lips and Tf-Cur-Lips. As a control, we measured the release rate of curcumin dissolved in PBS (pH7.4, 0.01Mol/L). The specific results are shown in Fig. 3. We know from the results that the release rate of curcumin solution dissolved in PBS is very rapid, which is close to 100% by 6 hours. Curcumin release rate for Cur-Lips and Tf-Cur-Lips was significantly slower than that of curcumin solution, and the cumulative release rate of Cur-Lips was 93% at 168h, while the cumulative release rate of Tf-Cur-Lips was 85% at 168h. The release rate of Tf-Cur-Lips was slower than that of Cur-Lips, and the difference was statistically significant. Initial burst release of drugs is a common phenomenon in many liposome delivery systems and also is an adverse factor in liposome delivery systems. In this study, it was found that no significant initial burst release effect was observed for either Cur-Lips or Tf-Cur-Lips. In addition, curcumin release curves of curcumin for Cur-Lips and Tf-Cur-Lips are quite close to zero-order release kinetics, indicating that curcumin release is a stable release rate for both Cur-Lips and Tf-Cur-Lips, which is also rare for liposome drug delivery systems.

Liposomes encapsulated drugs that are released in three ways: (a) the drug molecules adsorbed on the surface of liposomes are released via desorption upon contacting with release medium, (b) the encapsulated drugs are released by diffusion through the liposomes skeleton or/and (c) following the degradation or disintegration of liposomes [37]. We speculated the reasons for the reduced release rate of Tf-Cur-Lips was that transferrin may enhance the structural stability of curcumin-loaded liposomes to some extent after covalently bonding to the outside of curcumin-loaded liposomes. The enhanced structural stability of liposomes can slow down the disintegration of liposomes and slow down the release of drug carriers, which may be one of the reasons for the slow-release rate of curcumin-loaded liposomes after binding to transferrin. Another reason we analyzed may be that the transferrin on the surface of the curcumin-loaded liposome acts as a physical barrier to prevent the release of curcumin, which may lead to the sustained release of the liposomal curcumin after binding to the transferrin and prolonged in vitro time.

Pharmacokinetics of Cur-Lips and Tf-Cur-Lips in rats

In this study, we measured the changes of curcumin concentration in the plasma over time in the Cur-Lips and Tf-Cur-Lips groups and plotted the pharmacokinetic curve. The effect of transferrin modification on

curcumin metabolism in vivo was observed. Similarly, as a control, the metabolism of curcumin solution dissolved in pH7.4, 0.01Mol/L PBS in rats was also detected, as shown in Fig. 4. As can be seen from Fig. 4, the plasma concentration of curcumin solution at 2h after entering the plasma was only 0.8 ± 0.05ng/mL, while the presence of curcumin in the plasma could hardly be detected at 4h, indicating that curcumin solution was quickly metabolized after entering the blood. For the Cur-Lips and Tf-Cur-Lips, we found that the plasma concentration of curcumin can be maintained for a long time. At 2h, curcumin concentration in the Cur-Lips group was 76 ± 2.23ng/mL, while that in the Tf-Cur-Lips group was 93 ± 3.7ng/mL. The difference between the two groups was statistically significant. At 4h, curcumin concentration in the Cur-Lips group was 53 ± 1.32ng/mL, while that in the Tf-Cur-Lips group was 68 ± 2.31ng/mL. The difference between the two groups was also statistically significant. Curcumin was also detected in the plasma of the Cur-Lips and Tf-Cur-Lips groups at 48h, with concentrations of 3.6 ± 0.09ng/mL and 6.1 ± 1.05ng/mL, respectively ($P < 0.05$). However, curcumin could not be detected in the Cur-Lips group at 72h, and the plasma concentration of curcumin in the Tf-Cur-Lips group was 0.5 ± 0.07ng/mL. The results showed that both Cur-Lips and Tf-Cur-Lips had a slow-release effect compared with curcumin solution, but the Tf-Cur-Lips group had a stronger sustained release effect, and the circulation time of curcumin in vivo was longer, which also improved the bioavailability of curcumin. Like in vitro release experiments, we speculated that transferrin may enhance the structural stability of curcumin-loaded liposomes and acts as a physical barrier to prevent the release of curcumin which decreased curcumin metabolism in plasma.

Inhibition of Cur-Lips and Tf-Cur-Lips on HN4 cells proliferation

CCK-8 assay was used to detect the inhibition of Cur-Lips and Tf-Cur-Lips on HN4 cells proliferation, as shown in Fig. 5. Inhibition of HN4 cells proliferation by curcumin solution as a control was also examined. Results showed that the inhibition of curcumin solution on the proliferation of HN4 cells was weak, and the inhibition rate of HN4 cells was only 27% when treated with 80μmol/L free curcumin for 1d, and only 35% after 3d treatment, which may be related to the limited solubility and poor stability of curcumin solution. In contrast, Cur-Lips and Tf-Cur-Lips had significant inhibitory effects on HN4 cells proliferation and this inhibitory effect shows obvious concentration dependence. That is, the higher the concentrations of Cur-Lips and Tf-Cur-Lips were, the more obvious the inhibitory effect on HN4 cells proliferation was. The Cur-Lips with 80μmol/L of HN4 cells inhibited 52% of HN4 cells on day 1 and 78% on day 3. The inhibition rate of 80μmol/L Tf-Cur-Lips on HN4 cells was 68% on 1d and 93% on 3d. Compared with Cur-Lips, Tf-Cur-Lips showed no statistically significant difference in inhibiting the proliferation of HN4 cells at low concentrations of 10μmol/L and 20μmol/L at 1d. However, at high concentrations of 40μmol/L and 80μmol/L, Tf-Cur-Lips showed more significant cellular inhibitory effect than Cur-Lips and the difference was statistically significant. Moreover, compared with Cur-Lips, Tf-Cur-Lips showed more significant inhibition of HN4 cells proliferation at all concentrations during the treatment of 3d, which was statistically significant.

It is known from the results of this experiment that the liposomal curcumin prepared by us significantly increased the water solubility of curcumin, improved the stability of curcumin, and prolonged the time of action in vivo and in vitro, thus playing a better role in inhibiting HN4 cells. Results we found that the transferrin-modified liposomal curcumin has stable physical and chemical properties, uniform particle size, also has the very high rate of curcumin entrapment, and the surprise is through the in vitro release and in vivo pharmacokinetic experiments show that the transferrin-modified liposomal curcumin longer drug effect time in vivo and in vitro compared with liposomal curcumin. Therefore, transferrin-modified liposomal curcumin has a stronger inhibitory effect on HN4 cells than liposomal curcumin, which may be partly due to the sustained release effect of this drug. On the other hand, it is precisely because of modification of liposomal curcumin with transferrin that transferrin binds to transferrin receptors on the surface of HN4 cells and is actively absorbed into cells under the mediation of transferrin receptors, thus achieving the specific transport of curcumin to tumor cells. Therefore, the effect of transferrin-modified liposomal curcumin on inhibiting the proliferation of HN4 cells was significantly enhanced.

Extraction of total RNA, RT-PCR and real-time PCR

Figure 6 shows the expression of gene P53 and Fas in HN4 cells treated with Cur, Cur-Lips and Tf-Cur-Lips for 24h, respectively. The results showed that expression of P53 and Fas in Cur, Cur-Lips and Tf-Cur-Lips treatment groups increased 24h after administration compared with the control group. Among the four groups, the expression of P53 and Fas in the Tf-Cur-Lips group was the highest, followed by Cur-Lips. There were statistically significant differences in P53 and Fas expression between the groups. We demonstrated that the action of curcumin against HN4 cells can be achieved by up-regulating the apoptotic factors P53 and Fas, and that transferrin modification can improve this up-regulated effect. So, we speculated that curcumin inhibits HN4 cells process related to P53, and Fas gene related signaling pathways.

Conclusions

In this study, we successfully constructed transferrin-modified liposomal curcumin, which has stable physical and chemical properties and high rate of curcumin entrapment efficiency. To our surprise, the transferrin-modified liposomal curcumin can reduce the release rate of curcumin in vitro and prolong the curcumin blood circulation time in vivo compared with liposomal curcumin. Most importantly, the effect of transferrin-modified liposomal curcumin on inhibiting the proliferation of HN4 cells was significantly enhanced compared with curcumin solution and liposomal curcumin. Finally, we demonstrated that curcumin inhibits HN4 cells process related to P53, and Fas gene related signaling pathways. Although further efforts investigating the in vitro and vivo targeted antitumor effect of the transferrin-modified liposomal curcumin on HN4 cells are needed to support construct a long-term targeted curcumin delivery system against oral squamous cell carcinoma. We hope that this study will provide a new option for the targeted therapy and drug development of oral squamous cell carcinoma, provide a new idea for the application of curcumin in vivo, and provide a scientific basis for promoting the application of natural plant ingredients in cancer.

Abbreviations

Cur: Curcumin; Cur-Lips: liposomal curcumin; Tf-Cur-Lips: transferrin-modified liposomal curcumin; PBS: phosphate buffer saline; BSA: bovine serum albumin; COX-2: cyclooxygenase 2; iNOS: induced nitric oxide synthase; NF-κB: nuclear transcription factor-κB; MMP -9: matrix metalloproteinase 9; F68: Poloxamer 188; DLS: dynamic light scattering; ELS: electrophoretic light scattering; SEM: scanning electron microscopy; EE: encapsulation efficiency; GAPDH: D-glyceraldehyde-3-phosphate dehydrogenase; PDI: polydispersity index.

Declarations

Ethics approval and consent to participate

The experiment was approved by the Life Science Ethics Review Committee of Zhengzhou University.

Consent for publication

The author declare that consent this article for publication.

Available of Date and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

The author declare that has no competing interests.

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Authors' contributions:

Xue-Qin Wei designed research, performed the experiments, collected, and analyzed the date and wrote the manuscript.

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Figures

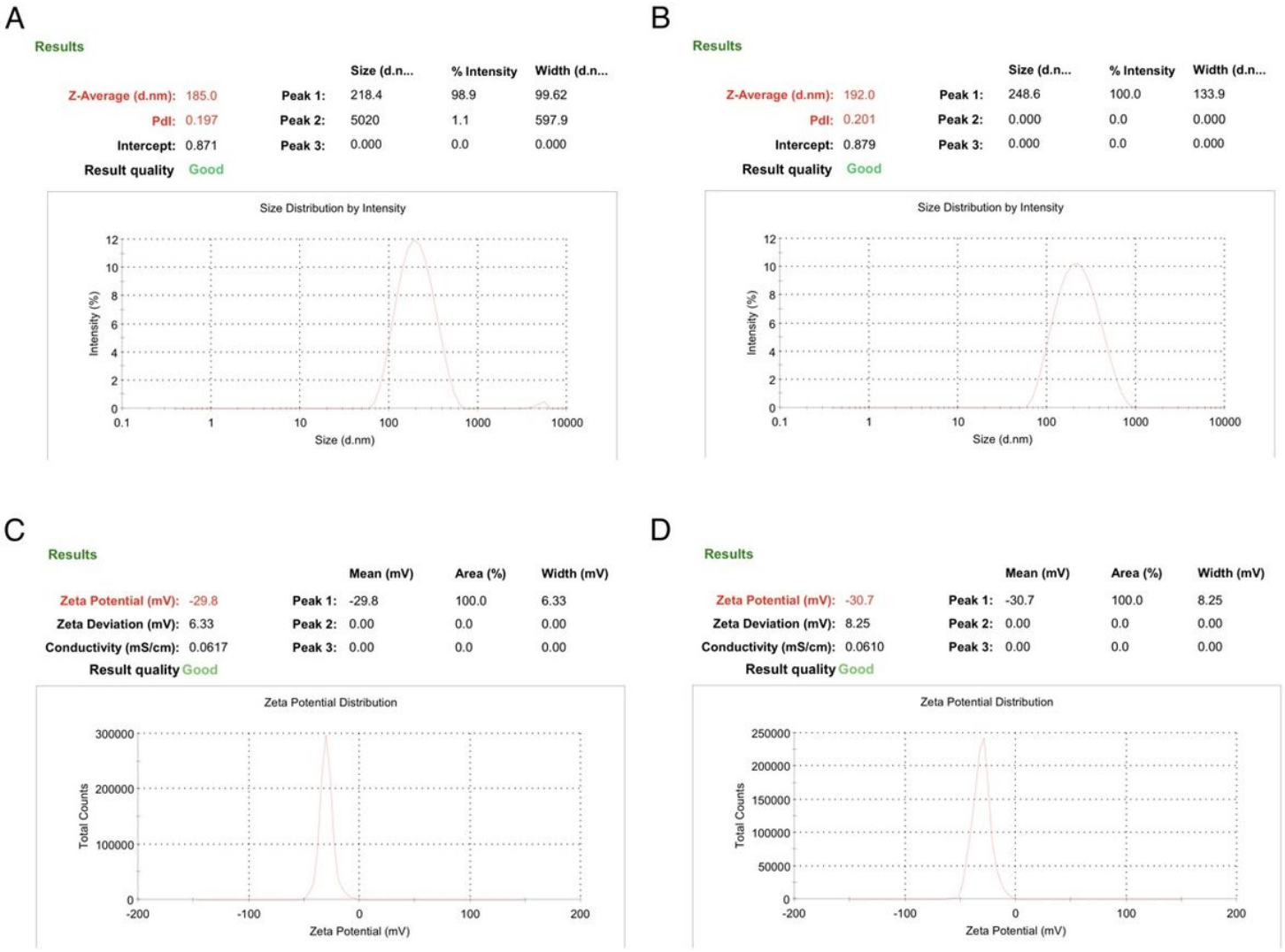

Figure 1

Particle size and potential profile. A and C are the typical particle size and ζ-potential distribution of Cur-Lips. B and D are the typical particle size and ζ-potential distribution of Tf-Cur-Lips.

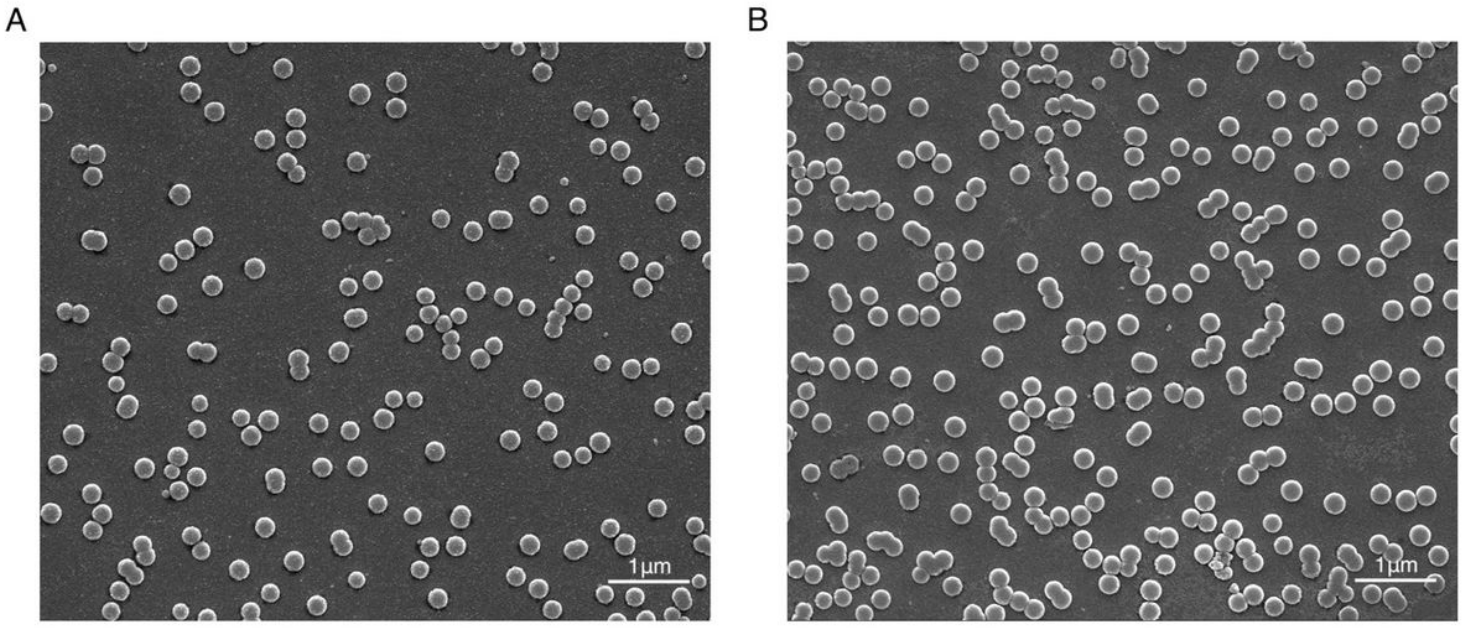

Figure 2

SEM photographs of Cur-Lips and Tf-Cur-Lips. A: Cur-Lips; B: Tf-Cur-Lips. Scale bar: 1μm

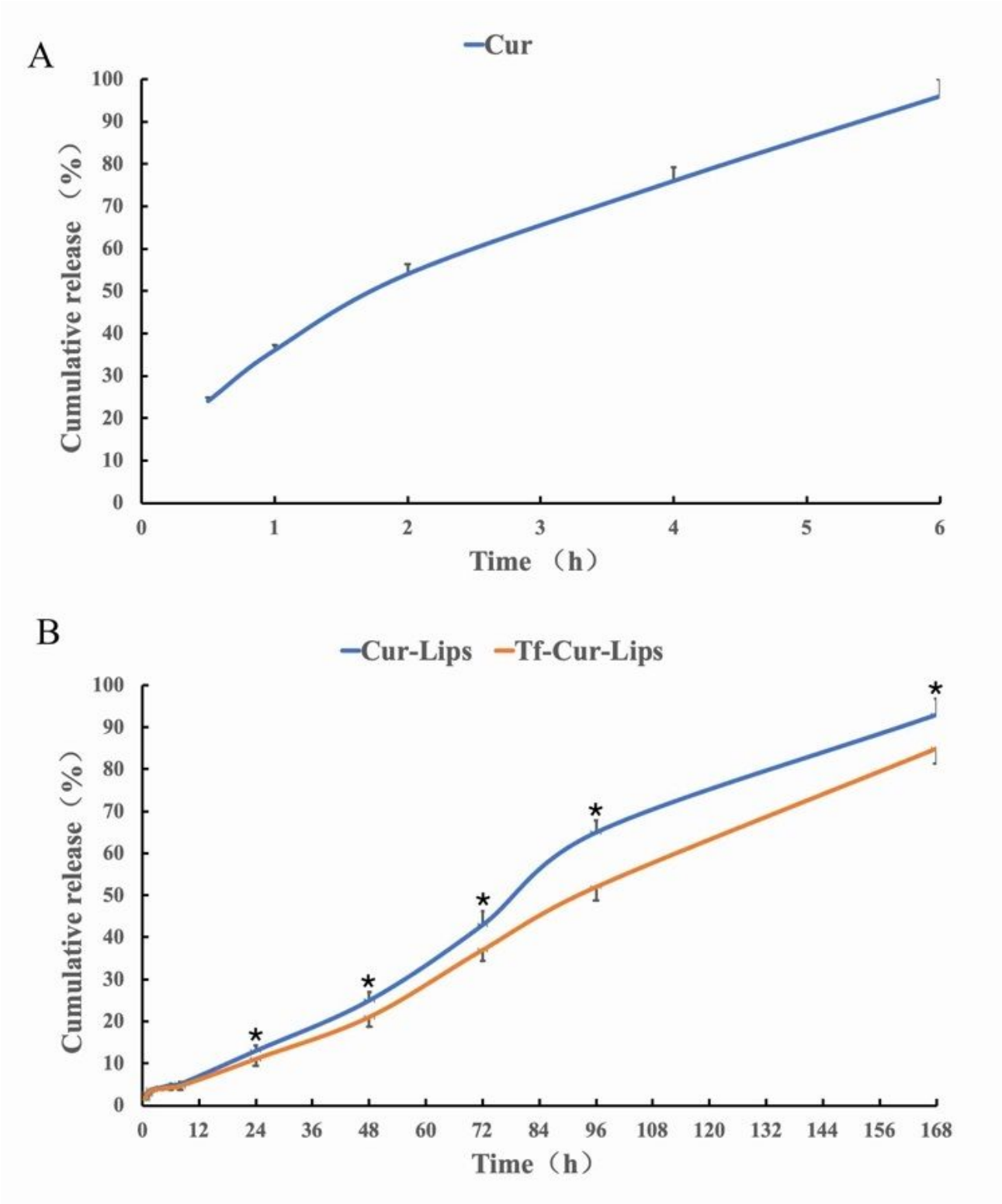

Figure 3

In vitro release profiles of different curcumin formulations in PBS containing 0.5% (m/v) of the Tween-80 (pH7.4). A: Curcumin solution, in which curcumin was dissolved in PBS (pH7.4, 0.01Mol/L). B: Cur-Lips and Tf-Cur-Lips in PBS (pH7.4, 0.01Mol/L). Data presented as the mean ± SD (n=3). *P* values < 0.05 were considered as statistically significant, as indicated by* for comparison in Cur-Lips vs. Tf-Cur-Lips.

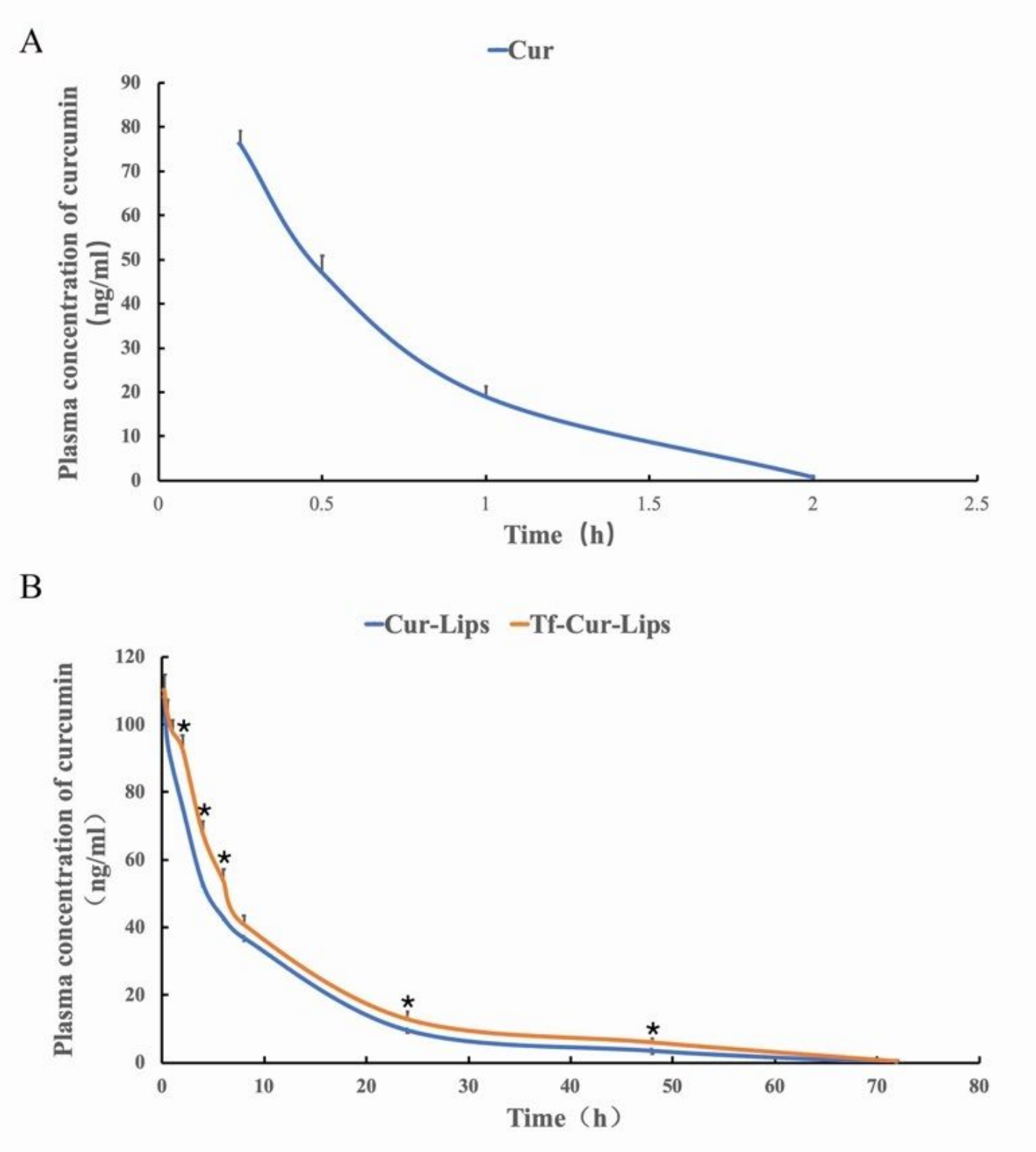

Figure 4

Pharmacokinetics profiles of different curcumin formulations after i.v. injection into the SD male rats at a dose of 200μg/kg. A: Curcumin solution, in which curcumin was dissolved in PBS (pH7.4, 0.01Mol/L). B: Cur-Lips and Tf-Cur-Lips in PBS (pH7.4, 0.01Mol/L). Data presented as the mean ± SD (n=6). *P* values < 0.05 were considered as statistically significant, as indicated by* for comparison in Tf-Cur-Lips vs. Cur-Lips.

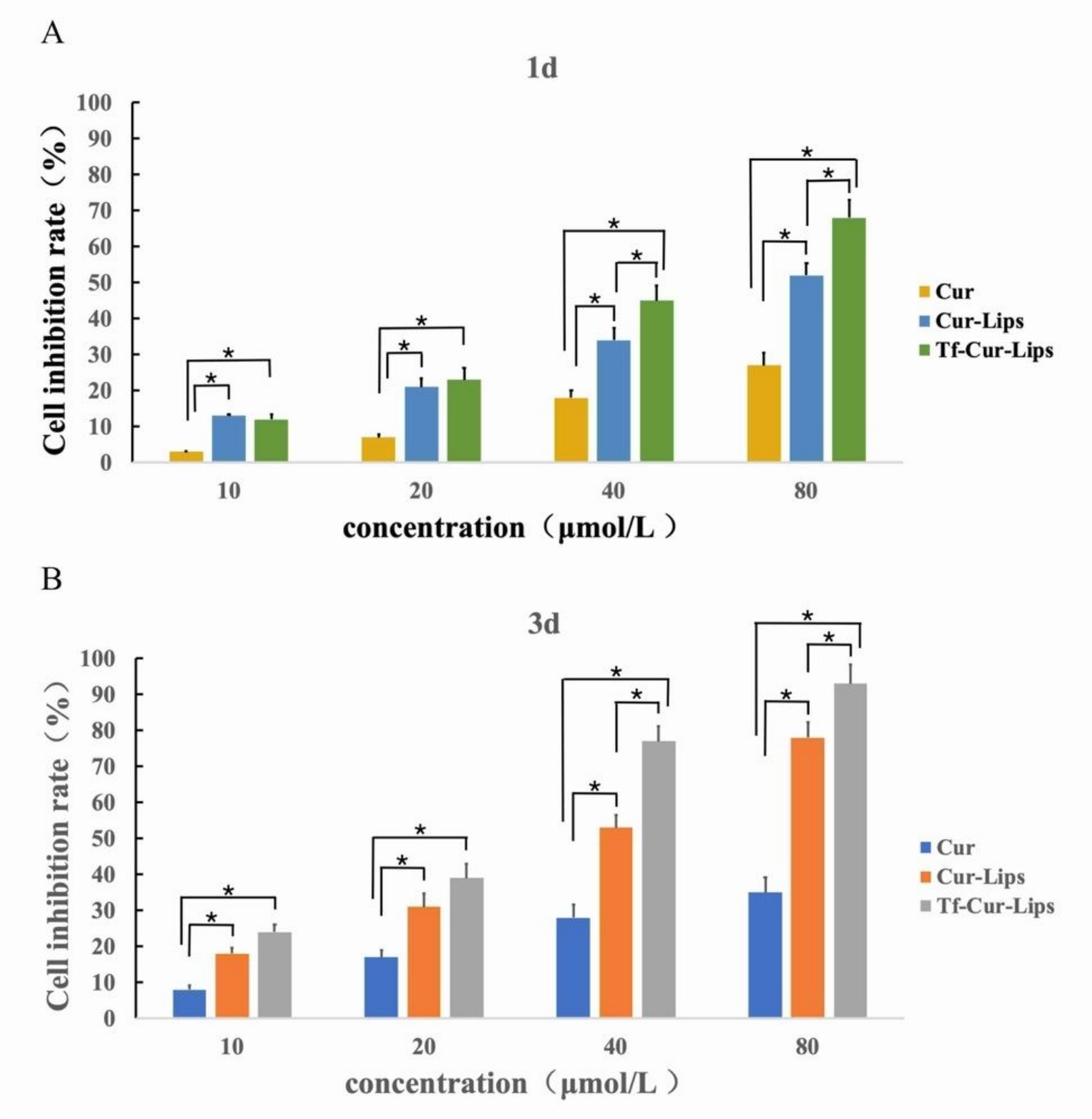

Figure 5

The inhibitory effect of Cur, Cur-Lips and Tf-Curl-Lips on HN4 cells proliferation at four curcumin concentrations. A: Incubation for 1d. B: Incubation for 3d. Data presented as the mean ± SD (n=5). *P values < 0.05 were considered as statistically significant.

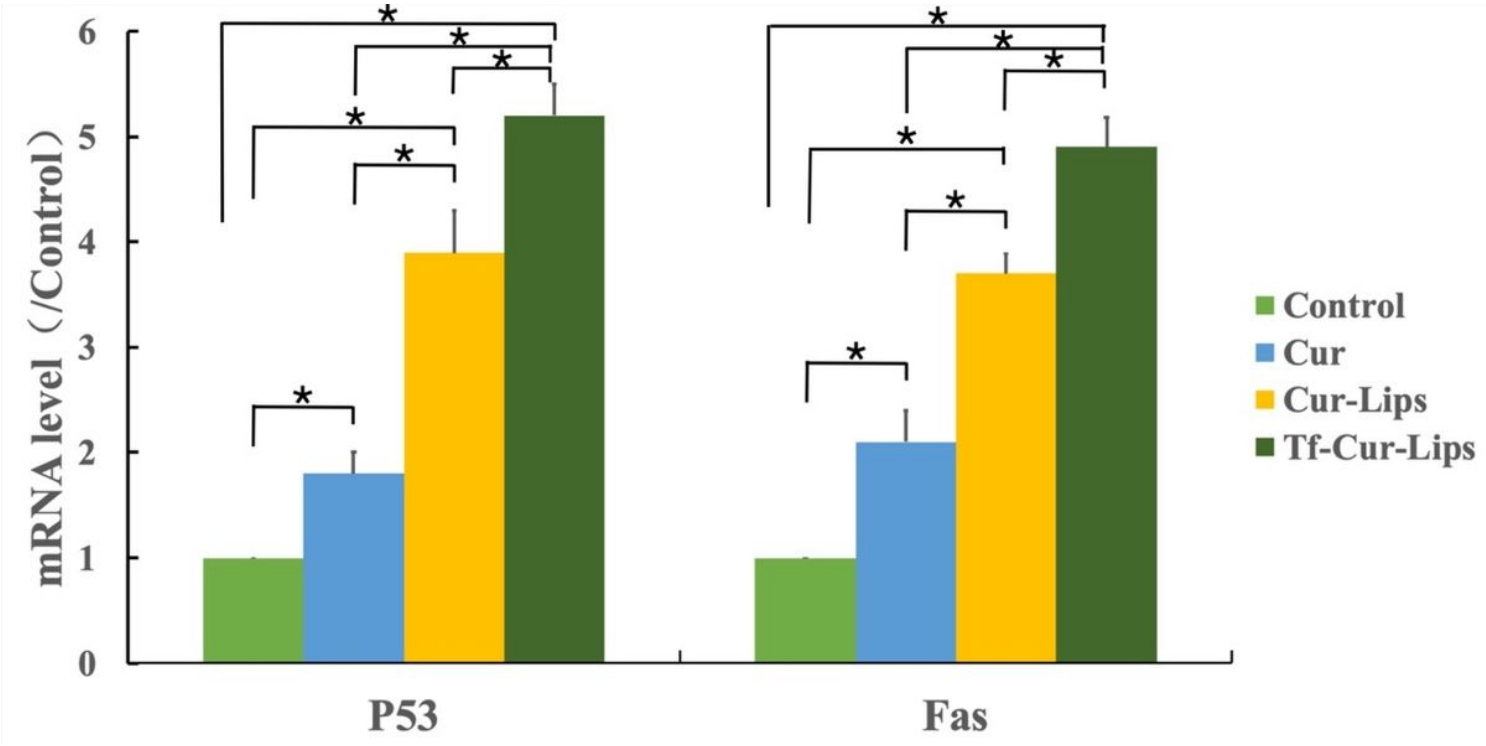

Figure 6

Effect of Cur, Cur-Lips and Tf-Cur-Lips on the expression of P53 and Fas gene of HN4 cells. Data presented as the mean ± SD (n=5). *P values < 0.05 were considered as statistically significant.